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Vaccines

VACCINES

Preventing Disease & Protecting Health

Ciro A. de Quadros, editor

Vaccines: Preventing Disease and Protecting Health celebrates the ways in which vaccines have played a role in improving the health of the world's populations. In early sections, the book relates successful efforts to fight disease with vaccines and looks at the challenges of using vaccines to cope with emerging and re-emerging diseases. In subsequent sections, the authors examine innovative efforts to test the efficacy of vaccines against diseases such as meningococcal infection in Africa, *Haemophilus influenza* type b, varicella, and hepatitis A, and look at efforts to develop a new generation of vaccines against cholera and typhoid, shigella, and *Helicobacter pylori*. The book also includes sections on the quest for vaccines against tuberculosis, HIV/AIDS, dengue, malaria, and hookworm; the use of vaccines to fight bioterrorism attacks; and regulatory, safety, and public health issues pertaining to vaccines.

The roster of authors reads like a "Who's Who" in vaccines and public health. Dr. Ciro A de Quadros, Director of International Programs at the Albert B, Sabin Vaccine Institute and Former Director of the Pan American Health Organization's Division of Vaccines and Immunization, ably edited the book and made valuable contributions to it.

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PREFACE

Throughout its history, the Pan American Health Organization (PAHO) has relied on vaccines to fight disease and improve health in the Americas. Early in the century, for example, there were impressive efforts to eradicate yellow fever and smallpox from the Region. But it was with the genesis of the Expanded Program on Immunization (EPI) in the late 1970s that the role of vaccines and immunization programs in improving the health of the people in the Americas took a quantum leap. Coverage rates skyrocketed, soaring from a paltry 10% to between 80% and 90%, on average; the number of vaccines routinely used in immunization programs steadily increased.

The countries of the Americas and PAHO, in a spirit of true Pan Americanism and in pursuit of equity, have worked through EPI to achieve dramatic successes. The Americas was the first region in the world to eradicate smallpox and polio, and measles is on the verge of being eradicated. These pioneering initiatives have made our Region a model and inspiration for the rest of the world. EPI has made invaluable contributions in terms of social mobilization and community participation, and it has left behind lasting lessons in developing models and tools for interagency cooperation. We will continue to strengthen EPI to ensure that its contribution to the health, information, surveillance, and local health systems endures well into the future.

The challenges ahead for vaccines and immunization programs are onerous. In the coming years, we will have to view infectious agents as natural risks to be dealt with in a globalized planet. We must move beyond simply trying to eliminate infectious agents to trying to reduce the vulnerability of individuals. Having attained survival through the natural selection of the few, we must now attempt to strengthen all in an equitable way. We must consider vaccination as a basic element in protecting health. In other words, we should not merely seek to alleviate suffering, we must aspire to improve the population's quality of life and well-being.

We also will have to face challenges in terms of the financial, political, and operational sustainability of immunization programs within complex and changing health systems. In this context, vaccines should become a basic right for our populations, not simply a tool for reducing illness. If we move in this direction, I have no doubt that vaccination programs and vaccine development efforts will gain new allies, thereby ensuring the po-

litical and financial sustainability of vaccines, and most especially, the ethical sustainability of vaccines.

VACCINES: Preventing Disease and Protecting Health looks at the success of historical immunization efforts; charts the future of vaccine development ventures targeting new diseases and involving new vaccine delivery systems; explores the role of vaccines in defending against bioterrorism; and examines regulatory, safety, and financing issues; as well as the future role of vaccines and immunization programs in public health. As such, it should become an invaluable weapon in the public health armamentarium for policy makers, academics, public health officers, scientists working in vaccine development, and, perhaps more importantly, for the indefatigable health workers and volunteers throughout the Region who have carried high the standard of public health's mission. Use it well.

—Mirta Roses Periago
Director
Pan American Health Organization

INTRODUCTION

The countries of the Americas have made tremendous strides in improving the health of the Region's peoples since the Pan American Health Organization was established just over 100 years ago. These improvements were due in great part to the implementation of national immunization programs. These programs, particularly those that operated over the last 25 years since the Expanded Program on Immunization was established in the Americas, have brought several vaccine preventable infectious diseases under control.

The Americas was the first of the world's regions to eradicate smallpox. Later, it also was the first to eradicate poliomyelitis, whose last indigenous case in the Americas occurred in Peru in 1991. This success led PAHO's Directing Council to set the goal of eradicating measles by the year 2000. As of this writing, more than one year has elapsed since the last indigenous case of measles was detected in Venezuela in September 2002. Recently, at its 44th Meeting, PAHO's Directing Council set a target for eradicating rubella from the Region by 2010.

Just as the disease eradication initiatives launched in the Americas have been expanded globally, innovative implementation strategies for immunization programs in the Region also have been emulated elsewhere.

Until a few years ago, immunization programs used just a few vaccines that had been developed several years ago. Among these were vaccines against diphtheria, tetanus, pertussis, tuberculosis, measles, and polio. Over the last decade, however, major advances in biotechnology made it possible to develop several new vaccines, and many candidate vaccines are now under way. Consequently, one of the challenges for health policy makers has been how to introduce these newly developed vaccines into national immunization programs. This is a particularly important issue, because new vaccines already available and those under development will certainly cost a great deal more than traditional vaccines already under use. A good example of this challenge has been the introduction of hepatitis B and *Haemophilus influenzae* type b vaccines, which were developed over 20 and 10 years ago, respectively, and only recently have started to be introduced in least developed countries. Latin American and Caribbean countries have pioneered the rapid introduction of these vaccines, thanks to the high-level political commitment of the governments and to financial mechanisms established by the PAHO Revolving Fund for Vaccine Procurement. The latter pooled the needs of all the countries,

thereby attaining economies of scale that allowed more favorable pricing. The Fund also allowed countries to pay off their debts in local currencies.

But the challenges ahead loom ever larger. Consider the vertiginous acceleration of vaccine development over time. For example, Jenner developed the smallpox vaccine in 1796, and it took about 100 years before Pasteur developed the rabies vaccine at the end of the 19th century. The first half of the 20th century, on the other hand, witnessed the development of several vaccines; the second half experienced an unprecedented leap in technology which allowed for the research and development of vaccines for more than 30 diseases, and real prospects for developing vaccines for diseases that were thought to be chronic and degenerative, but today are known to be the result of infectious diseases. Among these are vaccines targeting human papilloma virus, a major cause of cervical cancer, and *Helicobacter pylori,* which plays an important role in the pathogenesis of peptic ulcer and gastric cancer. The enormous progress in research and development in the field of vaccines makes us believe that the 21st century will be the "Century of Vaccines."

Given this accelerated progress, and to commemorate the Organization's first centennial, the Pan American Health Organization convened a conference so that experts at the vanguard in the field of vaccines and immunization could review the state of the art and look ahead to years to come. The conference, "Vaccines, Prevention, and Public Health: A Vision for the Future," was held at PAHO Headquarters in Washington, D.C., from 25 to 27 November 2002, and gathered more than 300 experts from the world over. The papers presented there marked the beginning of this book.

This book's chapters discuss progress made through vaccines used in most of the world's immunization programs, describe the status of introduction of the newest vaccines currently available to immunization programs, review progress in the development of vaccines against some bacterial and viral diseases that are responsible for much of mortality due to diarrheal and acute respiratory illnesses, as well as the quest for vaccines against HIV/AIDS, malaria, and dengue. A section addresses technological aspects of vaccine development, such as new concepts, including DNA vaccine technology, and new adjuvants and delivery systems. Diseases that may be used for bioterrrorism, such as smallpox and anthrax, also are discussed.

Because of the growing importance that regulatory issues bear in the development and use of vaccines and the increased interest of consumers in being better informed on the use of vaccines, the book presents a discussion on the regulatory and safety issues surrounding the development, production and utilization of vaccines.

In the last section, the book looks into the future, particularly to the economics of vaccines and immunization and the impact that some aspects of health reform processes may have in the sustainability of programs and the perspectives for future disease eradication.

This publication is a product of the work of the best scientists in their fields, who not only participated in the conference, but also gave of their

time and dedication to work on the chapters included in this book. The Pan American Health Organization, as a whole, and I, in particular, are grateful to them. PAHO also is grateful to the conference's sponsors and to all of those who helped make this book a reality.

In 1970, the Pan American Health Organization convened the "International Conference on the Application of Vaccines against Viral, Rickettsial, and Bacterial Diseases of Man." That Conference was the beginning of the Expanded Program on Immunization, the Children's Vaccine Initiative and the recently formed Global Alliance for Vaccines and Immunization. We hope that this book, likewise, will set the stage for several new initiatives in the field of vaccines and immunization, bringing more diseases under control and offering the world's peoples a healthier environment, as immunization is and will continue to be the most cost-effective health intervention in our medical armamentarium.

Finally, this book is dedicated to the thousands of health workers throughout the Americas, particularly those that deal with vaccines and immunization, who dedicate their lives to improving the lives of their fellow citizens.

Ciro A. de Quadros
Editor

Setting the Stage

VACCINES AND THE CHALLENGE OF EMERGING AND RE-EMERGING DISEASES: FROM HIV/AIDS TO BIOTERRORISM

Anthony S. Fauci[1]

As public health professionals know all too well, the threats posed by infectious diseases have not disappeared. World Health Organization (WHO) statistics indicate that infectious and parasitic diseases caused 26% of all deaths worldwide in 2001 (*1*). In terms of healthy life years lost, the situation is even worse. Infectious diseases strike the young disproportionately, causing approximately two-thirds of deaths among children younger than 5 years old (*2*).

Public health professionals also know that infectious disease threats constantly ebb and flow, as new diseases emerge and old ones reemerge in terms of their impact and geographic range (Figure 1). A brief consideration of four events that have occurred in the 100-plus years that PAHO has been in existence amply bears this out. Early in the last century, for example, the influenza A pandemic of 1918–1919 claimed more than 20 million lives worldwide. Today, the emerging HIV pandemic has yet to peak. One year before PAHO's 100th anniversary, the anthrax attacks of 2001 caused us all to confront the specter of the deliberate use of infectious agents to spread terror and death. Most recently, we were confronted by yet another newly emerging disease: the Severe Acute Respiratory Syndrome (SARS).

We have achieved remarkable successes with vaccines in the past, which have led to the eradication or near-eradication of several important diseases. We still confront two difficult issues, however. First, safe and effective vaccines are lacking for most emerging and reemerging infectious diseases, including those that may be used as agents of bioterrorism. Second, even when we have effective vaccines, they are not utilized worldwide as effectively as they might be. If we successfully meet these two challenges, we will significantly reduce the serious threats to public health that await us in the future.

This chapter will broadly discuss the fundamental importance of vaccines in meeting the challenges posed by emerging and reemerging diseases, as well as new strategies for vaccine development. It also will briefly discuss four emerging or re-emerging disease threats—HIV/AIDS, West Nile virus, bioterrorism, and SARS—and the role that vaccines will play in our efforts to cope with these threats.

VACCINE DEVELOPMENT

Vaccine development is an intensely collaborative endeavor. To move from concept to finished product requires the participation, to

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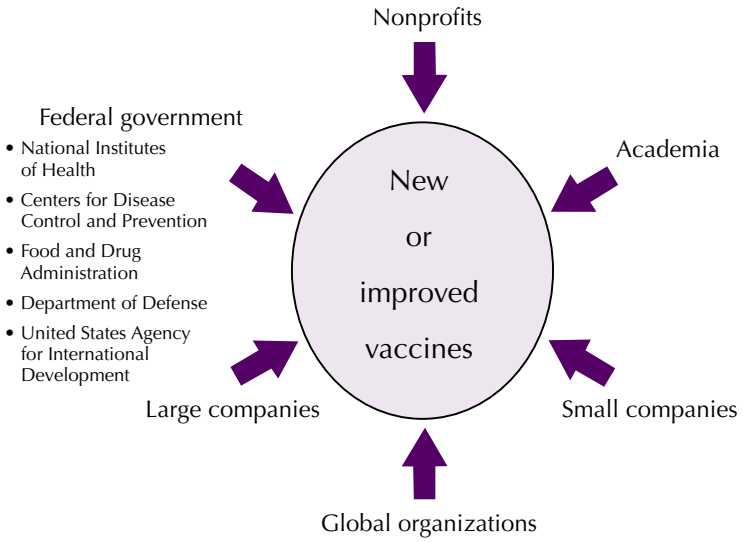
FIGURE 1. Worldwide geographic distribution of selected emerging and re-emerging diseases.

FIGURE 2. Players involved in vaccine development in the United States of America.



one degree or another, of government research and health agencies, nonprofit public health advocacy organizations, international groups such as PAHO, academic research centers, small biotechnology start-ups, and large pharmaceutical firms, among others. No single organization or group, acting alone, can possibly do all that needs to be done. Without extensive and productive teamwork among all groups involved, the efforts to develop safe, effective, and widely available vaccines that we need will not succeed (3).

The National Institutes of Health (NIH) and the National Institute of Allergy and Infectious Diseases (NIAID), in particular, play a significant role in this collaborative effort in the United States. The recently published 20th anniversary edition of NIAID's *The Jordan Report*, reviews recent developments in vaccinology and sets out a roadmap for the accelerated development of new vaccines in the future (4).

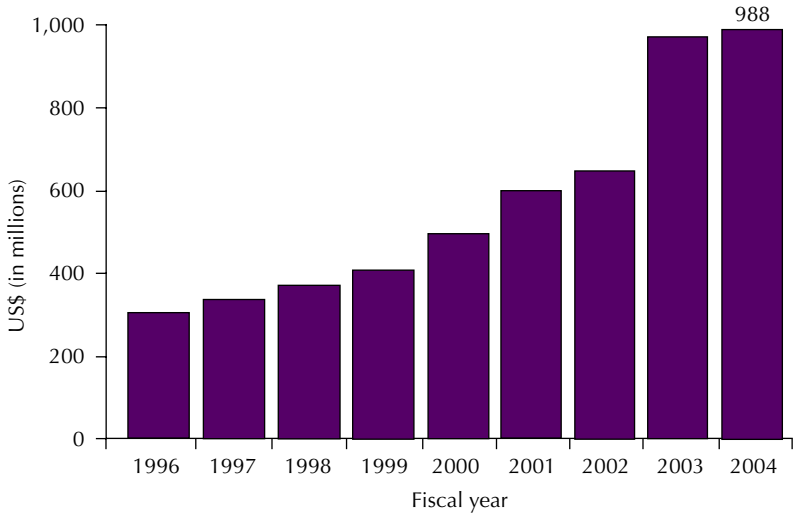
Developing new vaccines is a central part of the NIAID mandate. The foundation of vaccinology is basic research, and in that regard, NIAID is a major contributor. But many steps must be taken before a vaccine reaches field evaluation and product development. These steps require the participation of many other collaborators (Figure 2).

The NIH budget for vaccine development increased steadily in the mid-1990s, and accelerated sharply in fiscal year 2003 (Figure 3). The new resources for vaccine development in 2003 are available as a direct result of the growing understanding that bioterrorism is an extremely grave threat, and that vaccines are a vital part of our biodefense strategy.

New Strategies, New Opportunities

The increased NIH funding for vaccine research and development comes at an auspicious time. Vaccinology has taken a quantum leap in potential in just the past few years. As more resources are devoted to vaccine research and development, we are in an excellent position to capitalize on that new potential. New vaccine concepts go far beyond the classical approaches that were used so successfully in the past. Examples of new vaccine strategies include the use of recombinant proteins, noninfectious particles, replicons, recombinant viral vectors, peptides, and nucleic acid vaccines. The revolution in biotechnology also has

FIGURE 3. Growth of vaccine research funding at the National Institutes of Health, fiscal year 1996 to fiscal year 2004.



Note: Figure for 2003 is an estimate. Figure for 2004 is the funding level in the President's budget.

made available powerful new tools, notably whole organism genomic sequencing and post-sequencing functional genomics. Not only do we now have the full sequence of the human genome, but we also are rapidly sequencing a wide array of microbial pathogens. Consider the situation with malaria, for example, which kills more than one million people every year. We now have complete genome sequences for both *Plasmodium falciparum*, which causes the most severe form of malaria, and *Anopheles gambiae*, one of the most important mosquito vectors (*5*, *6*). Although these sequences will not provide answers in themselves, they will without doubt open many doors to new vaccine targets, not to mention better therapies and diagnostics.

Our growing understanding of the human immune system also is helping to accelerate vaccine development. This is especially true of recent insights into innate immune responses, which are evolutionarily older, less specific, and faster acting than the adaptive responses that have been the traditional targets of vaccines. As we come to understand innate immunity in more detail, and elucidate its relationship to the adaptive immune system, opportunities to create more effective vaccine adjuvants will emerge. For example, synthetic DNA sequences that contain repeated CpG motifs mimic the stimulatory activity that bacterial DNA fragments exert on the innate immune system. These sequences have recently shown promise as vaccine adjuvants that speed and strengthen immune responses (*7*). We can look forward to more progress of this kind as we continue to learn about the complex interactions between innate and adaptive immune responses.

HIV/AIDS

The HIV/AIDS pandemic is one of history's great scourges. At least 20 million people have already died of AIDS. More than 42 million people are currently living with HIV worldwide, according to the latest estimates of the Joint United Nations Program on HIV/AIDS (UNAIDS). The prevalence of infection in sub-Saharan Africa exceeds 30% in the adult population of some countries (*8*). The scope of the global pandemic is staggering, especially

when one considers that it is due to an emerging virus that was identified only 20 years ago.

The outlook for the future of this pandemic is grim if public health interventions to fight HIV/AIDS cannot be made more effective. Recent estimates suggest that by the year 2010 there will be 45 million new infections worldwide. By 2020, 70 million people will likely have died of the disease. The virus is set to expand explosively in many countries, including Russia, China, and India. In these populous and strategically important countries, even a modest increase in the infection rate would have potentially devastating consequences (*9*). In a country such as India, with a population of one billion people, for example, a change in the infection rate from 1% to just 2% would mean a huge increase in the number of people affected. The consequences would be devastating, and could dwarf what we already are seeing in sub-Saharan Africa. Such a scenario is likely if we stay on our current path.

The need for a safe and effective HIV vaccine, therefore, is clear. Such a vaccine would benefit countless individuals by preventing infection or by preventing, delaying, or ameliorating disease. A vaccine would have the obvious benefit to public health of slowing, if not reversing, this pandemic.

It is instructive to examine how the focus of HIV vaccine research has shifted since the epidemic began. HIV vaccine development started in the 1980s with monomeric envelope proteins intended to induce antibodies, sometimes coupled with novel adjuvants. We then moved to an increased emphasis on induction of cytotoxic T lymphocytes (CTLs), using recombinant viral vectors, DNA vaccines, and some novel peptides. Most recently, we have had encouraging preliminary results using other vectors, such as adenovirus and modified vaccinia Ankara (MVA). Thus, over the years the pendulum has swung from emphasis on antibodies without regard to CTLs, to perhaps too much relative attention to CTLs, to our current realization that both responses are very likely necessary to prevent infection and limit disease *(10)*.

A series of studies published over the past several years illustrates this pendulum swing clearly. For years, researchers have thought that a vaccine that could reduce an infected individual's viral load and that could slow progression to AIDS would be a very useful tool, even if it could not prevent infection. Three years ago, Barouch and colleagues (*11*) reported that a DNA plasmid vaccine was in fact able to prevent the progression of disease in macaques exposed to SHIV, a hybrid of SIV and HIV used extensively in vaccine research. All eight vaccinated monkeys became infected when challenged, but CTL responses soon reduced the viral load to very low levels, and prevented both the loss of CD4+ T-cells and the appearance of symptoms. Unfortunately, these same researchers reported last year that protection had failed in one case. A single point mutation appeared in a Gag protein CTL epitope that allowed the virus to escape suppression by CTLs. After this mutation appeared, the viral load increased, the CD4+ T cell count fell, and the monkey died (*12*). Similar "breakthroughs" in other vaccinated animals who previously kept the virus under control also have been reported by other groups.

These results are a reminder that we must not lose sight of the goal of inducing sterilizing immunity. Therefore, we must be even more aggressive in pursuing a combination approach to developing an HIV vaccine that is able to induce both protective antibodies and CTLs.

Another important issue in HIV vaccine research is the fact that there are multiple clades or subtypes of HIV around the world. Although there is some indication that antibodies directed against one clade or viral subtype can cross-react with epitopes from another, this is not likely to be sufficient to prevent infection. The NIH Vaccine Research Center took a significant step toward developing a multiclade vaccine, when it began a phase 1 study of a DNA vaccine containing *gag*, *pol*, and *env* gene sequences from the three most prevalent HIV clades in November 2002.

HIV vaccine research now faces several key scientific challenges. One is to improve vac-

BOX 1. The spectrum of HIV vaccine strategies.

- Viral surface proteins
- Live vecor viruses
- Combination of elements
- Naked DNA
- HIV peptides
- Live bacterial vectors
- Pseudovirions
- Replicons
- Whole, killed HIV
- Live, attenuated HIV

cine designs to elicit both broadly reacting neutralizing antibodies and cellular responses. Another challenge is to illuminate fully the correlates of immunity against HIV. Gaining a better understanding of why the immune system cannot contain HIV once infection occurs would be an important step forward. A third challenge is to continue moving forward with HIV vaccine efficacy trials. One candidate for phase 3 testing uses a prime-boost strategy, in which a recombinant poxvirus vector engineered to display HIV antigens is given first, followed by a boost with monomeric gp120 protein. Many other strategies to create an HIV vaccine also are being developed rapidly (Box 1), and the best way forward is not yet clear. We need to plan our next steps with great care, but also remember that the need to move ahead is urgent. We should, therefore, carefully consider conducting multiple efficacy trials simultaneously.

West Nile Virus

In the summer of 1999, West Nile Virus appeared in Queens, New York, and has since spread across the country (Figure 4). The arrival and rapid spread of West Nile virus has focused the attention of both the American public and the country's political leaders on the problem of emerging and re-emerging diseases. The speed with which West Nile virus advanced certainly was no surprise to those of us in the field of public health, but it did take most Americans by surprise. When we told political leaders that this virus was almost certainly going to march across the continent, they wanted to know how we could be so sure. The answer was simple. We knew that West Nile virus has been endemic in many developing countries for decades. Once the virus entered the United States, the virus, the vector, and the host were all present; therefore, predicting the outcome was not difficult. As the virus took hold in the United States, what we had predicted has in fact occurred: by October, 2003, the virus had reached 44 states. Statistics for the year 2003 from the Centers for Disease Control and Prevention (CDC) record 7,386 U.S. cases, with 155 deaths as of October 22. (*13*).

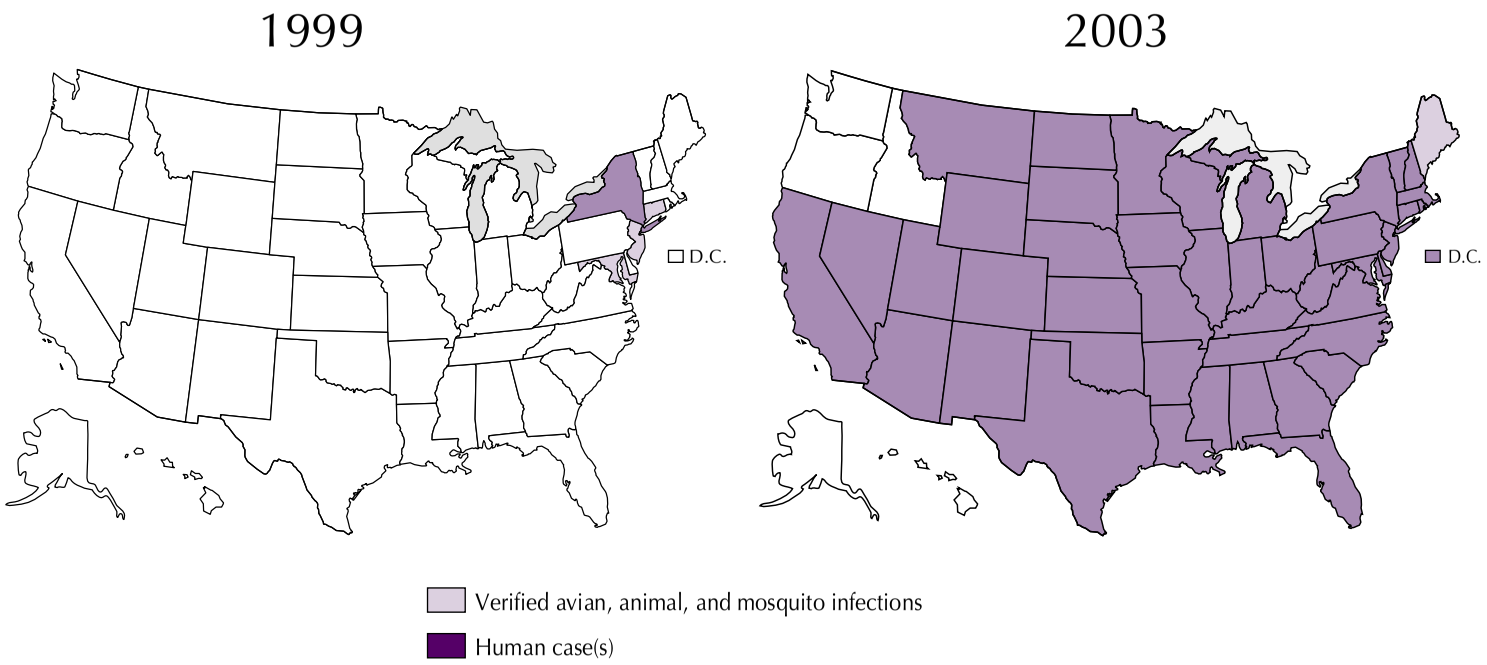
NIAID had a vigorous research program on West Nile and other flaviviruses before West Nile appeared in the United States, and this program has considerably increased recently. Our research agenda for West Nile virus includes basic research, antiviral therapy, vector biology, animal models, and rapid diagnostics. Development of a vaccine also will be a very important part of the overall effort. As an example of the rapid progress that can happen in vaccine development in the 21st century, we funded a fast-track project at Acambis to create a chimeric vaccine based on a yellow fever virus backbone engineered to display West Nile coat proteins *(14).* The preclinical data have been encouraging, and phase 1 testing in humans is imminent.

Biodefense

We now have yet another challenge before us, namely the threat of the deliberate spread of infectious diseases in the form of bioterrorism. This is a challenge not just for Medicine in the United States, but also for Medicine and Public Health throughout the world (*15*).

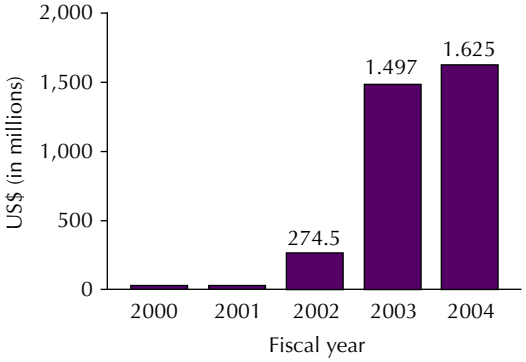
We already have accomplished a great deal. NIH funding for biodefense research has increased dramatically in just two years, going from less than US$ 275 million in 2001 to US$ 1.55 billion in fiscal 2003 (Figure 5). This

FIGURE 4. Spread of West Nile virus in the United States, 1999 to 2003.



Source: Centers for Disease Control and Prevention, Atlanta, Georgia.

FIGURE 5. Growth of biodefense research funding at the National Institutes of Health, fiscal year 2000 to fiscal year 2004.



Note: Figure for 2003 is an estimate. Figure for 2004 is the funding in the President's budget.

Source: National Institute of Allergy and Infectious Diseases (NIAID), Bethesda, Md.

represents the sharpest increase in support for any single discipline in the history of NIH.

I disagree with those who say this increase is inappropriately disproportionate. Instead, it serves to demonstrate what can be accomplished when the public and political leaders are motivated to address a serious public health threat. The growing awareness of the threat posed by deliberate use of pathogens for terrorist purposes also has served to help people understand the threat of other emerging and re-emerging microbes.

Effective use of such a massive increase in funding obviously requires careful planning, and NIAID has worked very hard to create strong strategic plans to guide our biodefense research efforts *(16).* As we execute these plans, however, we are faced with the question of how to apply classical public health research programs to biodefense. To that end, we are paying extraordinary attention to how best to translate basic research into useful products.

In discussions that I had in 2002 with President Bush and Secretary of the Department of Homeland Security, Tom Ridge, I was asked what NIAID could do with an infusion of more than a billion dollars. It was abundantly clear that my answer needed to go beyond promising to simply gain knowledge, to "learn a lot." Instead, we must use these funds to translate knowledge that we have gained through basic research into clearly definable end points, into products and procedures that make us more prepared to cope with a biological attack (*17*).

Bioterrorism holds some lessons for other emerging and re-emerging diseases. If anything good can be said to have come from the threat of bioterrorism, it is that it has reinforced the importance of developing vaccines for all groups of citizens, including the young, the old, the infirm, pregnant women, and immuno-suppressed people.

Our efforts to confront the threat of a deliberate release of smallpox reflect this growing awareness, and the urgency with which we are proceeding. The speed with which we have addressed the threat of smallpox is by any standards impressive. Consider our vaccine stockpile, for example. In late 2001, CDC held 15.4 million doses of Dryvax, a lyophilized preparation of vaccinia virus. We quickly showed that this material can be diluted fivefold and still maintain its potency; the data indicate that a ten-fold dilution would also induce an adequate immune response (*18*) (Table 1). These findings immediately increased our effective stockpile to at least 77 million doses. Aventis Pasteur then donated 75 million doses of another live vaccinia vaccine. Testing of this material indicates it, too, remains highly immunogenic, and that it can be diluted fivefold and retain its potency. We also quickly contracted to purchase more than 200 million doses of a second-generation smallpox vaccine based on live vaccinia manufactured with modern cell culture techniques. We expect delivery of this material by the end of 2003, and it should be fully licensed for use by mid-2004. This will bring our total stockpile to well over 600 million doses.

We are now struggling with the balance of risk versus benefit for live vaccinia vaccines, relying on data gained decades ago during the smallpox eradication campaign. The best historical data we have indicate that for everyone

TABLE 1. Rate of success[a] of initial and repeated vaccination with Dryvax.

Vaccine	No. of subjects	Success of initial vaccination (%)	Success of initial or subsequent vaccination (%)
Undiluted	106	97.2	97.2
1:5 dilution	234	99.1	100.0
1:10 dilution	340	97.1	98.8

[a] Success was defined by vesicle formation 7–9 days after inoculation.

Source: Frey SE, *et al.* Clinical responses to undiluted and diluted smallpox vaccine. *N Engl J Med* 2002;346(17):1265–1274.

million people vaccinated, between 14 and 52 people will suffer serious, life-threatening complications from the vaccine and one or two people will die (*19*). We have taken steps to augment our ability to treat vaccine complications by increasing the stockpile of vaccinia immune globulin, and we have data that indicate that cidofovir, a drug developed to treat cytomegalovirus infection in HIV-infected individuals, might be helpful in treating both smallpox itself and vaccine complications (*20*). The high rate of complications poses a difficult policy conundrum, however. If the vaccine had a better safety profile, a national vaccine program would almost certainly already have taken place and been completed. Clearly, we need a safer vaccine. One promising candidate is modified vaccinia Ankara (MVA). This is a highly attenuated live vaccinia virus that can be given by injection rather than scarification. MVA cannot replicate in most mammalian cell lines, although it elicits a significant immune response in animal models. Historically, it has an excellent safety profile, including when it is used in at-risk groups such as immuno-compromised people. Several other candidate smallpox vaccines are also in development, and NIAID will test the most promising at the NIH Vaccine Research Center and in the network of NIAID Vaccine and Treatment Evaluation Units.

SARS

In 2003, the world confronted yet another new infectious disease threat—Severe Acute Respiratory Syndrome (SARS), caused by a previously unidentified coronavirus. SARS was first reported in Asia in February 2003, although the first cases were thought to have occurred in the Chinese province of Guangdong in November 2002. Over the next few months, the illness spread to more than two dozen countries in North America, South America, Europe, and Asia. As of 26 September 2003, a total of 8,098 SARS cases and 774 SARS-related deaths had been reported to WHO (*21*). The SARS global outbreak of 2003 was contained; however, it is possible that the disease could re-emerge. Hence, concerted efforts are under way around the world to improve public health preparedness and to develop safe and effective SARS diagnostics, treatments, and vaccines. NIAID is supporting the rapid development of vaccines to prevent SARS through both extramural and intramural programs, including the NIAID Vaccine Research Center on the NIH campus. Our initial focus was on the development of an inactivated virus vaccine akin to those that have worked well against many other viral diseases. Other types of SARS candidate vaccines are also in the development "pipeline," including approaches such as vector-based and recombinant vaccines, and DNA-based vaccines *(22).* Fortuitously, vaccines against common veterinary coronaviruses are routinely used to prevent serious diseases in young animals, such as a vaccine given to pigs to prevent serious enteric coronavirus disease. Insight from veterinary coronavirus vaccines could prove useful as we develop vaccines to protect humans, and provide hope that a useful human SARS vaccine can be developed.

Conclusion

In closing, I want to remind us all that our need to create vaccines to counter infectious diseases will never be over. Years ago, my predecessor as Director of NIAID, Richard Krause, published a series of essays called *The Restless Tide* (*23*). In these essays, he makes the point that we are all, as members of the human species, continually vulnerable to a restless tide of emerging and re-emerging diseases. In our lifetime, we have first hand experience with this restless tide; the role of vaccinology in allowing us to navigate this tide is profound.

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A CENTURY OF VACCINES AND IMMUNIZATION IN THE AMERICAS

Ciro A. de Quadros[1]

This chapter discusses the immunization activities undertaken in the Region of the Americas over the last century, particularly those launched in the last quarter century, when the countries of the Americas accelerated their immunization-related activities. A century ago, in 1902, Walter Reed first identified that yellow fever was transmitted by a mosquito. The first yellow fever vaccine was developed in New York, by Max Tyler in 1937, and it was used in Brazil in the same year.

Subsequently, there were several disease eradication efforts initiated in the Region of the Americas (Table 1). General William Crawford Gorgas launched the first one in 1911, to eliminate yellow fever. It was followed four years later by the Rockefeller Commission's proposal for the global eradication of yellow fever. Fred Soper later proposed the eradication of smallpox in the Americas, and the Region became the first to eradicate the disease. The experience in the Americas led to an initiative for the global eradication of smallpox, which was successfully accomplished in 1977, after a ten year campaign spearheaded by Donald A. Henderson (*1*). More recently, the Region of the Americas successfully eradicated polio, and this major accomplishment led to the launching of a global polio eradication initiative. Finally, in 1994, the Ministers of Health of the Americas launched the measles eradication initiative, as a result of which, that disease is on the verge of being eradicated in the Region. The failure to eradicate malaria from the Region stands out among these decades of success in the efforts to eradicate disease in the Americas.

Immunization programs throughout the world, and particularly in the Americas, have been extremely successful in increasing immunization coverage. In 1970, the year that PAHO convened the International Conference on Vaccines Against Viral, Rickettsial, and Bacterial Diseases of Man, immunization coverage rates were under 10% for the scant vaccines that were being used in the Region's programs—basically DPT, BCG, polio, and tetanus toxoid. Today coverage hovers between an average of 80% to 90% for the vaccines being used, which now include many additional vaccines, such as measles, rubella, mumps, *Haemophilus influenzae* type b, and hepatitis B.

Ten years have elapsed since the last case of indigenous poliomyelitis occurred in the Region of the Americas (Figure 1) (*2*). In 2001–2002, there was a re-emergence of poliomyelitis in the Dominican Republic and Haiti. The small outbreak was due to a vaccine-derived polio virus, not a wild polio virus re-introduction; it was very quickly controlled. The challenge now is to sustain the political commit-

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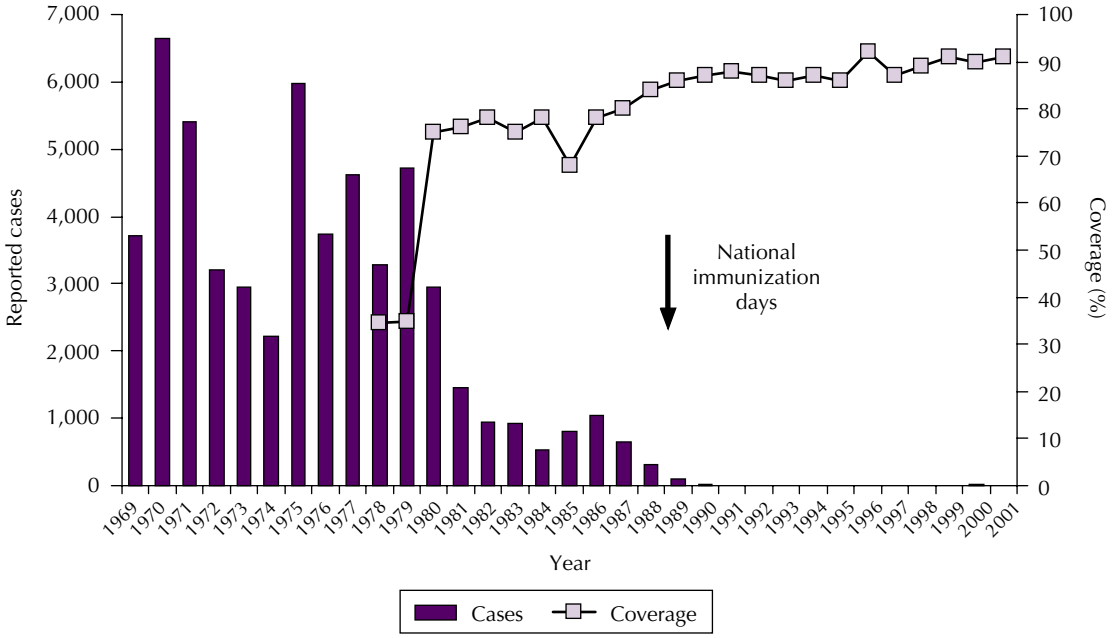
TABLE 1. Disease eradication initiatives, Region of the Americas and worldwide, 1911–1994.

Year	Initiator	Disease	Scope
1911	William Crawford Gorgas	Yellow fever	Region of the Americas
1915	Rockefeller Commission	Yellow fever	Worldwide
1950	Soper	Smallpox	Region of the Americas
1958	Viktor M. Zhdanov	Smallpox	Worldwide
1955	WHO	Malaria	Worldwide
1985	PAHO	Polio	Region of the Americas
1988	WHO	Polio	Region of the Americas
1994	PAHO	Measles	Region of the Americas

ment for continuing vaccinating against a disease that has already disappeared and strengthening surveillance so that events such as the one in the Dominican Republic and Haiti can be promptly detected and controlled (*3*).

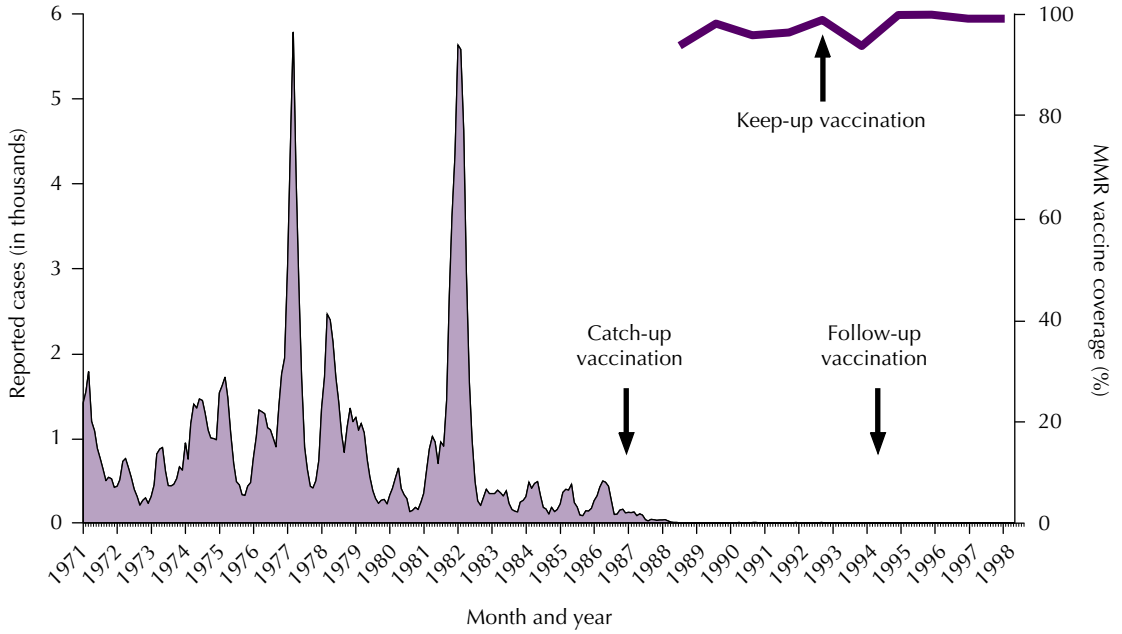
Measles is on the verge of being eradicated in the Americas. The strategy being utilized to eradicate measles in the Region was first tried by Cuba with a "catch up" vaccination campaign targeting all children 1 to 14 years old, "keeping up" with a very high level of coverage in new cohorts of children, and periodic "follow up" campaigns every four years targeting children 1–4 years old. The strategy is designed to prevent the accumulation of susceptibles as the vaccine is not 100% efficacious (Figure 2) (*4*). There were more than one-quarter of a million cases of measles in the Region

FIGURE 1. OPV3 vaccination coverage and incidence of paralytic poliomyelitis, Region of the Americas, 1969–2001.



Note: Coverage data are for children <1 year of age. Type 1 vaccine derived virus in 2000 and 2001.
Source: Pan American Health Organization, Immunizations Unit.

FIGURE 2. Reported measles cases, by month, Cuba, 1971–1998.



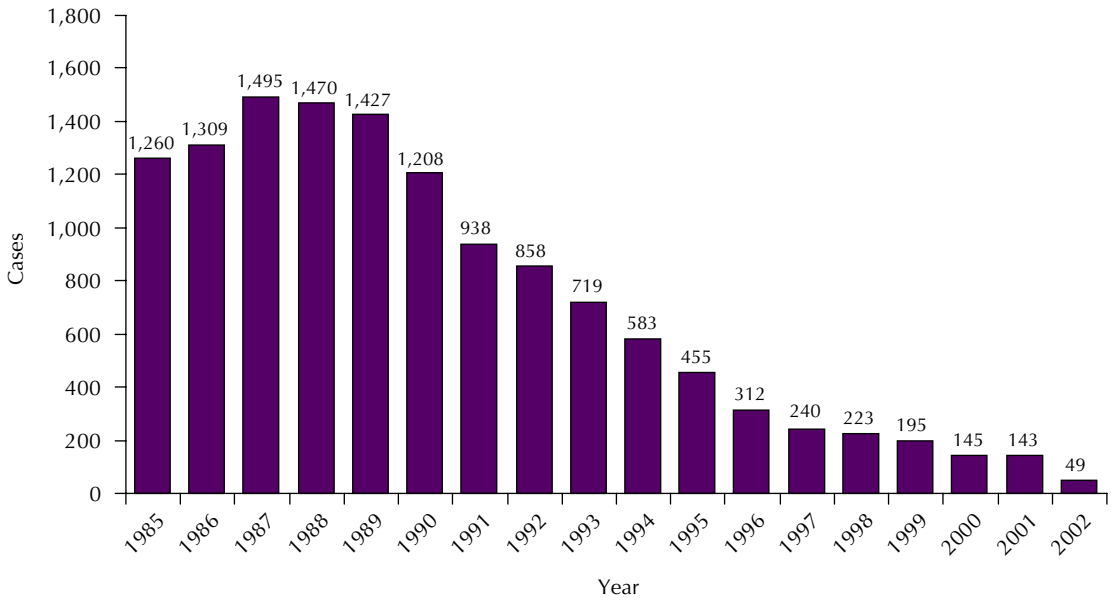
Source: Ministry of Health, Cuba.

of the Americas in 1990. Given inadequate surveillance, this number could well increase five- or tenfold. In 2001, there were only 545 cases in the Region, half of them in the Dominican Republic and Haiti, and another 25% in Venezuela. Venezuela, which had been free of indigenous measles transmission for more than two years, suffered an importation from Europe. This generated a sizeable outbreak because Venezuela's immunization program was not adequate at that time. In 2002, as of November 16, there were 2,548 cases reported, 94% of them in Venezuela and 5% in Colombia along the border with Venezuela, due to importation from the latter country. The few cases in other countries were all related to importations from Europe, Asia, or other regions. Thanks to the extraordinary efforts of the governments of Colombia, Venezuela, and of all the Region's countries in 2002, for the first time in history 10 weeks have elapsed without measles transmission detected anywhere in the Region. Colombia's last case occurred in week 36, and Venezuela's, in week 38. The Region of the Americas is on the verge of interrupting indigenous measles transmission.

Neonatal tetanus is under control in the Region, with an annual case average between 50 and 100 (Figure 3). These cases occur in fewer than 1% of the more than 13,000 districts in the Region. The Americas has been the first one to achieve the goal of less than one case per 1,000 live births in every district of each country that was set at the Children's Summit. Countries now are focusing on those few districts which still show cases and on the elimination of missed opportunities to vaccinate women of childbearing age. The latter is particularly important since most of the cases of neonatal tetanus are in multiparous women, indicating that they have visited health centers in previous pregnancies and were never vaccinated.

Cases of pertussis and diphtheria have declined steadily over the last few years. Rubella

FIGURE 3. Neonatal tetanus cases per year, selected countries in the Americas, 1985–2002.[a]



[a] Data as of week 26.

Note: Countries with cases in the last three years: Argentina, Bolivia, Brazil, Colombia, Dominican Republic, Ecuador, El Salvador, Guatemala, Haiti, Honduras, Mexico, Nicaragua, Panama, Paraguay, Peru, and Venezuela.

Source: Country reports.

and congenital rubella syndrome (CRS) are now under attack. Almost every Latin American country now includes vaccination against rubella in their national immunization programs, and the two or three countries that still do not include this vaccine have plans to introduce it next year.

Rubella and measles surveillance have been combined. A few countries already have succeeded in interrupting rubella transmission. Cuba was the first, when they used MMR vaccine in children 1 to 14 years old and rubella vaccine in the population 15 to 29 years old as part of their measles catch up campaign in the late 1980s. Then, in the early 1990s, countries in the English-speaking Caribbean vaccinated all males and females up to 39 years of age with MMR vaccine, and recently Costa Rica held a similar, very successful campaign (*5*). Other countries, like Chile and Brazil, have started vaccinating all women of childbearing age. During a recent meeting, the PAHO Technical Advisory Group on Immunization recommended that countries implementing mass campaigns against rubella should target both female and male populations as a way to interrupt rubella transmission. I believe that rubella will be the first disease to be eradicated in the Americas during PAHO's second centennial.

Hepatitis B is now under attack, with practically every country having included hepatitis B vaccine in their national programs, many of them using it in a combination vaccine with DTP and/or DPT/Hib.

When the Pan American Health Organization was created, one of the major issues was yellow fever, and the yellow fever vaccine was the first vaccine used in the Region. And now, 100 years later, yellow fever continues to be a threat in the Region. Cases have been declining over the past 15 to 20 years, however, due to major vaccination activities that have been conducted in countries like Bolivia, Brazil, Colombia, Peru, and Venezuela. In Brazil, for

example, nearly 80 million people have been vaccinated throughout the country in the past five years. The PAHO Technical Advisory Group recommends that vaccination coverage be maintained at a very high level in the enzootic zones, as well as in those contiguous areas that are infested with *Aedes aegypti*, which now can be found throughout the hemisphere. All travelers to those areas also should be vaccinated, particularly now, with the emergence of ecotourism. About one third of cases in Brazil have been tourists going to those areas. Surveillance must also be strengthened.

In the last five years, the *Haemophilus influenzae* type b vaccine also has been introduced (*6*). In 1996, the vaccine was used only in Canada, Chile, the United States, and Uruguay. Uruguay was the first Latin American country to introduce this vaccine, followed by Chile. The success of this vaccine in Uruguay and Chile showed that it was very efficacious, and this encouraged other countries to introduce it. At present, 90% of newborns in Latin America live in countries where this vaccine is used in the national vaccination schedule (Figure 4).

The model used to introduce *Haemophilus influenzae* type b vaccine in the Americas is important for the future introduction of new vaccines now in the pipeline. Components of this model included strong national and regional immunization programs, the population's high degree of awareness of the importance of immunization, a very safe and efficacious vaccine, and an awareness about the disease among health professionals and parents. The Pan American Health Organization's Directing Council approved a resolution urging governments to utilize the vaccine. The Directing Council also promoted the use of the vaccine through its Technical Advisory Group meetings and through publications on the impact of the vaccine. Last but not least, the use of the PAHO Revolving Fund for purchasing the vaccine led to economies of scale that made the vaccine available at an affordable price.

The result was the striking difference between the Americas and other regions of the world in the utilization of this vaccine. If we look back a few years, immunization schedules were very similar in the developed and developing worlds. However, as new, higher-cost vaccines became available, a gap started to open between these two groups of countries, with developing countries lagging behind in the use of these new vaccines. In the Region of the Americas, however, this gap has been rapidly closing due to the high commitment of the governments (Figure 5). This commitment is attested to by the fact that between 1987 and 1991 there was investment of more than US$ .5 billion in immunization programs in the Region, with about US$ 430 million coming from national budgets and about US$ 114 million coming from international partners and collaborators (*7*). In the next five-year period, between 1992 and 1996, the countries greatly increased their national budget contributions, thus diminishing the need for external support. This pattern was repeated in the next five-year period (1997–2001), and it is estimated that the trend will hold in 2002–2006, a period that will require even more resources, given the introduction of *Haemophilus influenzae* type b and hepatitis B, as well as influenza, pneumonia, and varicella vaccines that have been introduced in a few countries.

As we look into the future to PAHO's second century of work, there will be many more choices, because so many vaccines will become available. There will be new target populations for vaccination, from children through adults to grandparents. This will require better communication about the need for vaccination, its benefits, and risks, particularly now with the emergence of anti-vaccine lobbies that are questioning the use of the vaccines, especially as diseases are brought under control.

Among the challenges ahead is the need to have a legal basis for the financial and political sustainability of public health priorities at all levels. Vaccines should be seen as a public good with adequate and sustainable financing over time, with a specific line in the national budgets. In the Americas, over the last few years, many countries have enacted such laws to protect the budget for the national immu-

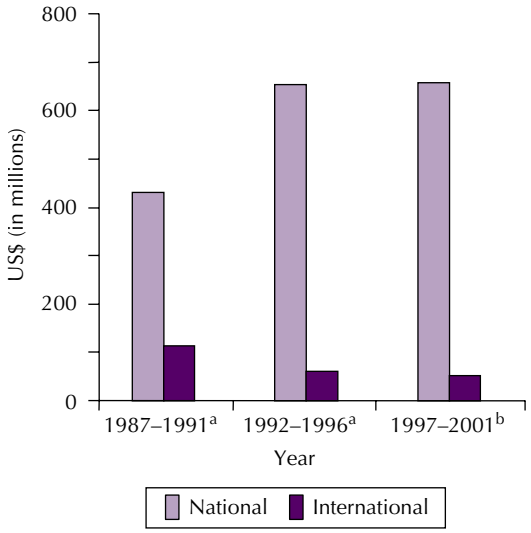
FIGURE 4. Introduction of *Haemophilus influenzae* vaccine in the Americas, 2001.[a]

[a] 15,889,000 vaccinations in 2001, representing
- 92% of all newborns in the Region and
- 89% of all newborns in Latin America

nization programs. Other challenges include the strengthening of the managerial capacity at the local level, particularly in an environment of decentralized health systems, and the use of indicators that can measure the impact of the program at the lowest level of the countries, in order that inequities can be promptly identified and acted upon.

For PAHO, the major challenge is to maintain the achievements attained by the countries so far, to make every effort to introduce the new vaccines as they become available, to

FIGURE 5. National and international expenditures in immunization programs in the Americas, 1987–2001.



[a] Includes polio eradication.
[b] Includes measles eradication (estimate).

move into adult immunization, and to look at the safety of vaccination.

Finally, the success of the immunization programs in the countries of this Region would have been impossible without a major partnership that included all the countries and territories of the Americas, countless institutions and organizations—nongovernmental, bilateral, and multilateral—and PAHO's regional coordination over the last 100 years, particularly over the last 25 years.

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Part I
The Present

POLIO: PRESENT STATUS AND POST-ERADICATION POLICIES

Daniel Tarantola[1]

The story of polio and its disappearance in many parts of the world is one that could not be told without speaking of the unique and historic partnership that has brought us to this critical stage: the final stretch of polio eradication. This core partnership consists of Rotary International, the U.S. Centers for Disease Control and Prevention, UNICEF, and the World Health Organization (WHO). There are also many others, such as the International Federation of Red Cross and Red Crescent Societies, whose millions of community volunteers have contributed to this work. The U.S. Agency for International Development and many other national and international entities have likewise provided valuable support to global polio eradication efforts. This chapter will describe the progress to date achieved by local, national, and international partners in interrupting polio transmission. It will also present various evolving issues related to global certification and will discuss the development of post-certification immunization policies.

Interrupting Polio Transmission

The Technical Consultative Group on the Eradication of Poliomyelitis (TCG), an independent body that oversees the work of WHO and its partners towards poliomyelitis eradication, has reminded us that interrupting polio transmission must remain our top priority (*1*). Since the polio eradication initiative was launched in 1988, the number of polio cases has been drastically reduced from the more than 350,000 cases reported that year. As of November 2002, for example, reported cases had dropped to 1,100, representing a greater than 99% reduction. By the same token, the number of polio-endemic countries also had dropped, from some 125 in 1988 to a mere 7 as of November 2002. As of this writing, 209 of the world's 216 countries, territories, and areas are polio-free.

Since the 1988 adoption of the World Health Assembly resolution to eradicate poliomyelitis (*2*), three WHO Regions have now been certified as free of poliomyelitis: the Americas was first in September 1994; followed by the Western Pacific in October 2000; and, most recently, Europe in June 2002. The number of reported cases worldwide had fallen to 483 in 2001, but rebounded to 1,127 in 2002. India and Nigeria together account for 85% of these cases. Most of the progress over the last couple of years has been achieved in the African Region. It is also important to note that Bangladesh and the Democratic Republic of the Congo have not experienced polio transmission for two years, which is quite remarkable, given the population sizes of these countries. Wild type 2 poliovirus has not been detected since October

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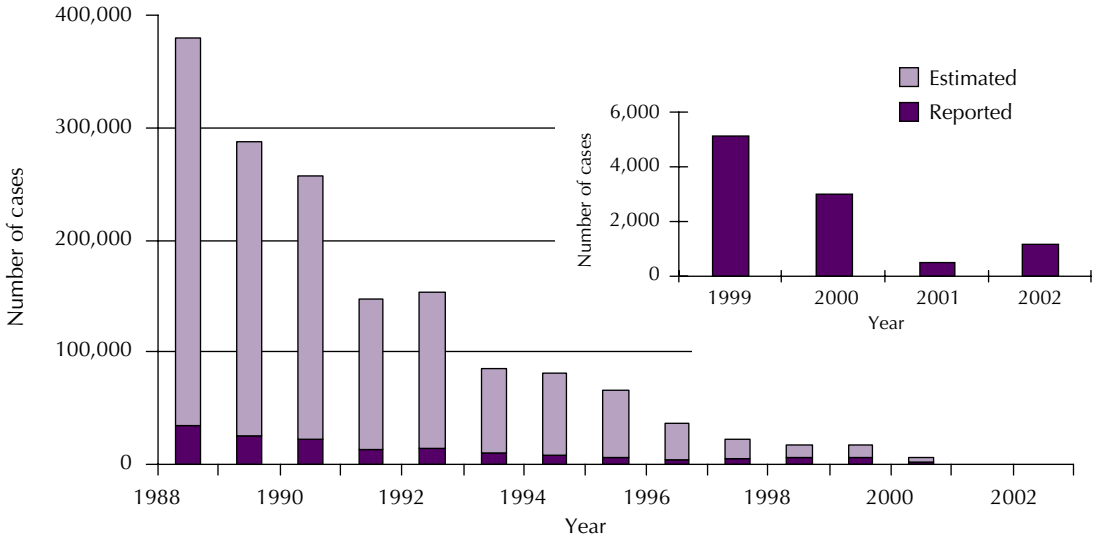
FIGURE 1. Number of estimated and reported[a] cases of polio worldwide, by year, 1988–2002.



[a] Reported cases in 2001, 483; reported cases in 2002, 1,127.

1999, indicating that the eradication of polio is feasible even in the most challenging settings. The emergence of vaccine-derived poliovirus (VDPV) type 2 in Madagascar in 2002, however, serves to remind us that as long as we have polio in the world and we use oral polio vaccine (OPV), there is always a risk of re-emergence of the poliovirus.

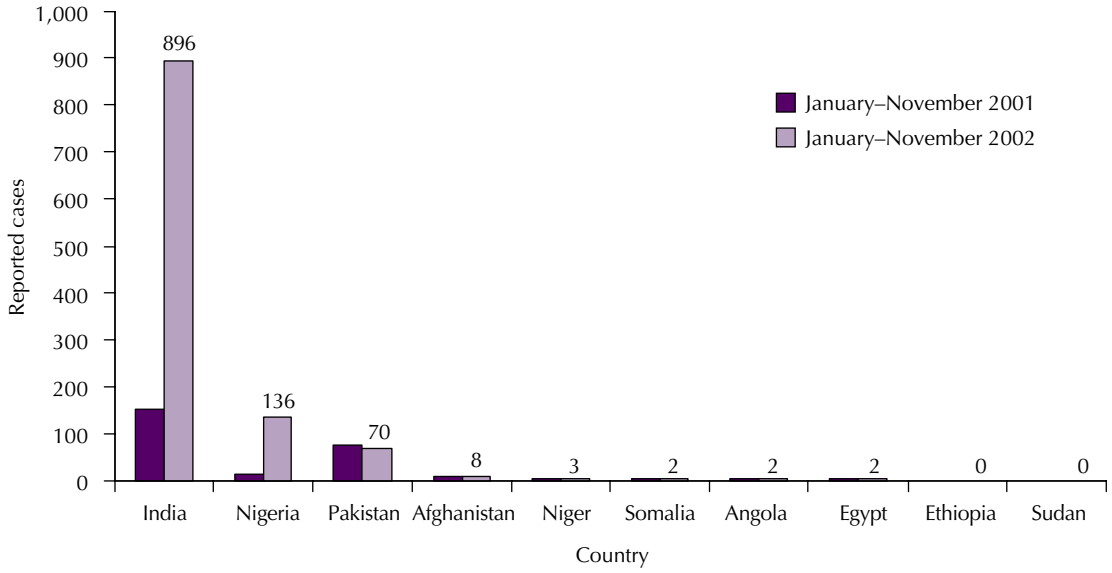
The number of polio cases fell dramatically between 1988 and 2001 (Figure 1). Then, in 2002, an upsurge in case numbers occurred that is related to low vaccination coverage, mostly in ethnic minority or other underserved populations. The high transmission areas continue to be India, Nigeria, and Pakistan. More than 80% of all reported cases in 2002 were found in just six of the 76 states/provinces in these three countries.

There are also some areas of low transmission. One is Egypt, where a new case was reported as of September 2002, after over a year without reported cases. Sewage samples have consistently shown that the wild poliovirus was circulating in the environment, however. A combined international-national evaluation has recommended that the quality of surveillance in Egypt should be improved, and this currently is being implemented.

It is important to note two new polio-free areas—Ethiopia and Sudan—where no cases have been found for at least 18 months (*1*). There are also areas where the number of cases is declining, including Afghanistan, Angola, Niger, Pakistan, and Somalia. The current concentration of cases continues to remain in northern India, particularly in the states of Uttar Pradesh and Bihar, and in the northern part of Nigeria, where Kano and Kaduna states account for over 50% of that country's cases. Hence, these areas will be a principal focus of the global partnership's efforts, particularly through the strengthening of immunization coverage and its extension to populations currently not being reached.

It is encouraging to note that tremendous efforts are currently under way in both India and Nigeria to reduce case numbers. India has developed programs to organize local health workers, who are now assigned to carry out social mobilization activities at both the district

FIGURE 2. Cases of wild poliovirus, selected countries, 2001 and 2002.[a]



[a] The comparison between 2001 and 2002 was made at 12 November each year.
Note: 80% of cases are in 6 of the 76 states/provinces of India, Nigeria, and Pakistan.

and block levels. It should be noted that the groups most disproportionately affected with poliomyelitis are minority Muslim communities living within a majority Hindu population. In an effort to reach this group appropriately and efficiently, Muslim leaders and grassroots organizations are being brought into the planning and implementation of supplementary immunization activities. In Uttar Pradesh, the number of vaccination teams with at least one female member is increasing, as is the number of teams with a third member being drawn from the community.

Figure 2 presents the relative contribution by country to the global burden of polio in 2001 and 2002 and illustrates that polio is currently more geographically contained than ever before. The major contributor to polio worldwide is India, followed by Nigeria and Pakistan. Several other countries have reported or detected fewer than 10 cases during this period. As stated earlier, more than 80% of all reported cases of polio for 2002 were found in just 6 of the 76 states/provinces of India, Nigeria, and Pakistan.

Until 2002, India had made tremendous progress in its efforts to eliminate polio. In 1999 there were 192 infected districts in the country. This figure plummeted to 89 in 2000 and to 62 in 2001, clearly demonstrating that it is possible to eliminate poliovirus from India. Unfortunately, however, the number of infected districts here had rebounded to 262 districts by the end of 2002.

At the global level, polio surveillance has improved considerably, yet signs of complacency remain in some areas. Surveillance indicator goals have been met in a large number of countries. However, in the Americas, polio surveillance deteriorated between 2001 and 2002, reminding all those committed to global polio eradication that there can be no room for complacency. Surveillance systems must remain consistently vigilant, although this sometimes presents unique challenges of its own in areas that have been free of polio for a long pe-

riod of time. The global partnership will be observing the Americas' situation closely so as to learn from its experience. Certainly in the future, PAHO and its Member Governments will be able to provide important lessons on how fatigue and complacency may be overcome to maintain high surveillance levels.

Other areas of concern remain, as well. One is the populated island of Papua New Guinea, where the combination of poor surveillance and a low vaccination rate greatly increase the likelihood of an emergency situation, such as an outbreak of VDPV. Another potentially dangerous situation exists in Madagascar, where the adequacy of stool collection varies from one region to the next. A third area of concern is South Africa, which was the first African country to eliminate polio, but which currently shows a tendency towards complacency and lack of compliance with epidemiological standards. Similarly, acute flaccid paralysis (AFP) surveillance still remains below standard levels in some parts of Central Africa.

Global Certification

The 51 countries of WHO's European Region were certified as polio-free in 2002, meaning three full years had passed without wild type 2 poliovirus emerging in the Region. In other parts of the world, Bangladesh and Democratic Republic of the Congo had reported having had two years without polio transmission as of 2002. Therefore, at the current time, there remain three critical reservoirs globally—northern India, northern Nigeria/Niger, and Pakistan/Afghanistan. These reservoirs are likely to sustain transmission throughout 2003, unless surveillance and immunization programs that have failed to meet global standards in the past are appropriately mobilized and revitalized.

In January 2002, the TCG recommended that polio-free countries which border endemic areas or have very low immunization coverage should continue to conduct National Immunization Days (NIDs) or Sub-National Immunization Days (SNIDs), as appropriate, on an annual basis. It further recommended that countries which have been polio-free for at least three years, but have not achieved or maintained greater than 90% coverage levels, should continue to conduct NIDs at least once every three years to prevent the accumulation of susceptibles. These recommendations were again reiterated at the most recent meeting of the TCG in November 2002. The TCG noted its extreme concern regarding the situation developing in India and Nigeria and set specific standards for the reinforcement of immunization activities.

It is estimated that more than one million childhood deaths and five million cases of poliomyelitis have been averted since the global eradication effort was launched in 1988. Evidence further suggests that polio eradication is feasible in the near future if there is sufficient and sustained national and international commitment. The single-most threatening obstacle to the eradication goal is a funding gap of US$ 275 million that will be needed to support polio activities for 2003–2005. The partnership's collective efforts to bridge that gap have shown promise, but the road will not be easy and members must remain tireless in their resolve to mobilize the necessary financial underpinning for the next steps in the eradication goal.

As previously noted, fatigue and complacency present additional risks. We need to be concerned with the legacy of polio eradication, which must be firmly established. In fact, these two issues are linked, and the best way to overcome the dangers they present is to build on lessons learned and the significant public health benefits that have accrued from our experiences with polio. In many countries, polio eradication activities have greatly strengthened the foundation of health care infrastructures in general. Surveillance capacity has dramatically improved, enabling health systems to utilize the epidemiological infrastructure created to detect polio to identify and investigate other important diseases. And at the same time that capacity grows to expand immunization services, new opportunities are being created to incorporate within these programs the

treatment of other childhood conditions, such as vitamin A deficiency, for example. Similarly, in Afghanistan consideration is being given to combining family planning services with polio outreach activities. All of these initiatives will ultimately have a multiplier effect on the target populations.

As additional WHO Regions and countries prepare to apply for polio-free status, a global structure has been set up to respond to these requests as they arise. In each of the six Regional Offices of WHO, a regional certification commission has been established. These, in turn, are supported by national certification committees composed of laboratory surveillance personnel. Regions may be certified as polio-free after the absence of wild poliovirus for at least three years in the presence of rigorous surveillance. The Global Certification Commission, established in 1995, expanded the criteria in 1997 to include the topic of regional containment. Global certification will therefore result from the combination of regional certifications—three of which, as we have noted, have already taken place—and regional containment, which is taking place at the current time.

Post-certification Immunization Policies

The risks of paralytic polio are central to the evolution of certification policies and post-certification immunization activities. For example, there are risks associated with the use of OPV, the vaccine which is associated with paralytic polio (VAPP). There are also risks associated with wild poliovirus emerging from inactivated polio vaccine (IPV) manufacturing sites or the intentional or unintentional release by laboratories harboring samples of wild polioviruses.

Table 1 shows the potential 10-year polio burden in the post-certification era, arising from a number of sources, assuming current OPV/IPV national policies and three yearly supplemental immunization activities (SIAs). Associated with the post-certification era are the options for stopping the use of OPV. These are real challenges during both pre- and post-certification periods, and yet it is important to highlight them at this juncture as a reminder that we will have to live with these activities and sustain them at very high standards for a number of years. The last occurrence of polio transmission will not signify the interruption of these activities, which, on the contrary, will need to continue for a number of years into the future. In this sense, maintaining population immunity against polio by sustaining very high polio coverage will be critical. Rigorous surveillance standards must also remain in place. At the same time, polio surveillance should also be combined with that of other diseases, as is the case in Africa where, for example, surveillance of measles, yellow fewer, and meningitis is combined with polio surveillance.

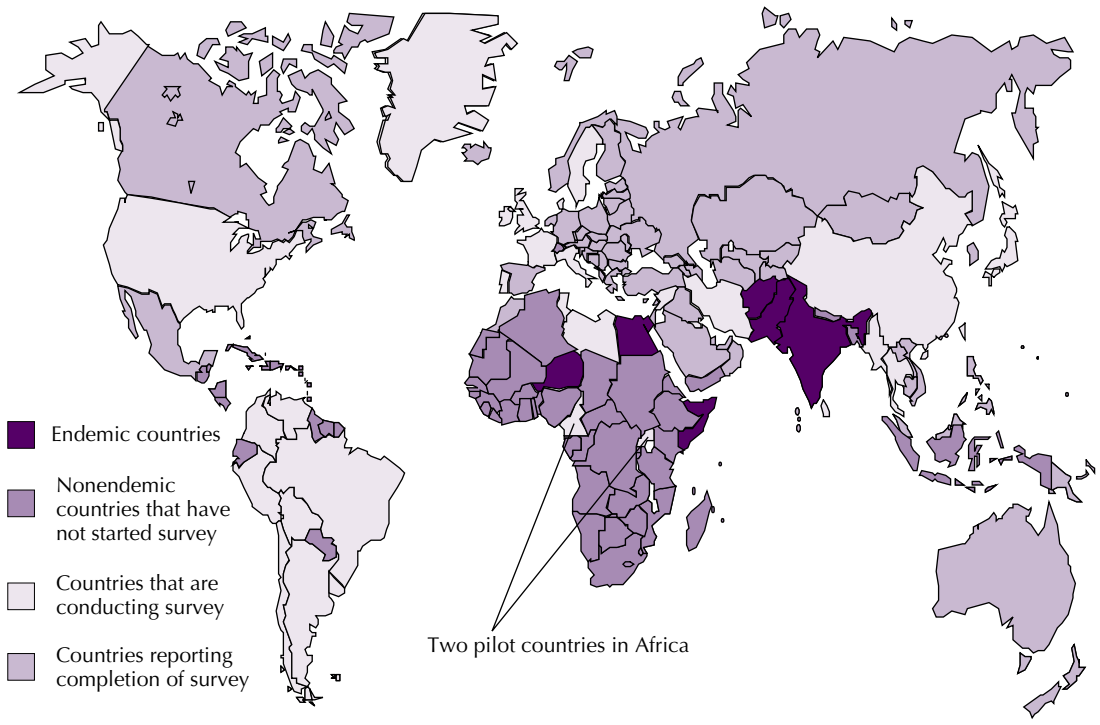
In moving towards certification and containment, it will be important to establish a focal point and task force to oversee the process of surveying laboratories that may harbor the wild poliovirus, the subsequent de-

TABLE 1. Ten-year polio burden projections in the post-certification era.[a]

Source	Cases	Prevention	Mechanism
VAPP	3,750	Stop OPV	NA
cVDPV	250–500	Stop OPV	Pulse OPV
iVDPV	10	Stop OPV	Screen IDs
IPV manufacturers	0	Containment	Local IPV
Inadvertent release	0	Containment	Local IPV
Inadvertent release	0	Containment	Local IPV
Intentional release	0	Containment	Universal IPV

[a] Projections assume current OPV/IPV national policies and three-yearly supplemental immunization activities.

FIGURE 3. Progress in the survey and inventory phase of the polio containment strategy, 2002.



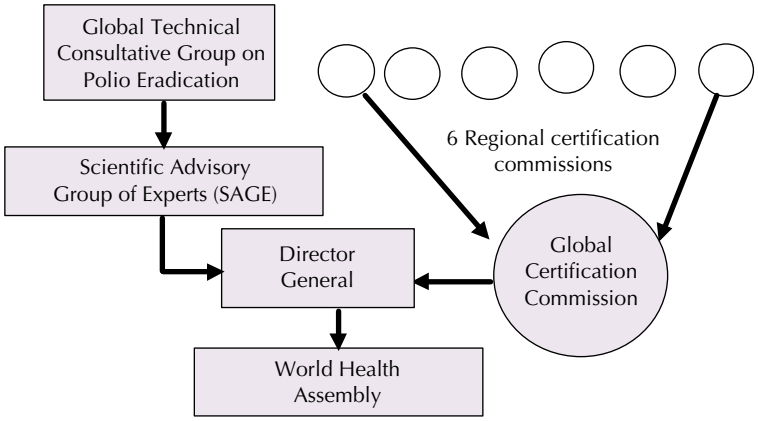
struction of these specimens, and the application of biosafety standards. The Western Pacific Region was the first to start this process, and progress is now under way in Europe.

Figure 3 shows countries that have yet to begin their survey, those whose surveys are under way, and those that reported completion of the survey in 2002. It is expected that by 2003, all countries will have completed their surveys, except for countries in Africa other than Morocco, most Southeast Asian countries, and Yemen, Syria, Jordan, Iran, and Afghanistan in the Middle East.

A steering committee currently guides WHO's work in relation to the poliomyelitis research agenda. In April 2002, the committee provided valuable direction to the Organization in the development of post-certification immunization policies. In November 2002, when the steering committee met again, it observed that significant progress had been achieved in bridging some of the earlier gaps noted just six months previously. It is important to highlight that no policy development can happen without a strong evidence base. The evidence base provides the foundation upon which to develop this policy. WHO, as a technical agency, will rely heavily on the results of this research to shape future policies, including post-certification immunization policies. The research needs that have been identified include antiviral therapy, exploratory uses of IPV, and the stockpiling of monovalent or trivalent OPV.

Developing simplified decision models for policy decisions is the next goal. During 2003, WHO expects to see very intense activities in the field of policy development. Milestones are being adjusted and will eventually be forwarded to the World Health Assembly for consideration by WHO Member States. But past experience cautions us that WHA resolutions

FIGURE 4. Polio immunization policy-making process.



are not always binding. Examples include a resolution calling for the temporary retention of the smallpox virus and another that dealt with the international marketing of breast milk substitutes, which benefited from very strong input from nongovernmental organizations. For practical purposes, these resolutions should be viewed more as an accord by which the countries agree to abide by proposals put forth by the Assembly. In contrast, the Framework Convention on Tobacco Control will be binding after signature and ratification by the Member States, as will the International Health Regulations (IHR), which are under revision and will go to the World Health Assembly in 2003. It is important to note these other examples, because, within the framework of the post-certification polio eradication policy, the IHR will provide a model that will incorporate binding commitments on the part of the States. There are two processes that will inform the World Health Assembly on post-certification immunization policy, as illustrated in Figure 4.

In conclusion, the progress made since 1988 toward polio eradication has been nothing short of remarkable. Yet today we are at the most critical phase of this long journey. The 2002 upsurge in cases in India should not distract us from the main task ahead, which is to improve and sustain immunization coverage levels and the quality of all immunization and surveillance activities. The certification process is in motion, and three WHO Regions are now certified as polio-free. The containment process has been initiated, and post-certification immunization policies are being addressed. Support of these activities is a top priority for WHO, and it will be critical for the Organization to provide updates from time to time and to consult with its Governing Bodies. We remain committed to the task and hope that all the WHO Regions will have completed or will be in the process of completing their contribution to global certification in 2005.

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POTENTIAL FOR CIRCULATION OF VACCINE-DERIVED POLIOVIRUSES

Philip Minor[1]

INTRODUCTION

The global program for the eradication of poliovirus has made extraordinary strides towards the goal of removing disease due to the wild type virus from the world. At the present time, polio is restricted to a few countries in Africa and Southeast Asia. While there have been setbacks in 2002–2003, particularly in northern India, where more cases were reported for 2002 than in the preceding 12 months, there is no technical or logistic reason why the goal should not be achieved by the end of 2003. One of the most striking illustrations of the success of the program is that the last natural wild type 2 virus was isolated in October 1999. At least one of the three serotypes, therefore, appears to be extinct in the wild. This raises the question of the course of action to be followed once all wild type viruses have been eradicated, and, in particular, how or if vaccination can be safely stopped at some point in the future.

The vaccines used in the eradication program are derived from the live attenuated strains developed by Albert Sabin, which induce immunity by imitating natural infection. All three, one of each serotype, are derived ultimately from wild type circulating strains by laboratory adaptation (*1*) and are capable of causing poliomyelitis at a very low frequency, estimated at one case per 750,000 first-time vaccine recipients or one case per two million recipients overall (*2*). The vaccines are also capable of causing disease in contacts of vaccinees. The issue to be considered in stopping vaccination is, therefore, whether the rate of paralysis due to the vaccine strains and the rate of transmission from one person to another are sufficiently low to permit vaccination to stop safely. In this respect, the polio vaccine is very different from the smallpox vaccine, which has more common serious side effects, but is incapable of causing smallpox.

EVOLUTION OF VACCINE STRAINS IN HEALTHY VACCINES

From the first use of polio vaccines, it was known that they changed on replication in the human gut and could regain virulence, either wholly or partially. The sophistication and precision of the process was only revealed when molecular studies were performed. In the 1980s and early 1990s, molecular biological approaches elucidated the basis of the attenuation of the Sabin live attenuated strains of poliovirus (*3*). In general, few mutations were involved; all three serotypes had mutations in the 5′ non-coding region of the virus in very similar regions and either one or only a few

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other mutations located in the structural proteins. These studies were based on identifying mutations which affected virulence in animal models when the vaccine strains were compared with their virulent precursors or an isolate from a vaccine-associated case of poliomyelitis; changes in isolates from other vaccine-associated cases were consistent with the conclusions in that the mutations were either reverted or indirectly suppressed. When healthy vaccine recipients were examined, however, the same kind of mutations were observed (*4*). For the type 3 strain, mutations were lost in a well-defined order: first that in the 5′ non-coding region within two to three days, then that in the structural proteins at day 11. At the same time, as the last mutation was lost, the excreted virus became a recombinant in which the structural proteins derived from the type 3 strain and the nonstructural proteins from the type 2 or 1 strain. Changes in antigenic sites known to be the target of neutralizing antibodies also occurred. In part, the changes clearly affected the growth properties of the virus; for example, by changing its optimum growth temperature from 35 °C to closer to 37 °C as found in the human gut. Many of the other changes remain of unknown effect, although they are selected with great reproducibility. Similar changes occur in all three serotypes to some extent, and it is clear that the virus is able to adapt to the human gut in a very rapid and effective manner. It therefore seems likely that it would respond to any appropriately applied selection pressure with the same speed and effectiveness.

The Molecular Clock and the Dating of Common Ancestral Viruses

The sequences of polioviruses change rapidly as a result of adaptation to the gut in which they are growing, but they also drift in a steady and apparently random way during epidemics, or during the rare instances of long-term infection of individuals. The rate of drift is remarkably constant, although it has been expressed in different ways. It is apparently independent of the serotype or strain. An example of drift in an epidemic came from a trial of a novel type 3 vaccine strain in Poland in 1968 (*5*). The strain was being developed as a replacement for the type 3 Sabin strain, which proved difficult to produce consistently. The novel strain had excellent laboratory properties in terms of the stability of its attenuation when it was grown in culture and the immunity induced in recipients. In Poland, a few children were vaccinated without ill effects, but some six months later there was an epidemic of poliomyelitis in a nearby city. The isolates were shown to be extremely closely related to the vaccine strain used. In addition, if the sequences of the region of the genome encoding the structural proteins were compared, only taking into account third-base, non-coding changes, which are assumed to have a negligible effect on virus growth, the rate of change was perfectly linear at about 2.7% per year.

Some individuals lacking humoral immune responses can become long-term excreters of poliovirus if inadvertently immunized. Vaccine-derived polioviruses from immunodeficient, long-term excreters (iVDPVs) are discussed in more detail below, but the rate of drift in virus isolates from such individuals is virtually identical to that in epidemics. In one case the accumulation of third-base mutations in the region encoding the structural proteins was again perfectly linear over a two-year period at 2.7% per year (*6*).

These observations provide the tool to provide an accurate date for the divergence of related viruses from a common recent ancestor and from a more distant ancestor in a more approximate way. Thus, if a vaccine-related isolate is made, it is possible to say how long it has been replicating in humans since the vaccine was given.

Circulating Vaccine-derived Polioviruses

In 2000–2001 there was a small outbreak of poliomyelitis on the island of Hispaniola, comprised of the Dominican Republic and Haiti (*7*). There were just over 20 cases in all. The last

outbreak due to wild type strains had occurred in the mid-1980s, and the whole of the Americas had been declared free of poliomyelitis in 1994. The strains causing the outbreak were clearly freely transmissible and closely related to the Sabin type 1 strain, from which they differed by only about 2% overall. This corresponds to about two years of circulation as all bases were considered, and not just those in third-base positions. Upon closer examination, all the strains were found to be recombinant viruses in which the structural proteins were derived from the vaccine strain and the larger part of the nonstructural proteins from a virus other than either the type 2 or type 3 Sabin strain. It is assumed, since there is believed to be no wild type poliovirus in that geographical region, that the partner virus is an enterovirus of group C, which includes viruses such as some of the coxsackie A strains, as well as polio. The compatibility of the genomes of the group C enteroviruses in this way raises other issues for the cessation of vaccination, as discussed below. In fact, several different recombinant strains were identified, implying multiple recombination events. Recombination between polioviruses is clearly common, as shown in vaccinees; this observation implies that it may also be common between enteroviruses of the same group.

Other outbreaks involving strains derived from the Sabin vaccine strains have been reported in Egypt, where the circulating type 2 strain for about five years was vaccine-derived; the Philippines, where a very small outbreak of type 1 was reported in 2001; and in Madagascar, where an outbreak caused by two separately derived type 1 strains was reported in 2002. In all cases, the strains were recombinants where the partner was not identified but was assumed to be a group C enterovirus, and circulation had been undetected for about two years. There is no known virological reason why the strains should have to be recombinants but in the well-documented cases, so far they have been. The reason for the development of the circulating strains is not proven, but a highly plausible model is that both surveillance and routine vaccine coverage are likely to decline after polio has been eradicated and other health issues take priority. Thus, a small proportion of infants may receive the vaccine and shed live vaccine virus while mixing with their unvaccinated peers. This provides virologically ideal conditions for the selection of transmissible strains which may persist for years. Under these conditions, circulating vaccine-derived polioviruses (cVDPVs) seem almost inevitable.

Vaccine-derived Polioviruses from Immunodeficient, Long-term Excreters

Patients who are deficient in humoral immunity have been known to have particular difficulties with enteroviruses and poliovirus, in particular, for some time. In one clinical trial in the United Kingdom in the early 1960s, 30 such patients were given live vaccine in an attempt to induce some sort of immunity and, in any case, to reduce their chances of being infected by wild type virus. Most of them excreted virus for the normal length of time, which is on average five to six weeks for the first dose of vaccine. One vaccinee excreted type 1 virus for about three years, and another, type 3 virus for nearly two years, as described above (*8*). Chronic excretion of virus is not common, although the incidence is not known since the patients are not routinely screened. There are about 20 cases reported, most of whom were identified because they became paralyzed. The remainder were discovered by chance (*9*), including one British subject who is known to have been excreting type 2 virus for eight years, based on isolates being available from 1995 to 2003. In fact, the patient had been excreting virus for far longer than this, and the extent of drift of even the 1995 isolates from the vaccine strain is very high. Based on the individual's vaccination history, it is believed that excretion of virus has been continuous since the age of 11, or for over 20 years. The virus is highly virulent in animal models and has lost all molecular markers of attenuation. It is also excreted at a titer comparable to that found in most vaccinees or those infected with

wild type strains. Such individuals could form the focus of an epidemic if those around them are not immunized and if they fail to follow good hygienic practices.

Treatments attempted on the British subject have included the administration of immunoglobulin by the oral route, which appeared to have some effect on the titer of virus excreted in the stools. Chemotherapeutic agents are not well established for such patients, although some have been considered. The virus excreted is resistant to the most commonly considered drug. The patient was given milk from a breast milk bank in the region, and this did appear to have an effect, although when the treatment stopped, virus excretion rapidly reached the same high titers. There is currently no treatment for such patients. Some stop excreting virus spontaneously for unknown reasons, but the numbers concerned are currently unknown.

Possible Solutions to the Issues of Vaccine-derived Polioviruses

It seems reasonable that cVDPVs such as those which caused the outbreaks in Hispaniola and other regions could be avoided if routine immunization programs were either maintained at a high level of excellence or were abandoned altogether after one last blanket coverage effort to immunize the population. The assumption would be that the virus would die out faster than the susceptible population needed to maintain it could build up. Possibly inactivated vaccine could be used to maintain protection while the environment was monitored to see if poliovirus was dying out.

New iVDPVs could be avoided by stopping the use of oral polio vaccine, but at the present time there is no cure for the existing cases, which are, at any rate, probably rare.

While they are not vaccine-derived, it is possible that a group C enterovirus could evolve to fill the niche left by poliovirus after its eradication (*10*), and there are other scenarios by which the virus could re-emerge, including escape from laboratories or manufacturing facilities or through acts of bioterrorism. The issues raised by the eradication of poliovirus are potentially difficult, and some strategy must be available to deal with its possible re-emergence. It seems unlikely that an eternal vaccination program against a nonexistent disease is an acceptable answer.

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IS GLOBAL MEASLES ERADICATION FEASIBLE?

Ciro A. de Quadros[1]

BACKGROUND

Measles is one of the most infectious diseases. Before the introduction of the measles vaccine, practically all children contracted measles in the long run. Human beings are the only reservoir of measles, although other primates, such as monkeys, also can have the infection. The most infectious phase is the prodromic one, before other symptoms, such as fever and exanthema, appear. The communicability diminishes rapidly after the appearance of exanthema (*1*).

At the end of the 1970s an attenuated live measles virus vaccine, which was authorized for use in the United States in 1963, had already been widely disseminated in several parts of the world. It has been documented that this vaccine protects for more than 20 years, but it is believed that the immunity conferred by the vaccine lasts for a lifetime (*2*). Its effectiveness is around 90% to 95%. Due to the interference of maternal antibodies, the effectiveness of the vaccine increases after the first 6 months, reaching the maximum level from 95% to 98% at 12 to 15 months of life (*3*). By the end of the 1980s, most countries of the world had incorporated measles vaccines into their routine vaccination programs, and immunization coverage with this vaccine has increased considerably. By 1990, reported coverage of children aged 2 years was approximately 70% worldwide.

Data from the World Health Organization (WHO) indicate that measles is responsible for 10% of deaths worldwide in children under 5 years of age. Worldwide, some 40 million cases and 800,000 deaths due to measles still occur every year; more than half of the deaths occur in Africa. The eradication of measles would, therefore, play an important role in improving child survival.

To answer the question posed in this chapter's title it is necessary to review the experiences with measles eradication in the Region of the Americas. To that end, what is briefly described here are the strategies being implemented in the Americas to interrupt indigenous measles transmission, as well as the results achieved so far.

MEASLES ERADICATION IN THE WESTERN HEMISPHERE

The goal to eradicate measles from the Western Hemisphere was set by the Pan American Sanitary Conference in 1994, at the same time that the International Commission for Certification of Poliomyelitis declared the Region polio free (*4*). The rationale for the strategy employed to achieve this goal was based on the epidemiology of measles before and after the vaccine was introduced. Before the vaccine was introduced, measles epidemics occurred

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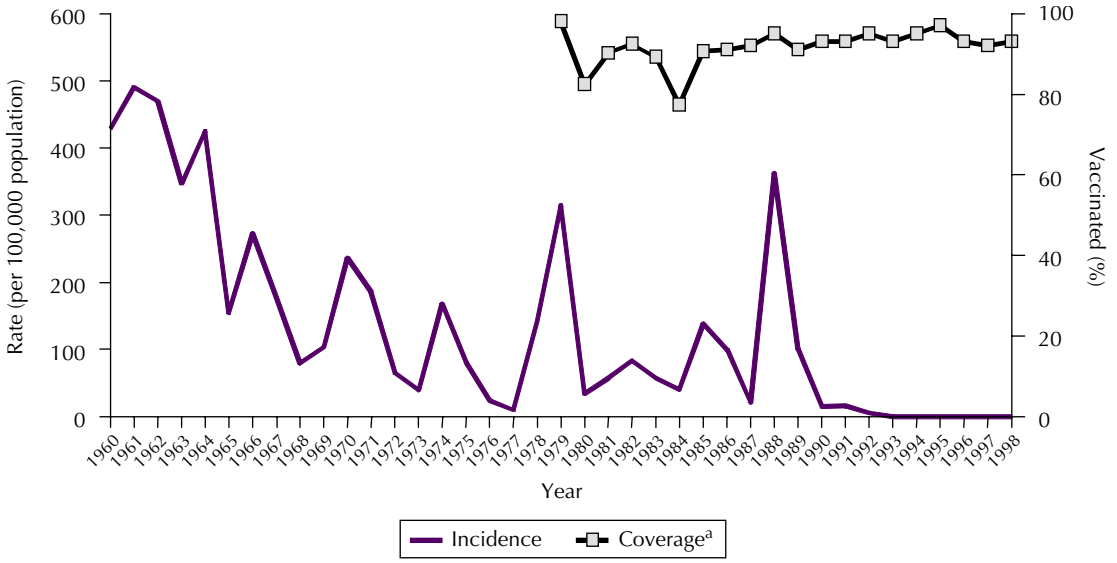
FIGURE 1. Measles interepidemic periods, post-vaccine era, Chile, 1960–1998.



[a] Vaccination coverage in children <1 year of age.
Source: Immunizations Unit, PAHO.

every couple of years, emerging as soon as a pool of susceptibles provided by every birth cohort was available to fuel transmission when the virus was introduced in a given population. After the introduction of the vaccine and with subsequent increases in vaccination coverage, the interepidemic periods lengthened, sometimes stretching for several years between one epidemic and another. For example, the interepidemic period spanned nine years in Chile (Figure 1) and 12 years in the United States.

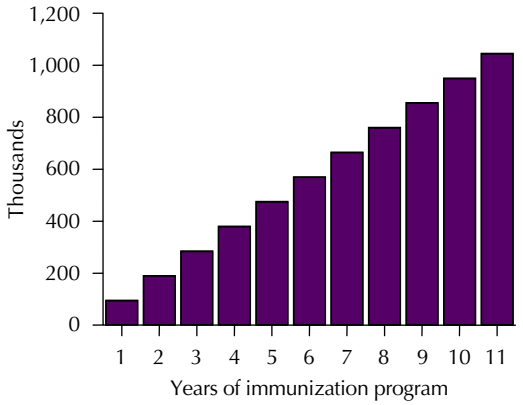
Furthermore, in the pre-vaccine era, measles cases occurred in very young children, and by age 5 almost all had already suffered the disease. With the introduction of the vaccine, and with increased coverage, the age specific rate increased in older children, and even young adults and adults suffered measles (*5*).

Another important factor to consider is that a considerable number of children remain susceptible because they never received the vaccine. In addition, because vaccine effectiveness is not 100%, a small proportion of those vaccinated who were primary failures also remain susceptible. The result is that over a few years, even with a very good immunization program in place, accumulation of susceptible children will occur (Figure 2). In other words, vaccine coverage does not equal population immunity.

Strategies

Given this background, the strategy recommended by the Pan American Health Organization called for high vaccination coverage of the susceptible population at all times, effective surveillance to detect measles transmission, and an adequate response. The vaccination strategy (*6*) is three-pronged. First, a one-time-only "catch up" campaign, implemented during the low season, targets all children 1 to 14 years of age, to attempt interruption of all chains of measles transmission. This age group was chosen because it was among them where more than 90% of the cases were

FIGURE 2. Accumulation of susceptibles while an immunization program is in place.



Note: 500,000 newborns, vaccine coverage = 90%, vaccine efficacy = 90%.

Source: de Quadros, CA, et al. Measles elimination in the Americas. Evolving strategies. *JAMA 1996;* Jan. 17;275(3): 224–229.

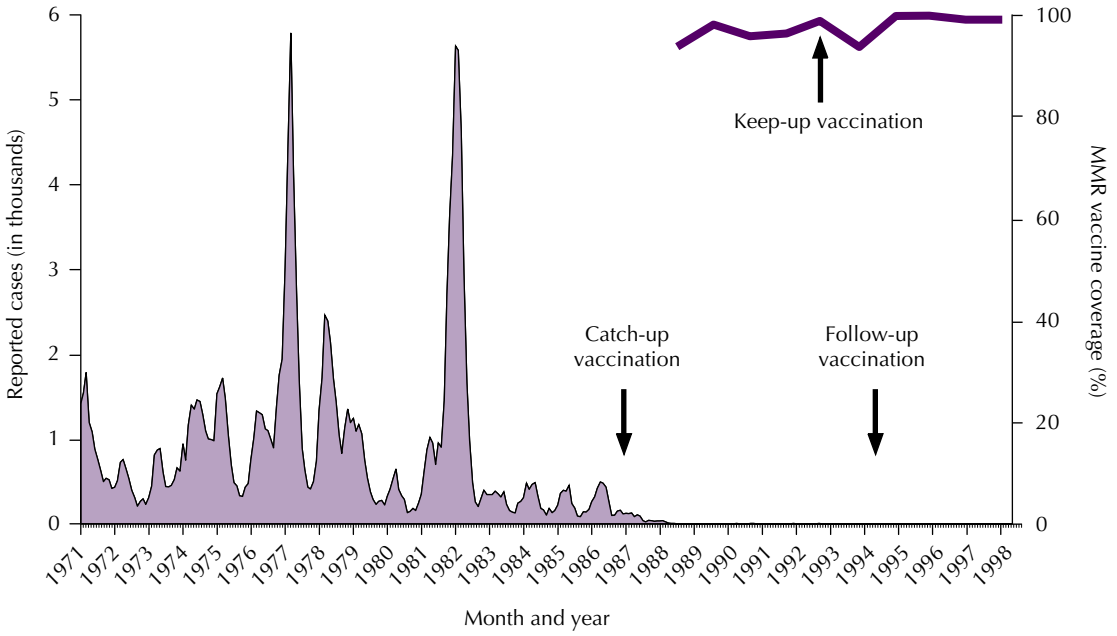
occurring by the time this program started in the Americas. The strategy's second component is to "keep up" with vaccination in routine services to achieve the highest level possible of coverage in the new birth cohorts in every district of every country in order to delay the accumulation of susceptibles.

However, even with high coverage in every district, susceptibles will accumulate because some children will be missed and a few that received the vaccine are primary failures, as indicated above. With average vaccination coverage of 80%, it is estimated that it takes about five years for the accumulation of susceptible children to be equivalent to one birth cohort. When this number is reached, it is suggested that a "follow-up" campaign be undertaken in all children aged 1–4 years, regardless of previous vaccination status. This, then, is the third component of the vaccination strategy—"follow-up" campaigns designed to address the accumulation of susceptibles. These campaigns are conducted every four years and target all children 1–4 years of age, regardless of previous vaccination status. The campaigns' main objective is to reach those children that never received a single dose of measles vaccine, but those children that did receive a previous dose will benefit from a second dose. This strategy offers children a "second opportunity" to receive their first measles vaccine dose. The first country to utilize this strategy in the Americas was Cuba, which successfully interrupted measles transmission in the late 1980s (Figure 3).

The surveillance component was designed to be very simple and timely, as well as sensitive to detect outbreaks and to be understood by every health worker, allowing for a prompt and adequate response (Figure 4). Basically it works this way: if a health worker suspects measles, the suspected case should be visited by a trained epidemiologist who decides whether the case should be classified as a suspected measles case requiring further investigation and collection of a blood specimen for confirmation through an IgM capture test. If no adequate specimen was taken but there was an epidemiological link with a lab-confirmed case, the case also would be lab-confirmed, otherwise it would be clinically confirmed. This last category of cases resulted from deficiencies in the surveillance system.

In the beginning of the program, a major proportion of cases were clinically confirmed, while at present nearly 100% of cases are discarded because they have adequate specimens and negative lab results. Surveillance was integrated with rubella surveillance to maximize the activities related to rubella control. If a suspected measles case is lab-negative, tests are performed to investigate for rubella, and vice-versa. Management indicators have been introduced, such as the proportion of suspected cases investigated within 48 hours of reporting; the proportion of adequate specimens collected and sent to the lab; and for each outbreak, taking of urine samples for virus isolation. The proportion of lab results that are available within five days of receipt at the lab serves to measure the lab network performance. Active search for cases also is conducted periodically in areas that have suffered recent outbreaks or have low coverage, have

FIGURE 3. Reported measles cases, by month, Cuba, 1971–1998.



Source: Ministry of Health, Cuba.

FIGURE 4. Surveillance strategy for measles cases.

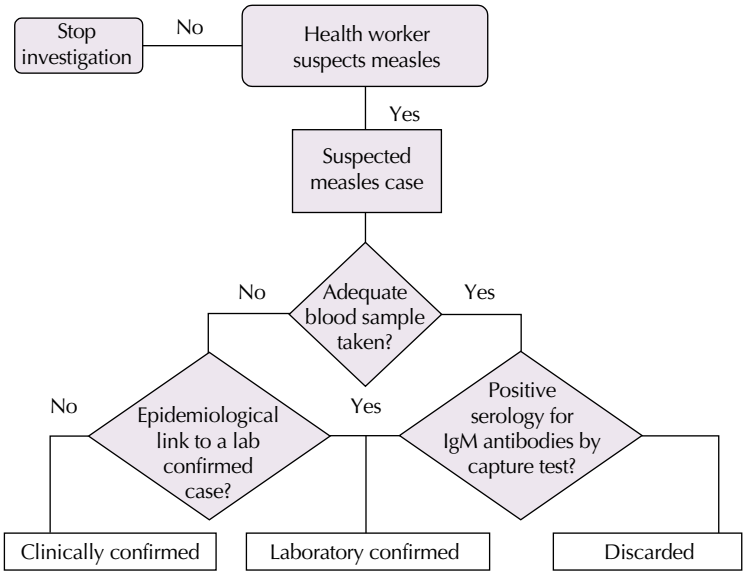
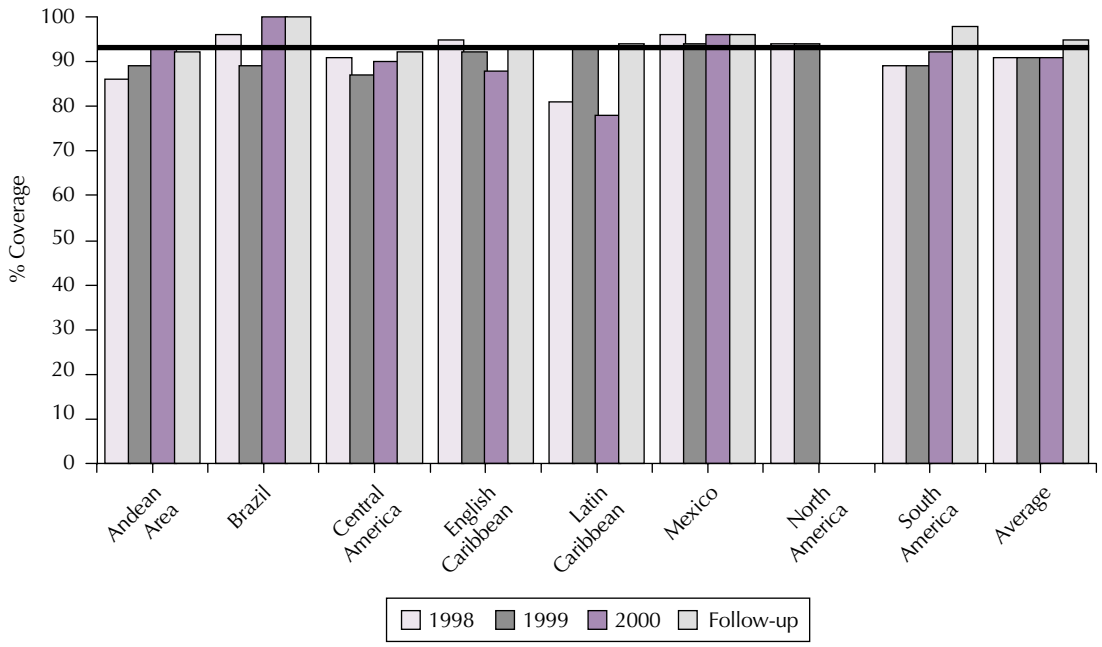


FIGURE 5. Measles vaccination coverage, by subregion, 1998–2000, and last "follow-up" campaign.



Source: Country reports. U.S. data are from national survey for children ages 19–35 months.

reported suspected cases for some time, or where the population has low access to health services.

Progress to date has been remarkable. Most of the countries have conducted "catch up" campaigns with very high coverage levels, and now most of them are in the phase of implementing "follow up" campaigns. These campaigns usually have achieved very high coverage, more than 90% at the national level (Figure 5).

Districts that are below the national average are identified, and additional "mopping up" campaigns are then implemented in districts at risk.

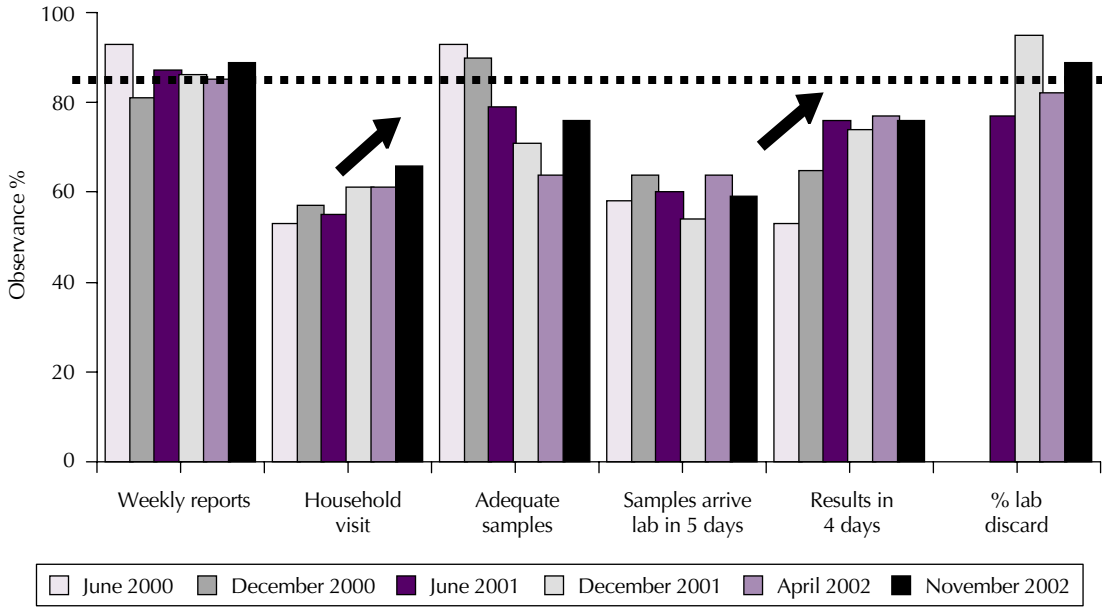
Surveillance indicators have been kept at acceptable levels (Figure 6). Lab response within five days has improved, and the laboratory discarded cases now reach over 80% (*7*).

Results

In 1990, there were more than 240,000 cases reported in the Region. In 1996, only 2,106 cases of measles were reported in the Western Hemisphere. Of these, some 50% were laboratory-confirmed. By the end of 1996, the number of measles cases in the Americas had been reduced by 99%, compared with 1990. In 1997 there was a resurgence of measles in São Paulo, Brazil, that country's only state that did not implement a follow up campaign in 1996. An outbreak that started in early 1997, originating from a probable importation from Europe, spread to other states and to several other countries in the Region. By the end of 1997, more than 50,000 cases were reported in the Americas, with more than 90% originating in Brazil (*8*).

In 1998, the number of cases declined to 14,000 cases, following the epidemic generated in Brazil in 1997, with subsequent spread to Argentina, Bolivia, and eventually to the Dominican Republic and Haiti. During 2001 only 545 cases were reported in the entire Region, with epidemic transmission at the end of 2001 only in Venezuela and a few importations into the northern border areas of Colombia.

FIGURE 6. Average observance, measles surveillance indicators (%), Region of the Americas, July 2000–November 2002.



Source: Country reports, data as of November 15, 2002.

Transmission in the Dominican Republic and Haiti was interrupted in mid-2001. The majority of cases reported in 2002 were from Venezuela, with other countries reporting a few cases related to importations from other Regions of the world (Figure 7).

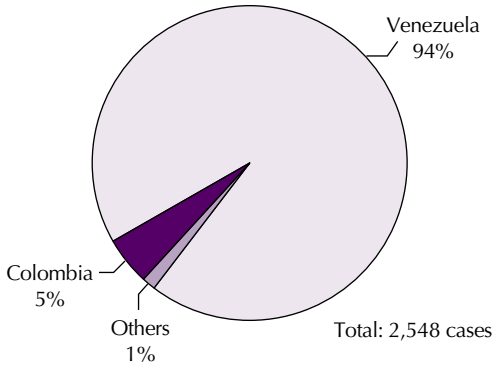
The last indigenous cases in the Region were in Colombia in week 36 and in Venezuela in week 38. As of today, four months have elapsed without indigenous transmission being detected anywhere in the Western Hemisphere.

Lessons Learned

In summary, the "catch up" campaigns, the "keep up" activities, and the "follow up" campaigns have been successful in interrupting measles transmission in the Americas. Measles is no longer an endemic disease in the Americas, and interruption of transmission has been documented in most countries. Thirty-eight out of 47 countries and territories have been free of indigenous measles transmission for more than two years. The Americas suffered a reemergence of measles in 2001/2002 because of failure to fully implement the recommended strategy. In that instance, most cases were seen in vaccinated pre-school aged children and in unvaccinated young adults, with health professionals playing a very important role in the chain of transmission. A similar reemergence of measles occurred in 1997 and 1998 in Brazil for the same reason—failure to fully implement the strategy.

Importations of measles into countries that have followed the PAHO recommended strategies did not generate epidemics, and only occasionally generated a few secondary cases. This happened in El Salvador, for instance, which had its last case in 1996. In May 2001 two young adults that were traveling in Europe, returned infected with measles, probably acquired in Switzerland. There was no second-

FIGURE 7. Distribution of confirmed measles cases, Colombia, Venezuela, and all other countries, Region of the Americas, 2002.[a]



[a] Data as of 16 November 2002.
Source: Pan American Health Organization, Immunizations Unit.

ary transmission in spite of an active search conducted throughout the country in which basically every household was visited. Peru suffered several importations from neighboring Bolivia during the outbreak during 2000. Only in a few instances were there secondary cases within the household where the importation had occurred. Cases in Canada and the United States of America also have been linked to importations from Europe. In Mexico, two cases were imported from Japan, into Cancún, a very busy tourist resort, but with no spread into the overall community.

Surveillance has considerably improved throughout the Region, and active search has not detected transmission in any country. In the Dominican Republic and Haiti there were house-to-house vaccinations to control a vaccine-derived polio outbreak that occurred in 2000/2001. This polio outbreak was concomitant with the importation of measles into both countries, therefore the vaccination campaigns used polio and measles vaccines. Furthermore health workers were offered a US$ 100 reward if they found a case of polio or measles during the house-to-house visits. No cases of either disease were found.

Although the resurgence of measles in the Americas during 1997 represented an important increase compared with the number of cases reported in 1996, the total of 53,000 cases represents only about 10% of the cases reported in 1990. Nevertheless, important lessons can be extracted from this experience.

First, the lack of a timely "follow-up" vaccination campaign in 1996 in São Paolo for children 1–4 years old, combined with low coverage of routine vaccination ("keep-up") of infants with at least one dose of measles vaccine, allowed for a fast and dangerous accumulation of susceptible children. Second, the presence of a great many young adults who were not exposed to the natural infection and had never been vaccinated exacerbated the risk of an outbreak. Third, the measles virus was most likely introduced from Europe into São Paulo. Finally, the city's great population density facilitated contact between infected persons and the susceptible population.

Surveillance data for measles, combined with the results of molecular epidemiology studies, indicate that the countries of the Americas are continually exposed to measles virus from other Regions of the world where measles continues to be endemic.

As of today, four months have elapsed since the detection of the last case in Venezuela. The eradication of the clade 9 of the measles virus that was imported into Venezuela has been documented (*9*).

Conclusion

The experience of the last five years with the measles eradication program in the Americas shows that measles transmission can be interrupted and interruption can be sustained over a long period if countries fully apply the strategy of vaccination recommended by PAHO for all the countries of the Region.

The experience described indicates that the PAHO strategy can effectively achieve and sustain the interruption of epidemic transmission in a very large geographical area, such as the Western Hemisphere. From this experience

we believe that global eradication is feasible if an appropriate strategy is implemented. We also believe, and the experience in the Americas proves this, that the current measles vaccine, although not perfect, has been adequate to stop measles transmission. The eradication of measles will have a major impact on childhood morbidity and mortality. Even in a new paradigm in which eradication is not followed by the discontinuation of vaccination, eradication of measles will be a good investment to avoid expensive epidemics of measles, but most importantly, to save the almost one million children that die every year due to infection with the measles virus.

However, before a global initiative on measles eradication is launched, it is necessary to demonstrate that poliomyelitis has been eradicated. There also will be programmatic, political, and financial obstacles that will need to be overcome before global measles eradication is launched. Partnerships will be essential to support governments embarking on it.

It is not a dream to imagine a world free of measles by the year 2015.

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NEW MEASLES VACCINE FORMULATIONS AND DELIVERY SYSTEMS AND THEIR POTENTIAL CONTRIBUTION TO REDUCING MEASLES MORTALITY WORLDWIDE

Maria Teresa Aguado[1] *and Ana-Maria Henao-Restrepo*[2]

INTRODUCTION

Although substantial progress has been made in controlling measles worldwide, in 2000 there were an estimated 39.9 million cases of measles, resulting in 777,000 deaths (*1*). Children under 5 years of age represent nearly 75% (587,000) of the estimated measles deaths that occurred that year, and approximately 60% of the total measles deaths in the World Health Organization's Africa Region. Measles accounted for 46% of all estimated deaths among children due to vaccine-preventable diseases in 2000 (Figure 1) and was the fifth leading cause of childhood mortality, accounting for 5% of all deaths among children under 5 years of age (*2*).

Recommended strategies for measles control include increasing routine vaccination coverage to at least 90% with the first opportunity for immunization in each district and nationally, providing a second opportunity for measles immunization to all children through routine immunization or supplemental immunization campaigns, and improving surveillance with laboratory confirmation of suspected measles cases (*3*).

Failure to deliver at least one dose of measles vaccine to all infants remains the primary factor in the high measles morbidity and mortality rates. In 2000, only 74 countries (35%) reported measles vaccination coverage above 90%, with 16 countries (with a combined population of 12 million children under 1 year of age) reporting coverage below 50% (*4*).

Supplementary vaccination campaigns to provide a second opportunity for measles immunization have been conducted in several countries pursuing either measles mortality reduction or measles elimination (*4*). The number of children immunized during mass measles vaccination campaigns has increased gradually since 1992, when countries in the Region of the Americas began efforts to interrupt indigenous measles transmission. Following WHO's recommendation to provide a second opportunity for measles immunization, the number of children receiving measles vaccine during mass measles vaccination campaigns increased from

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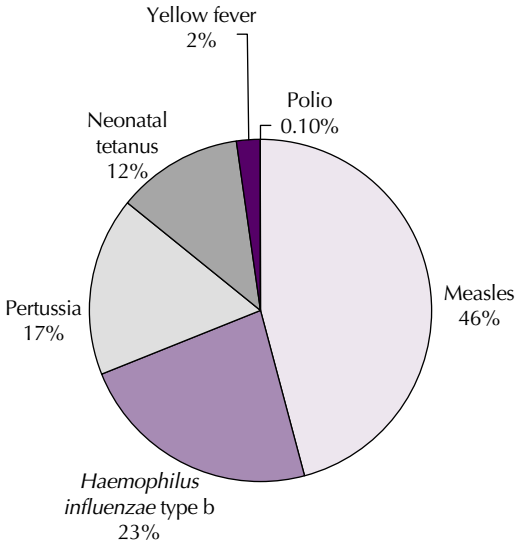
FIGURE 1. Proportional mortality of the 1.7 million childhood deaths due to vaccine-preventable diseases among children worldwide, 2000.



Source: Henao-Restrepo AM, Strebel P, Hoekstra EJ, Birmingham M, Bilous J. Experience in global measles control, 1990–2001. *J Infect Dis* 2003 May 15;187(Suppl 1):S15–21.

approximately 50 million in 1999 to nearly 120 million (Figure 2) in 2000. In 2001, approximately 110 million children were vaccinated during mass measles vaccination campaigns, 21 million of them during mass campaigns conducted in 8 African countries. This number is predicted to increase in future years (*4*). Despite this progress, there is a need to develop approaches to facilitate the full implementation of the recommended strategies, particularly in developing countries, where the disease burden is high and resources are limited.

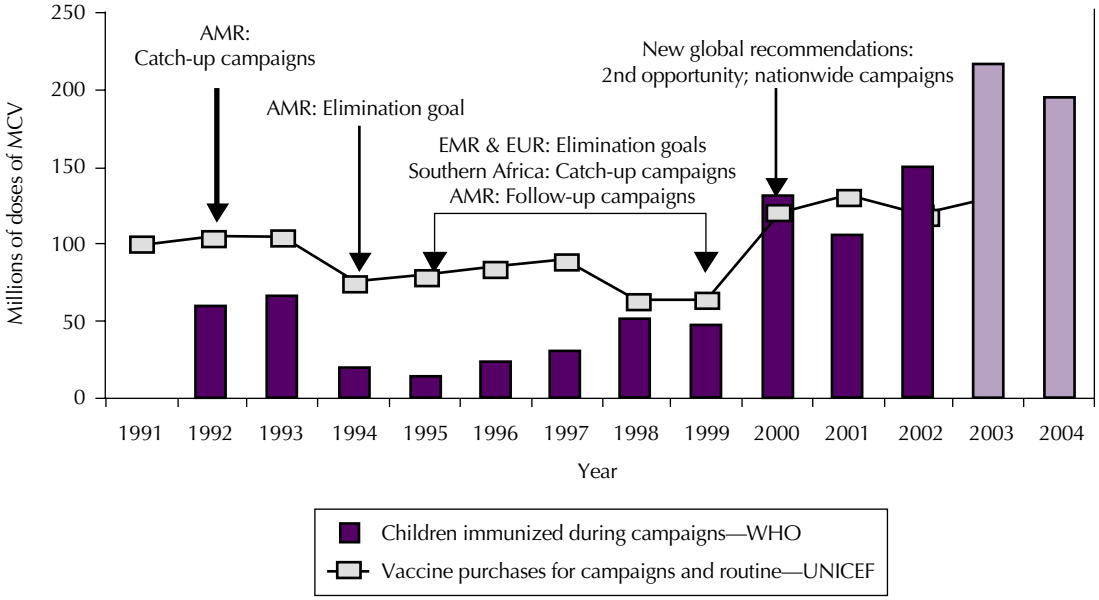
This chapter reviews the case for new, needle-free measles vaccine delivery devices and new vaccine formulations. It focuses primarily on such needle-free vaccine delivery devices as jet injectors for delivering parenteral measles vaccine, and on alternative routes for measles mucosal immunization (aerosol-delivered and nasal vaccines). It also outlines the optimal profile for new products, summarizes the state of the art of these products, and considers their suitability and practicality for use in measles immunization programs, particularly in developing countries. Finally, this chapter presents an update on a special project that has been designated a high priority for WHO—the Measles Aerosol Project.

Defining the Product Profile

It is important to bear in mind that the current measles vaccine is safe and effective. Measles vaccine has been licensed for nearly 40 years and has an excellent safety record (*5*, *6*), good stability (*7*), and a low cost. Moreover, there is abundant evidence of its effectiveness. Worldwide, measles vaccine prevents an estimated 80 million measles cases and 5 million measles deaths annually (*4*). Furthermore, implementation of the WHO-UNICEF recommended strategies has resulted in a dramatic reduction in measles mortality and morbidity. From 1990 to 2001, measles cases in the Region of the Americas declined by more than 99% and transmission is now limited to a few countries (*8*). In southern African countries, after nearly 24 million children aged 9 months to 14 years were vaccinated, with an overall vaccination coverage of 91%, reported measles deaths declined from 166 in 1996 to 0 in 2000 (*9*). Elsewhere, countries that have implemented the recommended strategies in recent years have observed a marked decline in reported cases and deaths (*4*).

Given this situation, why are new measles vaccine formulations or delivery systems needed? New vaccine delivery systems may help to accelerate measles control efforts by simplifying administration techniques and reducing the need for trained health personnel, thereby facilitating efforts to expand immunization coverage. Even today, the routine delivery system in many countries fails to reach many children with the first opportunity to receive measles vaccine at the recommended age (i.e., 9–12 months). Between 1990 and 2000, reported global routine vaccination coverage with one dose of measles vaccine among infants remained at approximately 80% (61%–

FIGURE 2. Measles-containing vaccine (MCV) doses administered (and planned) globally during mass campaigns and UNICEF doses of MCV purchased (and forecast), 1991–2004.



Note: World Health Organization regions: AMR—Americas, EMR—Eastern Mediterranean, EUR—European. Data reported to WHO headquarters by October 2002.

Source: Costa A, Henao-Restrepo AM, Hall SM, Jarrett S, Hoeskstra EJ. Determining measles-containing vaccine demand and supply: an imperative to support measles mortality reduction efforts. *J Infect Dis* 2003;187(Suppl 1):S22–28.

83%) (*4*). This coverage gap reflects the limitations of the health services infrastructure and the lack of adequate financial and human resources in some countries, which result in a failure to fully implement planned strategies.

In addition to the challenges of reaching every child, injection safety is a recognized problem, particularly in developing countries. Limited availability of staff trained to administer injections safely and improper injection practices involving reuse of nonsterile needles and syringes may result in abscesses and pose a risk of transmission of bloodborne pathogens (*10*, *11*). In 1995, WHO reported that up to a third of immunization injections in four of its six regions were unsterile, thus posing the risk of iatrogenic infections, including fatal septicemia, and transmission of bloodborne pathogens (*12*). New delivery systems may help make measles immunization safer. Technological developments, such as auto-disable syringes and safe disposal boxes, prevent the reuse of syringes and make injections safer (*13*, *14*). However, they produce infectious waste and, in some countries, ensuring proper collection and destruction of used needles and syringes is difficult (*15*). These concerns are especially important during mass immunization campaigns, when millions of doses are administered in a short period of time (*16*).

Taking these issues into consideration, a new delivery system or a new formulation of the vaccine should be at least as effective and safe as the currently licensed vaccine. It should also be at least as heat stable and have a comparable cost. Furthermore, the new vaccine formulation or delivery system should be easier to administer and less invasive (e.g., ad-

ministered by trained non-health staff and needle-less, if possible).

In addition, some authors argue that a new formulation of the vaccine should achieve immunity among young infants with maternal antibodies and/or an immature immune system, and be able to prime the immune system at a younger age, thus reducing the risk of infection among infants (*17, 18*). Finally, a new vaccine formulation should demonstrate that it does not predispose the vaccinee to atypical measles (*19, 20*).

Beyond the potential positive impact that a new measles vaccine formulation or a new measles delivery system may have on the efforts to control measles, these new approaches could also be applied to other vaccines in order to improve safety and facilitate vaccine delivery (*21*).

New Measles Vaccine Formulations

Different measles vaccine approaches include peptides, immune-stimulating complexes (iscoms), DNA vaccines, bacteria vectors, and virus vectors (e.g., adenoviruses, poxviruses, and alphaviruses). Studies with all of these approaches indicate that they are immunogenic in mice, but this does not ensure that these approaches will have similar results in monkeys, which constitutes the next research development step. This chapter focuses on the efforts carried out by two key groups of researchers.

Researchers at the Johns Hopkins University, in Baltimore, Maryland, U.S.A., have been working on a DNA vaccine and one delivered by an alphavirus vector. Studies using juvenile macaques vaccinated intradermally with DNA vaccines including fusion (F), hemagglutinin (H), or H+F gene resulted in antibody and cytotoxic T lymphocyte (CTL) responses, but the magnitudes of the responses were marginal when compared with the response needed to confer protection. Some monkeys were protected against disease and no evidence of atypical measles was detected (*22, 23*). For the alphavirus approach, they used a small RNA virus with nonstructural proteins and structural proteins of two different promoters, so that the measles virus protein could be inserted at the structural protein site. The candidate vaccine can be administered via the respiratory or parenteral route. Preliminary results suggest that infant macaques respond at low levels and very slowly (over months), and that the vaccine induces a CTL response. The vectors are continuously improving and some researchers believe that this approach seems promising.

A different approach, called DNA prime/live vector boost strategy, is being tested at the University of Maryland's Center for Vaccine Development (CVD), also in Baltimore. This approach involves priming the immune system with attenuated *Shigella* mucosal live vector strain CVD 1208 carrying a DNA vaccine encoding measles hemagglutinin (H) and fusion (F) proteins. This attenuated strain, which harbors deletion mutations in guaBA and the genes that encode *Shigella* enterotoxins 1 (set) and 2 (sen), is well tolerated and immunogenic when administered as a live oral *Shigella* vaccine (*24*). The immune response is then boosted with attenuated measles vaccine administered via aerosol. Since attenuated *Salmonella typhi* and *Shigella flexneri* can deliver measles DNA vaccines mucosally in cotton rats, inducing measles immune responses (including neutralizing antibodies) and protection, boosting strategies can now be evaluated in animals primed with measles virus DNA vaccines (*24*). Dr. M.M. Levine has pointed out that in a preliminary study, three of four monkeys developed high titers of neutralizing antibodies (Dr. M.M. Levine, Center for Vaccine Development, University of Maryland, personal communication, 2003).

All of the above approaches are at early stages of development. WHO's Initiative for Vaccine Research and its different advisory groups on new technologies and research related to measles are following up on or actively supporting some of these developments.

New Delivery Systems for Measles Vaccine

Jet injectors were widely used for vaccine delivery until the 1990s, when multiple-use noz-

zle jet injectors were found to transmit such bloodborne pathogens as hepatitis B virus and HIV (*25, 26*) and their use in immunization programs was no longer recommended. A large number of jet injectors for vaccine administration are available on the market (*27*). Jet injectors are known to induce immune responses comparable to those obtained when the vaccine is administered using syringes and needles. Jet injectors are particularly suitable for mass measles vaccination campaigns because they permit the immunization of up to 600–1,000 individuals per hour using the same dose chamber. Jet injectors do not pose the risk of accidental needle stick and reduce infectious waste. Also, their potential low cost per dose administered makes them more cost effective than other delivery mechanisms (*28*). However, safety concerns have been a major barrier to their acceptance. Some reports indicate that their use may be accompanied by higher rates of local reaction. Lastly, some of the current models of jet injectors require dedicated trained vaccination teams and daily cleaning and sterilization, which may limit their introduction in certain settings. Following is a summary of the progress of two models of jet injectors.

A high workload jet injector is currently being developed by Felton International in partnership with the Program for Appropriate Technology in Health (*29*). This device is targeted for use in mass vaccination campaigns and its designers are attempting to address the known environmental, logistical, technical, safety, and regulatory issues. The design incorporates a disposable cap through which the vaccine passes during injection in order to reduce the risk of transmission of bloodborne pathogens from person to person. The current model requires the presence of an intact cap that autodisables after use. The caps are low-cost and burn without releasing toxic fumes. Further studies to validate its safety performance are required.

A needle-free injector with on-site ampule filling techniques (LectraJet™) is being developed by DCI (*29*). This injection system consists of ampules that are filled with vaccines and are disposed of after a single use. Different models include both manual and electric injectors, two types of magazines for cartridges, and two types of filling stations. The filling system is being designed so that it can be used to fill ampules in the field. However, ampules could also be filled by vaccine manufacturers. The ampules are estimated to cost a few U.S. cents. They have demonstrated proof-of-principle, built a prototype device, conducted bench performance testing, and performed animal testing for depth and dispersion.

WHO is currently developing the policy and specification guidelines regarding the use of jet injectors. It will include provisions to ensure that the safety of these devices is demonstrated before they can be recommended for use in immunization programs. Ongoing work on the development of sensitive methods to assess contamination and cross infection of these devices is being supported by WHO (*30, 31*). These should be followed by studies to assess safety in humans and usability under field conditions, as well as potential economic benefits from their introduction.

Alternative Routes for Immunization

Alternative routes for measles vaccine administration, using the currently existing measles vaccine, may facilitate further progress in controlling the disease and reducing the related disease burden (*31*). An aerosol vaccination method that uses currently licensed measles vaccine and a suitable device is thought to be adaptable to mass vaccination campaigns and routine immunization and would avoid the risks associated with injections. There are a number of alternative methods of administration of currently licensed measles vaccine, including jet nebulizer systems, ultrasonic nebulizer systems, and nasal spray systems. Also, a dry powder measles vaccine could be delivered using aerosol systems (*32, 33*), with the added advantage of being heat stable, thus avoiding the need for cold chain.

Jet Nebulizer Systems

Jet nebulizers are devices in which the driving gas passes through a very narrow hole from a high pressure system. Changes in pressure suck up the liquid into fine ligaments that collapse into droplets and are then atomized (*34*). A review of the studies on measles aerosol immunization using jet nebulizers indicates that seroresponse rates in infants and schoolchildren after aerosol immunization are at least as good as the subcutaneous route. Good response rates were reported when measles aerosol vaccine was administered to children 3 to 6 months of age and to seronegative children 9 months of age or younger (*31*). Schoolchildren vaccinated with EZ vaccines by aerosol had higher seroconversion rates, higher geometric mean titer, and a smaller percent seronegative compared to subcutaneous vaccination (*31, 35–38*). Recent studies in Mexico have shown seroresponse to rubella vaccine given by aerosol (as MR) to be comparable to subcutaneous vaccination (*39*).

The decrease in vaccine dose volume (i.e., up to five times more children could be vaccinated using the same amount of vaccine) and elimination of syringe and needle costs, including disposal, could result in important savings in supply costs. No significant increases in adverse events in either recipients or vaccinators have been noted, although the follow up period was sometimes short or not described (*31*). Adverse events have been reported as significantly reduced in schoolchildren receiving measles vaccine by aerosol (*31*). Jet nebulizers have not been widely used because of several disadvantages. The current models may be cumbersome and require outlet or car battery electricity to power the air compressor and crushed ice to prevent loss of vaccine potency. It is difficult to precisely measure the dose delivered and some vaccine strains are reported to lose potency in the nebulizer even in the presence of crushed ice. There are also some concerns about reflux of respiratory pathogens into the device with subsequent transmission to other vaccinees.

Despite the fact that these devices use the currently licensed measles vaccine and the evidence of the safety and effectiveness of this administration route, it constitutes a new route of administration. Therefore, all the preclinical and clinical testing required for licensure must be completed before its widespread use is recommended.

Ultrasonic Nebulizer Systems

Ultrasonic nebulizers use a rapidly vibrating piezoelectric crystal to produce aerosol particles. Ultrasonic vibrations from the crystal are transmitted to the surface of the solution, where standing waves are formed. Droplets in the crest of these waves are atomized and released as aerosols (*34*). The U.S. Centers for Disease Control and Prevention (CDC), in collaboration with Creare, a bioengineering firm, have developed a portable, handheld ultrasonic nebulizer. This device has been designed to mimic the output of the jet nebulizers used in the previous measles aerosol trials in terms of particle size distribution, airflow, and rate of aerosol delivery (*40*). The device is designed to deliver a continuous aerosol stream of reconstituted measles vaccine and no loss of vaccine potency has been observed in laboratory tests. It uses rechargeable batteries, with one charge estimated to administer up to 1,200 doses. It has a replaceable nasal prong tip for directing the aerosol stream into the nostril of the recipient and a reusable "ice pack" designed to maintain vaccine potency for at least 8 hours. This device appears to be suitable for use by lay persons and the estimated cost per dose administered is expected to be low. Preliminary bench studies and studies in macaques indicate that this device may be a promising candidate for measles vaccine delivery; however, the major disadvantage of this device is that it has not been tested in humans.

Nasal Spray Systems

In contrast to the jet nebulizers, intranasal immunization of measles vaccine has not been

studied extensively. Although a number of studies have been published, most studies involve small numbers of subjects and a variety of administration methods (e.g., drops, swab, spray, atomization). In many of the studies, the administration techniques are poorly described and are inadequately standardized. Another limitation of these studies is the lack of systematic testing for optimal dose, strain, and route; the differences in the definition of response; and the nonuniformity of the assays performed or the follow-up period (*31*).

The advantages of the nasal spray method are that there are no power requirements and that it can potentially be used by trained nonmedical personnel. Unlike the aerosol devices, there are no equipment costs with the nasal spray method. However, as each syringe is used only once, disposal costs may be higher. Immune response results vary. None to very low levels of immune response (<50%) were observed in studies among 2-week to 2-year-old children in the U.S. using swabs (*41*, *42*), 9- to 23-month-old Kenyan children using drops (*43*), 6-month-old Thai children using sprays and drops (*44*), and 23-month-old Mexican children using drops. Greater immune responses (>90%) were reported in studies among children in former Yugoslavia who were immunized using nasal drops (*45*). Recently, Liashenko et al. (*46*) reported that immune responses among children 6 to 7 years old and adults immunized with measles nasal sprays were similar to those observed after subcutaneous immunization.

The biggest drawbacks to nasal spray systems are that upper respiratory tract infections might reduce their immunogenicity and that their effectiveness and safety have not been fully assessed for measles vaccine.

Measles Aerosol Project

In 2001, the WHO Steering Committee on research related to measles vaccine recommended that WHO organize a product development group (PDG) for measles aerosol vaccine in order to accelerate its development and licensure (*47*). In early 2002, WHO convened the PDG and invited a group of experts on aerosol science, aerosol immunization, device development, vaccine trials, and regulatory issues to become members. The PDG is assisting WHO's Initiative for Vaccine Research to define the licensing strategy and product profile, and to ensure that the Measles Aerosol Project remains focused. The PDG also contributes to the development and implementation of realistic development plans, identifies critical issues, and finally, ensures that the development plan is implemented to international standards of good practice.

Since there was abundant evidence of the safety and immunogenicity of measles aerosol immunization, and considering that a concerted effort with a clear regulatory pathway was required to ensure speedy licensure of this route of administration, WHO organized the Measles Aerosol Project in 2002 and gave it priority. The Measles Aerosol Project is being supported by a partnership of CDC, the American Red Cross, and WHO. Financial resources from the Bill and Melinda Gates Foundation have been granted to WHO to ensure the implementation of all activities needed for the development and licensure of this administration route. The goal of this project is to license at least one method for respiratory delivery of currently licensed measles vaccines, which will provide a means of administering measles vaccine that is cheaper, safer, and easier than injection. At least three devices for aerosol administration of reconstituted measles vaccine, and if feasible in the time frame, a dry powder device, will enter the initial studies. The technical assumptions of the Measles Aerosol Project are that the aerosol vaccination devices will use current vaccines, focus initially on the measles component, and will be aimed at children 12 to 59 months of age for routine vaccination and 12 months to 18 years of age for mass measles vaccination campaigns (*48*). Considering that there is evidence that this aerosol route could be equally safe and effective for rubella vaccines, it is thought that in coming years the project will begin to address

the steps required for the development and licensure of this route for rubella vaccines. Since the inception of the project, preliminary bench studies to assess different delivery devices and animal studies in monkeys to evaluate immunogenicity and safety have been completed. The regulatory pathway has been outlined and preliminary design of the clinical trials is well advanced. Clinical trials are planned to begin in early 2004 and it is expected that clinical testing could be completed by 2007.

Conclusions

Because measles continues to be a major childhood killer in developing countries, countries and their partners have given a high priority to reducing measles mortality and important progress in reducing measles mortality has been made in recent years. Nevertheless, high levels of population immunity will be required to maintain the elimination of the circulation of the measles virus and/or sustainable levels of measles mortality reduction.

To address this challenge, needle-free vaccine delivery systems and new vaccine formulations may help to facilitate measles immunization efforts, especially during mass measles vaccination campaigns. Licensure of any new product must be achieved as soon as possible, since mass measles vaccination campaigns are already being implemented in several high disease burden countries. Indeed, new needle-free vaccine delivery devices would help long-term sustenance of measles elimination and mortality reduction goals by allowing developing countries to increase measles vaccine coverage and protect their children from measles.

The recent experience with the Measles Aerosol Project has highlighted the fact that funding for an organized, comprehensive approach to testing the effectiveness and safety of these methods should accelerate the development process and lead to the licensure and wide use of at least one method.

Acknowledgments

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THE BURDEN OF CONGENITAL RUBELLA SYNDROME

Louis Z. Cooper[1]

INTRODUCTION

More than half of the pediatricians in the United States are too young to remember such vaccine-preventable diseases as polio, measles, and rubella. Current challenges involve not only persuading parents that their children need vaccines, but, just as important, imparting to today's clinicians the same emotional understanding, the same passion, that has driven those of us for whom the diseases were a source of real fear. This is true in the United States, both in the public and private sectors. We depend heavily on the private sector to deliver vaccines in the United States. The success of our immunization programs has been built on public-private collaboration, with approximately 85% of vaccination occurring in private settings, although more than half of vaccines are now being purchased from public funds.

The major historic events in our understanding of rubella and congenital rubella syndrome (CRS) are well known, to wit:

- in 1815, George Maton describes distinct illness;
- in 1866, Henry Veale coins euphonious name;
- in 1942, Norman Gregg describes congenital rubella;
- in 1962, Paul Parkman, Edward Buescher, Malcom Artenstein, Thomas Weller, and Franklin Neva isolate the virus;
- in 1963–1965, the United States suffers a major rubella pandemic;
- in 1969, the rubella vaccine is licensed in the United States—HPV-77 and Cendehill >>RA27/3.

The rubella story is a classic example of the sort of situation where generations of doctors overlook a disease because it is beyond their conception. If three mothers, each with a young infant with cataracts, had not met in Dr. Norman Gregg's waiting room, and in conversation noted that each had had rubella early in their pregnancies, and had they not shared that with Gregg, who was a good listener, how long would we have waited to learn that rubella early in pregnancy poses a high risk of congenital defects (*1*)?

The last major rubella pandemic swept across the United States in 1964, just three years after rubella virus was successfully isolated in tissue culture (*2*, *3*). Armed with new tools for virus culture and serologic studies, a number of investigators were able to add to our understanding of the spectrum of outcomes associated with rubella in pregnancy (*4*). In New York, which had the only program with rubella virus diagnostic capacity in a metropolitan area of 20 million people, our team at Bellevue Hospital-New York University Medical Center had the opportunity to evaluate hundreds of preg-

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nant women and/or their infants for suspected rubella in pregnancy/congenital rubella syndrome. The material described here, most of which has been reported elsewhere, represents the work of a large, interdisciplinary team—the Rubella Birth Defects Evaluation Project, known as the Rubella Project. The findings, some quite surprising at the time, have been confirmed by many other investigators (*5*). These findings also helped to emphasize the clinical astuteness of Gregg and others who worked without virologic confirmation. This chapter summarizes the differences between the usually benign illness—rubella—as seen in children and adults, and the serious consequences of rubella in pregnancy—congenital rubella syndrome.

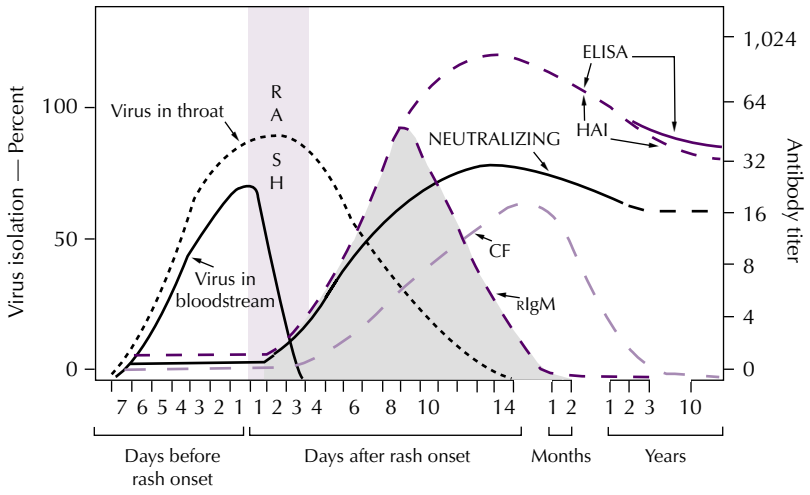
Rubella

For children and adults, rubella is most commonly a mild, three-day exanthem, although the spectrum of infection ranges from being completely subclinical to an illness more typical of measles. Low-grade fever, mild malaise, and adenopathy, particularly posterior cervical and post-auricular, represent a typical picture. However, the maculopapular rash has no distinguishing feature, and even the presence of post-auricular adenopathy is not pathognomic. Adults are more susceptible to transient arthralgia or, occasionally, arthritis, but complications are uncommon. Patients are most contagious from a few days before to 7–14 days after the rash. Before rubella was identified in the laboratory, the frequency (perhaps 25%–50%) of asymptomatic rubella—rubella without rash—complicated control measures and assessment of risk of fetal infection. In a controlled environment, the Krugman team characterized the pattern of virology and immune response to rubella, laying the groundwork for evaluation of rubella vaccines (Figure 1). In controlled challenge studies, any level of pre-existing antibody protected against clinical disease and viremia, the major issues in terms of the disease in pregnancy.

Congenital Rubella Syndrome

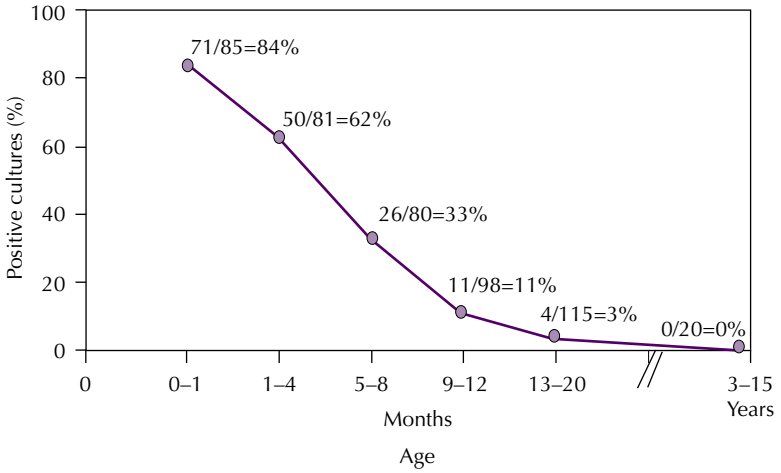
Whereas postnatal rubella is typically mild, self-limited, and without lasting sequellae, the

FIGURE 1. Natural history of rubella: Pattern of virus excretion and antibody response.



Source: Adapted from Cooper LZ, Krugman S. Clinical manifestations of postnatal and congenital rubella. *Arch Ophthalmol* 1967;77(4):434.

FIGURE 2. Incidence of virus excretion in infants and children with congenital rubella syndrome, by age.



Source: Adapted from Cooper LZ, Krugman S. Clinical manifestations of postnatal and congenital rubella. *Arch Ophthalmol* 1967;77(4):437.

ravages of congenital rubella syndrome (CRS) in an 11-month-old infant are painfully obvious. Growth retardation and profound developmental retardation, microcephaly, cataracts, persistent hepatosplenomegaly, cardiac disease, deafness, and ongoing meningitis are accompanied by continuing rubella virus infection and contagion. The pattern of virus excretion and antibody response to infection beginning in utero also is dramatically different. Whereas pharyngeal shedding of rubella virus may last less than two weeks in rubella, an infant with CRS may remain contagious for months (Figure 2). Most newborns with CRS make detectable levels of rubella-specific IgM (RIgM) prenatally and in the early weeks of life. Rubella-specific IgG then becomes dominant, and in approximately 80% to 90% of CRS patients, persists indefinitely.

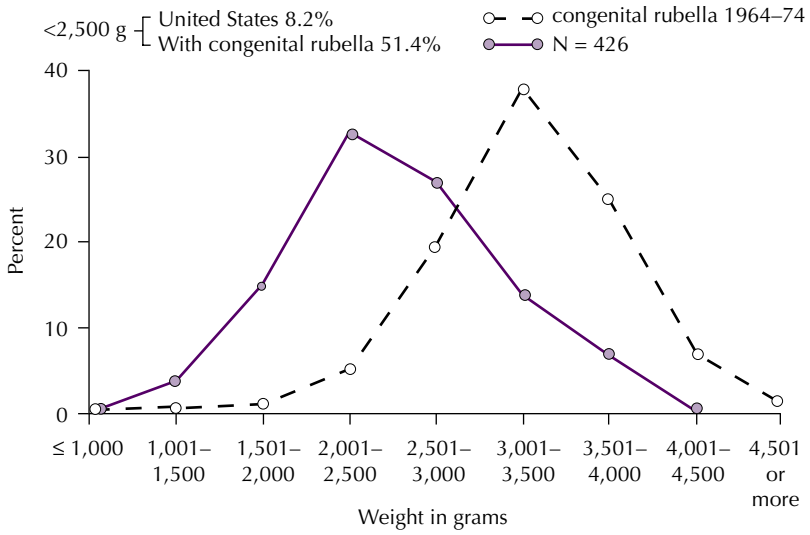
Studies of fetal tissue obtained for abortion due to maternal rubella demonstrated rubella virus in virtually every organ. It should not have surprised us, then, that CRS would have such widespread clinical pathology. In vitro study of rubella-infected cells in culture and histopathologic study of clinical specimens have demonstrated that clinical lesions reflect disturbance in cell growth more than inflammatory response.

Seroepidemiology of rubella has confirmed that, in temperate climates, it is a universal illness that peaks primarily among children in early school grades. Epidemics in the United States occurred at irregular six-to-nine-year intervals. The major pandemic of 1963–1964 was the first after virologic tools became available. It became clear that at least 1% of pregnancies during the epidemic period were rubella-damaged, with 20,000 children surviving with CRS.

Neonatal Manifestations of CRS

The combination of a major epidemic and laboratory confirmation of rubella infection led to recognition of clinical syndromes not well-appreciated before 1964. While many newborn infants with CRS appear to be normal at birth, others have impressive, transient neonatal manifestations, such as thrombocytopenic purpura, hepatitis, and radiographic evidence of disturbed bone growth. These so-called "blueberry muffin" newborns often had multi-organ disease and poor prognosis. The thrombocytopenia and bone lesions (originally

FIGURE 3. Comparison of birthweights of infants with confirmed congenital rubella, 1964–1974, with birthweights in the overall population, 1967, United States of America.



Source: Cooper LZ. Congenital rubella in the United States. In Krugman S, Gershon AA (eds). *Infections of the Fetus and Newborn Infant.* New York, Alan R Liss, 1975.

indistinguishable from those in congenital syphilis) resolved completely in infants who survived.

Recognition that many infected infants appear to be normal has been important in understanding the full impact of CRS, since their hearing loss or neurodevelopmental disability could not be recognized in infancy. Surprisingly, both clinically ill and normal-appearing infected infants were contagious for close contacts while still shedding virus in pharyngeal secretions (Figure 2).

Intrauterine growth retardation is a feature of CRS. The distribution of birthweights among such children is clearly lower than that of the general population of newborns in the United States (Figure 3). Although many CRS infants had birthweights in the normal range, when sibling weights were available for comparison, the CRS infants were lighter.

Pathogenetic mechanisms in CRS fall into three categories. Those noted above, found in the newborn period, are transient in survivors and probably reflect high levels of active virus infection, perhaps abetted by the infant's emerging immune responses. The remainder of this chapter illustrates the major, permanent structural disease in CRS and late emerging manifestations. Some are common; others, rare.

Eye Lesions in CRS

Rubella cataracts, the lesions that attracted Norman Gregg's attention, are bilateral half the time. Although these dense, nuclear lesions are easy to recognize and frequently were detected by parents, the newborn's eyes may look perfectly normal at birth and for the early days of life. The cataract may be associated with microphthalmia in the same eye and severe myopia in the other. Since these children are frequently deaf, detecting and correcting the myopia is particularly important.

Congenital glaucoma in CRS is a phenocopy, again hard to miss, but not always present at birth and presenting in the early weeks

of life. Like the cataracts, management is surgical, but where ophthalmologists may temporize in the early weeks before cataract surgery, for congenital glaucoma, surgery is best done as soon as arrangements can be made with an ophthalmologist experienced with this rare condition. In CRS, cataract is tenfold more common than congential glaucoma. Management in both instances requires careful postoperative lens fitting.

The most common rubella eye lesion is neither cataract nor glaucoma, but it is clumping of the retinal pigment layer. This so called "salt-and-pepper retinopathy" is not a retinitis, because it is not an inflammatory process, but another example of altered growth. These lesions have not been shown to have functional significance, but are a useful diagnostic clue, especially when evaluating a child with hearing loss or brain injury.

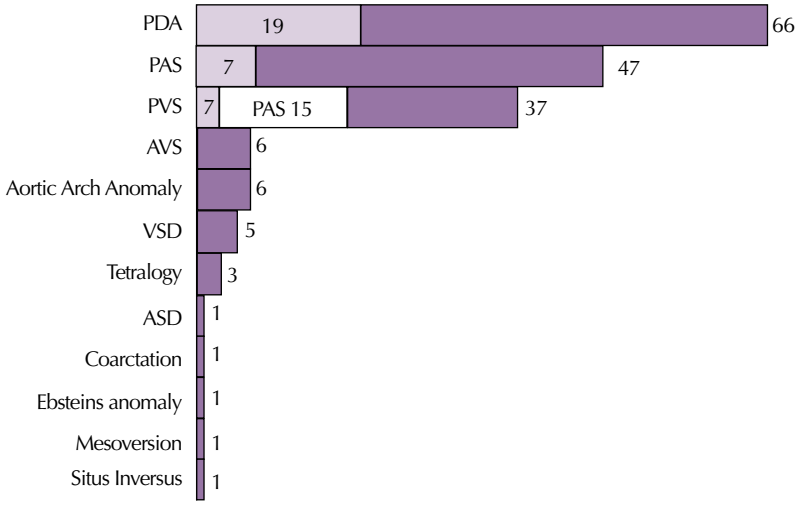
Cardiac Lesions in CRS

Congenital heart disease has contributed to mortality in CRS. Although the mechanisms remain unknown, the heart disease, as are the eye lesions, is very targeted. Patients have either cataract or (primary) glaucoma, never both, and the cardiac lesions are localized to a specific region, around the pulmonary outflow track (Figure 4). The classic cardiac lesion in CRS is patient ductus arteriosis, with or without pulmonic. Most of these lesions now can be corrected with surgery.

Deafness

The most common rubella defect is hearing loss. The reason for that is straightforward. Cardiac disease, cataract, and glaucoma are consequences of maternal rubella, only occurring before the ninth or tenth week of pregnancy because by that gestational age, organogenesis is then complete. The inner ear remains susceptible to damage from rubella well into the fourth gestational month, however. The hearing loss is sensorineural. It may be unilateral or bilateral, mild to profound, and has no characteristic audiometric configuration. All too often, however, it is severe or

FIGURE 4. Heart disease manifestations in 96 children with congenital rubella heart disease,[a] ages 0–5 years.



[a] Virology and cardiology laboratory-confirmed.

profound. Absent early detection and intervention, deafness is a major hazard to language development. The technology for diagnosis and management of congenital deafness has improved dramatically since epidemic rubella existed in the United States. Congenital hearing impairment remains the most frequent major birth defect, and its early detection and intervention remain a clinical and developmental challenge. Since control of rubella by immunization, however, the number of congenitally deaf children in the United States has been reduced dramatically.

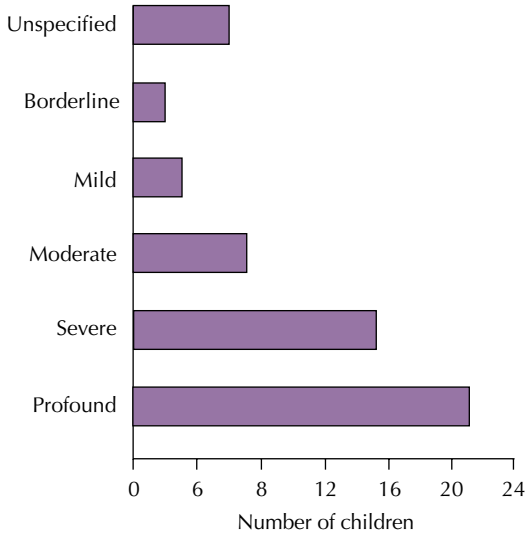
Central Nervous System Infection in CRS

The most frustrating and devastating of rubella's lesions are those that affect the developing brain. In contrast to the virus being very specific with regard to its cellular targets in the eye and in the heart, the virus appears to be shotgunned into the brain. Brain injury consequent to that unpredictably scattered rubella virus infection is equally unpredictable—and the brain infection may be clinically evident even throughout early infancy while brain development is so rapid and critical.

Profound global mental retardation may affect every aspect of development, and is the most devastating of rubella's effect on children. Mental retardation as a manifestation of CRS follows the pattern typical of early, prenatal biologic insult, with profound or severe retardation being most common (Figure 5). On the other hand, children may have normal intelligence, but debilitating motor defects such as spastic diplegia.

A great surprise was recognition that CRS is a cause of autism. The characteristic autistic behavior was first noted by the Rubella Project clinical and educational team, and then was well-characterized by the behavioral research team (led by Stella Chess). The latter demonstrated that 7% of the study children had typical or atypical autism, a frequency approaching 100-fold greater than expected in the general population at that time.

FIGURE 5. Degree of mental retardation in 54 mentally retarded children, out of 210 children with congenital rubella.



CRS was and remains the only documented cause of autism. In 1975, when these observations were reported, they flew counter to prevailing psychiatric views that autism resulted from abnormal parenting. That CRS is not a major cause of autism or autism spectrum disorder (ASD) is obvious. It is ironic that while rubella vaccine has virtually eliminated indigenous CRS in the United States, measles-mumps-rubella vaccine (MMR) is believed by some to be a cause of ASD—even in the face of an increasing number of reports that show no such association.

Endocrine Disorders in CRS

There are other biologic surprises related to late emerging manifestations of CRS. Most striking has been the appearance of insulin-dependent diabetes mellitus (IDDM), which occurs in approximately 20% of Rubella Project patients by adulthood. This prevalence is more than 100 times that observed in the general population. Studies of HLA type indicate that congenital rubella syndrome patients with di-

abetes have the same frequencies of selected HLA haplotypes as diabetic patients without the syndrome (e.g., increased HLA DR3 and decreased HLA DR2). The presence of pancreatic islet cell and cytotoxic surface antibodies in children with CRS does not appear to be related to any specific HLA type. It has been postulated that congenital infection increases the penetrance of a pre-existing susceptibility to diabetes in these patients.

Thyroid dysfunction has been reported in about 5% of patients, and manifests itself as hyperthyroidism, hypothyroidism, and thyroiditis. Autoimmune mechanisms appear to be responsible for these abnormalities. The presence of rubella virus antigen has been demonstrated in the thyroid gland of a symptomatic adolescent with CRS.

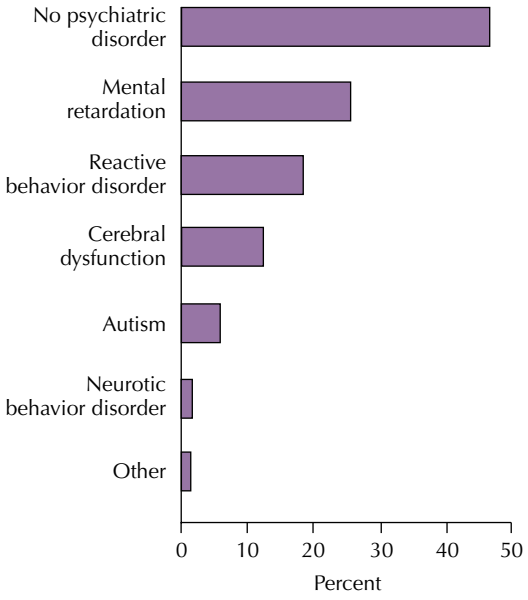
Although growth hormone deficiency has been reported among one group of eight growth-retarded older children with CRS, no evidence was found of functional abnormality in the hypothalamic-pituitary axis, and normal or elevated levels of somatomedin C were seen. Growth patterns in a group of 105 late adolescents revealed three patterns: growth consistently below the fifth percentile; growth in the normal range, but with early cessation, usually with final height below the fifth percentile; and normal growth. Growth failure correlated closely with the magnitude of the cognitive deficits.

Community and Family Consequences of CRS

The frequency of multiple organ damage in CRS challenged the state of the art in special education almost 40 years ago, and does so even now. However, the prognosis for individual children has been quite variable and not predictable solely on the basis of impaired vision or hearing. A major determinant has been the extent of the brain injury.

Federal statutes passed in response to the crisis of so many deaf-and-blind children after the 1964 epidemic became the forerunners of early intervention and of the Individuals with Disabilities Education Act that now defines the federal role for all children who require special education in the United States. The Preschool for Multihandicapped Children (established by the Rubella Project at Bellevue Hospital in 1967) was a model that now is widespread and has been superseded by programs that attempt to enroll infants as soon as developmental disability is recognized, even in infancy.

FIGURE 6. Percentage of psychiatric diagnoses in 210 children with congenital rubella.



What happens to children with CRS? Of the group of 270 children that were followed from the first years of life to age 10 years, only 20% of them were in regular school or in regular school with help (Figure 6). Many children required significant special education, both for single handicap and multiple handicaps. By age 10, a large number of them were placed in institutions. The Rubella Project no longer follows the cohort in an organized way. However, an informal approximation when the survivors were in early adulthood revealed that only one-third were functioning independently in the community, another one-third required considerable family support at home, and the final third required care in institu-

tional settings such as group homes. The economic and social burden of caring for these survivors has been enormous.

Final Perspectives on the Magnitude of the CRS Problem prior to Control by Vaccine

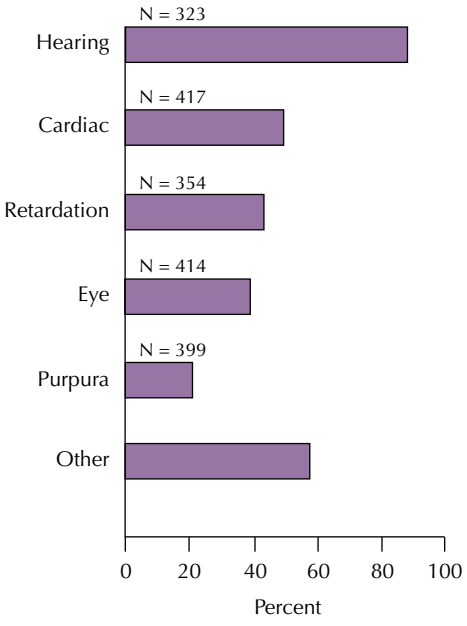
The full impact of the last major epidemic of rubella in the United States cannot be precisely defined. In New York, the Rubella Project data reveal the spectrum of common manifestations among 429 children with CRS (Figure 7). These data do not reflect the distribution of clinical disease among all children with CRS, because infants were enrolled in the Project because of recognized damage (e.g., cataract or cardiac lesion). Hearing loss is certainly underrepresented in this group, because it is often detected in children older than those enrolled in the Project as infants.

We have not lost sight of the fact that of the approximately 400 pregnant women who were reported to the New York City Department of Health because of rubella in pregnancy during the epidemic period, three-fourths of the pregnancies were terminated even prior to the Roe v Wade Supreme Court decision. Certainly, had those pregnancies been continued, the number of cases of CRS in New York would have increased substantially.

We now know that when rubella occurs early in pregnancy, the risk of fetal infection and CRS is very high, probably exceeding 80% in the first eight gestational weeks and tapering almost to zero after sixteen weeks. For individual pregnancies, however, the outcome remains unpredictable, as illustrated by a set of twins followed in the Rubella Project. One was smaller and deaf from CRS; the other, not infected, was normal.

The bottom line is clear. CRS has been a tragic cause of morbidity and mortality. We are grateful that where immunization has become routine in childhood—primarily as MMR vaccine—CRS now is rarely seen.

FIGURE 7. Major clinical manifestations in 429 children with congenital rubella.[a]



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ACCELERATED CONTROL OF RUBELLA AND PREVENTION OF CONGENITAL RUBELLA SYNDROME: EXPERIENCES IN THE AMERICAS

Gina Tambini,[1] *Carlos Castillo-Solórzano,*[2] *Mónica Brana,*[3] *and Ciro A. de Quadros*[4]

In response to the ongoing circulation of rubella virus and the potential for major rubella epidemics in the Region, the Pan American Health Organization (PAHO) Technical Advisory Group (TAG) on Vaccine-preventable Diseases recommended in 1997 the implementation of a Regional initiative to strengthen rubella and congenital rubella syndrome (CRS) prevention efforts. The initiative included the introduction of a rubella-containing vaccine into routine childhood immunization programs; vaccination of women of childbearing age; formulation of specific vaccination strategies for accelerated rubella control and CRS prevention; development of integrated surveillance systems for measles and rubella; implementation of a CRS surveillance system; and support for enhanced laboratory capabilities in rubella virus isolation.

In 1986, 16 years after the rubella vaccine was licensed, six countries (Canada, Costa Rica, Cuba, Panama, the United States, and Uruguay) had introduced measles, mumps, and rubella (MMR) vaccine into their childhood immunization programs. It was only in January 2003 that 42 of the 44 countries and territories in the Region of the Americas had finally introduced a rubella-containing vaccine (measles and rubella [MR], or measles, mumps, and rubella [MMR]) into their national childhood immunization programs.

The remaining two countries, the Dominican Republic and Haiti, will follow suit between 2003 and 2004. Cuba was the first country to eliminate rubella and CRS using a combined strategy that targeted adult women and children with a rubella-containing vaccine; the last case of CRS was reported in 1989, and the last rubella case in 1995. This goal was achieved largely through the implementation of two mass vaccination campaigns in 1985 and 1986, the first targeting women aged 18 to 30 years, and the second targeting children aged 1 to 14 years.

At the 1999 TAG meeting, held in Canada, an accelerated rubella control and CRS prevention strategy was developed for the Region, based on the experience of the English-speaking Caribbean countries and Cuba in conducting adult mass vaccination campaigns against rubella. The strategy rests on vaccination of adult men and women, coupled with

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the introduction of rubella vaccine into national childhood immunization programs. This combined vaccination strategy seeks to achieve rapid reduction of rubella virus circulation, while preventing the shift of disease burden to susceptible young adults, particularly women of childbearing age, thereby avoiding the incidence of CRS.

The principal rationale of an accelerated vaccination strategy is to reduce the time it takes to interrupt rubella virus circulation and prevent the occurrence of CRS. Most countries in the Region have already implemented routine childhood rubella vaccination. Given that the strategy aims to principally target for protection the child population, but not women of childbearing age, it will take over 20 years to control CRS.

Cuba's experience and that of the English-speaking Caribbean countries have helped shape the accelerated control initiatives in Chile, Costa Rica, Brazil, and Honduras (Figure 1). These four countries have conducted adult mass vaccination campaigns for accelerated rubella control and CRS prevention. Brazil (2001–2002) and Chile (1999) have targeted these campaigns to women, achieving high coverage of over 95% (Figure 2). Targeting both men and women, Costa Rica (2001) achieved almost 100% coverage, and Honduras (2002), 80%.

Mass vaccinations of heterogeneous population groups including men, women, and adolescents have achieved high coverage. In Costa Rica, for example, 42% of the population (1.6 million persons) were immunized within one month. The mass vaccination of 28 million women in Brazil against rubella has also provided important lessons on the vaccination of large population groups. Cuba, Brazil, and Honduras used MR vaccine; Chile used the monovalent rubella vaccine.

The experience of the English-speaking Caribbean countries has also offered useful insights into the cost-benefit of immunizing against rubella infection. These studies show that the benefits of accelerated control vaccination far outweigh the costs associated with CRS treatment and rehabilitation. The cost-benefit ratio was estimated at 13.3:1 for interruption of rubella and CRS prevention in the entire English-speaking Caribbean. The cost-effectiveness of mass campaigns has been estimated to average US$ 2,900 per case of CRS prevented. Barbados and Guyana estimated their own costs for interruption of transmission with a cost-benefit ratio of 4.7:1 for Barbados and of 38.8:1 for Guyana, and a cost-effectiveness of US$ 1,633 per CRS case prevented.

The impact of accelerated rubella vaccination strategies on the rapid reduction of CRS morbidity in Cuba, the English-speaking Caribbean, and Chile is being documented, as is the rapid interruption of rubella virus transmission in Costa Rica. CRS is now recognized as a serious public health problem. Still, limited surveillance data remain a source of concern, providing only a partial view of the real disease burden and the success of initiatives. In response, additional tools that can enhance the identification of suspected CRS cases are being implemented. These include collaboration with the Perinatal Information System from the PAHO/WHO Latin American Center for Perinatology and Human Development and the Latin American Collaborative Study of Congenital Malformations. Information collected includes the history of maternal exposure to rubella; clinical illness of the mother during pregnancy; vaccination status of the mother; laboratory confirmation of maternal rubella; and any congenital malformations, hepatosplenomegaly, or purpura in the newborn.

As countries in the Region of the Americas embark on the accelerated control of rubella, documenting the endemic strain in each country will become critical in determining whether the case is imported or not. As with measles, even though a country succeeds in eliminating rubella, importations of the virus may occur and can be avoided only when other regions worldwide take similar measures. Laboratory confirmation of the diagnosis is therefore recommended. For patients with rash and fever, if a serum specimen is negative for measles IgM, it is recommended that it be tested for rubella IgM. For infants with CRS, rubella IgM is readily detected in

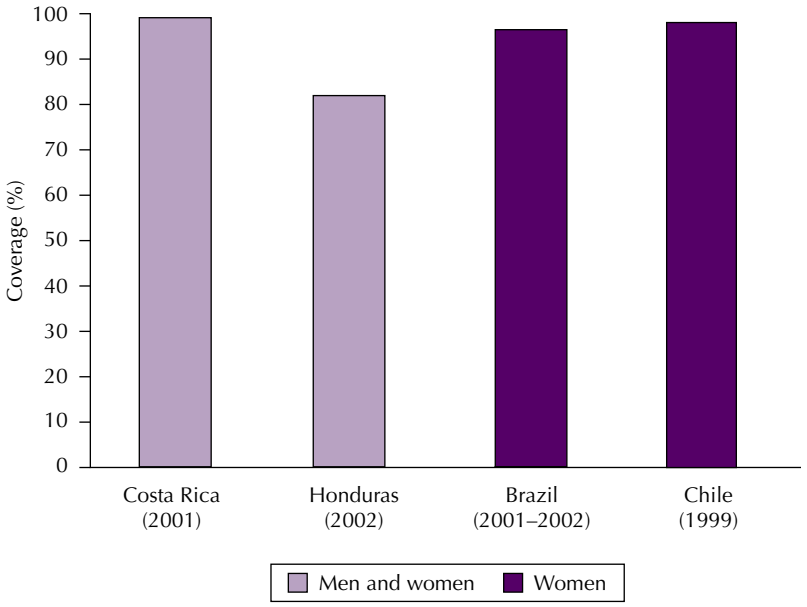
FIGURE 1. Countries with accelerated rubella/congenital rubella syndrome (CRS) control programs in the Americas, by strategy, December 2002.

serum collected during the first 6 months of life.

Rubella virus may be isolated from a nasopharyngeal swab obtained up to 12 months of age. Currently, however, few clinical cases of rubella or CRS are being confirmed by laboratory testing, and few virological specimens are being submitted for molecular typing. As countries establish accelerated rubella control and CRS programs, these areas will need to be strengthened. Molecular typing of viral isolates will permit the identification of the source and propagation of rubella outbreaks and CRS cases, as well as the determination of variations of rubella strains.

Countries that are already applying an accelerated rubella control strategy will need to maintain effective surveillance systems. The

FIGURE 2. Rubella vaccination coverage, Brazil, Chile, Costa Rica, and Honduras.



surveillance of rash and fever is currently the most effective tool. Surveillance systems and adequate laboratory diagnosis will allow detection of rubella activity and document the impact of the rubella vaccination strategy being implemented, as well as the investigation of each confirmed case, rather than simply tracking the location where the virus is circulating. Emphasis should be placed on laboratory confirmation of all suspected rubella cases.

Countries in the Americas are reporting great progress in their efforts to control rubella and prevent CRS. Health authorities in the Region have embraced the challenge by providing key political support at the country level. At the 26th Pan American Sanitary Conference, in September 2002, PAHO's Governing Bodies approved a resolution calling for Member States to undertake accelerated control of rubella and CRS prevention initiatives, and to continue improving epidemiological surveillance of rubella and CRS, as well as laboratory diagnosis and investigation procedures.

The Region of the Americas is providing excellent information on the range of issues faced by countries introducing rubella vaccine, including strategies for vaccine delivery, the importance of surveillance coupled with laboratory confirmation of cases of rubella and CRS, and the value of health economics studies. All these advances are possible because of the partnerships that have been established among PAHO, the March of Dimes, the U.S. Centers for Disease Control and Prevention, the Latin American Center for Perinatology and Human Development, the Latin American Collaborative Study of Congenital Malformations, and the International Federation of Gynecology and Obstetrics.

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THE CHALLENGE OF YELLOW FEVER

Thomas P. Monath[1]

Yellow fever is the original viral hemorrhagic fever—a frightening and life-threatening illness. During the late 19th century, its scourge became so serious in some parts of the Americas that the disease served as a principal catalyst for the birth of the Pan American Health Organization (PAHO) in 1902.

Today the threat of yellow fever persists in tropical areas of Africa and the Americas. Approximately 15% of those infected by the bite of a mosquito carrying the virus develop the hepatitis syndrome, and 20–50% of these patients succumb to the disease. Although much attention had been given to Ebola and other emerging diseases in recent years, the incidence, morbidity, and mortality associated with yellow fever far surpass those of the other viral hemorrhagic fevers. The etiologic agent, yellow fever virus, probably diverged from an ancestral flavivirus lineage about 3,500 years ago. Unlike other RNA viruses, it appears to have a rather stable genome. There are seven recognized variants or genotypes, two in South America and five in Africa. Fortunately, from the perspective of disease control, the genotypic differences distinguishing geographical strains do not translate into antigenic differences, and a single yellow fever vaccine developed from the West African genotype virus has been shown to protect against all strains of the virus. Evolutionary changes have been rather slow to affect yellow fever virus compared to other flaviviruses, probably because of the highly selective requirements for primate hosts and specific mosquito species in the transmission cycle, which restrain genetic drift.

Figure 1 shows the annual incidence of officially notified cases in South America and Africa from the 1960s to the 1990s. The endemic zones in tropical areas of South America and sub Saharan Africa are regions that sustain the enzootic/endemic transmission cycle involving nonhuman primates and tree-hole breeding mosquitoes. The recent outbreaks of the disease in 1999–2001 remind us that, despite the availability of a highly effective vaccine for over 65 years, yellow fever remains a continuing public health concern on both continents. To illustrate how intrusive yellow fever infection can be, data from recent outbreaks in Africa show an attack rate of 3–5%, an incidence of infection of 20%, an inapparent:apparent infection ratio of approximately 7:1 (extremely low, measured against the standard of most infectious diseases), and a case fatality rate of 20% (*1*, *2*). Yellow fever is not an eradicable disease, because it has a sylvatic maintenance cycle involving wild vertebrates and mosquitoes. Therefore, continuous preventative immunization of children born in endemic regions is the most effective measure against this disease.

An important factor in the epidemiology of yellow fever is that many countries contain regions that are either within or outside of the

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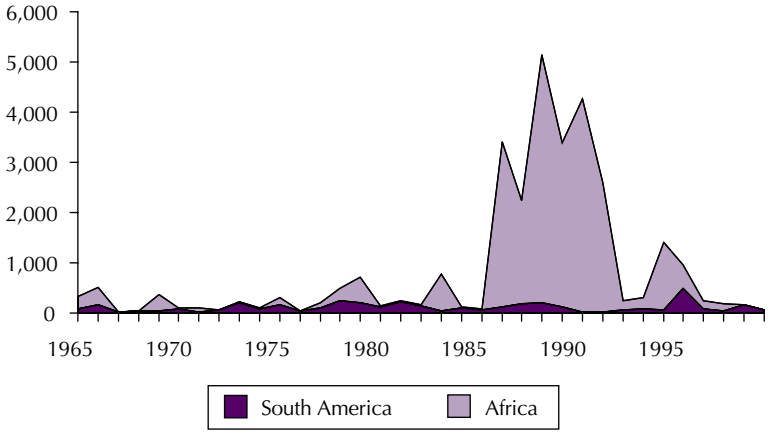
FIGURE 1. Annual incidence of officially notified cases, South America and Africa, 1960s–1990s.



Note: Dark areas represent recent outbreaks of the disease in 1999–2001.

zone of endemic transmission. This situation creates some vaccination policy questions, made increasingly difficult by both changes in human demography and the ecology of yellow fever vectors. In South America, the coastal regions were for several decades free from infestation by the urban yellow fever vector, *Aedes aegypti*, which is capable of sustaining epidemic, interhuman virus transmission. The regions outside the endemic zone are densely populated but have not been historically subject to vaccination campaigns because freedom from *A. aegypti* precluded risk of epidemic transmission. However, as will be pointed out later, this situation has changed dramatically in recent years.

During the final 20 years of the 20th century, the world saw a reemergence of yellow fever, mainly due to a failure to implement effective vaccination strategies, but also to human population growth in what were previously rural locations. In the case of Africa, burgeoning human populations and dwindling habitat for nonhuman primates in these rural areas are gradually changing the landscape of yellow fever ecology, with humans serving increasingly as the host in transmission cycles.

Yellow fever has a complicated epidemiology that is dependent upon rainfall, temperature, and other factors that influence vector biology. Most of our knowledge about yellow fever's ecology is based on studies conducted between 1930 and 1960. Understandably, there is little interest today in funding field studies on a disease that has faded from scientific view and is potentially controllable with an existing vaccine. However, it should be emphasized that our current understanding of the ecology of yellow fever is exceedingly superficial. The intricate subtleties of vector-host interactions, the mechanism for survival across seasons of prolonged dry weather, influences of the El Niño phenomenon on transmission cycles, mutations in the virus influencing virulence or transmission, and the dynamics at the interface of the sylvan and urban cycles are poorly understood, if at all. Also, as emphasized later on, our understanding of the influence of cross-reactive heterologous flavivirus immunity on disease expression and transmission is similarly incomplete. Those of us in a position to influence priorities for research should not lose sight of the importance of these fundamental questions.

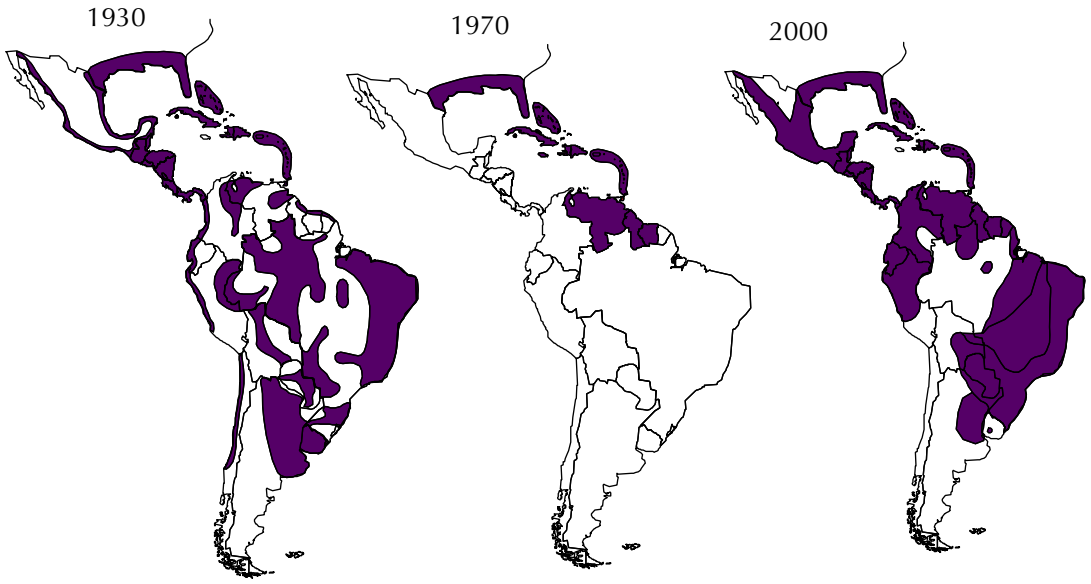
Surveillance, which is so critical to describing the medical impact of any disease and thus to the formulation of health policy, is quite incapable of identifying endemic yellow fever, and it is insensitive for detection of epidemic yellow fever, as well. It has been estimated that < 1% of cases are actually detected by existing surveillance systems (*3*). Yellow fever occurs in remote areas where communications and health services are rudimentary and outbreaks may proceed for weeks or months before they are recognized. The lack of specific diagnostic methods and facilities and the intensity of transmission are always underestimated. In Africa, the greatest impact is on children (*2*, *4*). Since 1988, the World Health Organization has reiterated a recommendation for incorporation of yellow fever vaccine into the Expanded Program on Immunization (EPI) (*5*), and in the last two years, the Bill and Melinda Gates Foundation has supported vaccine purchases for this purpose. Despite these efforts, vaccine coverage is still too low to preclude epidemic disease, with rates < 50% in most countries. Dynamic modeling and direct epidemiologic observations suggest the prevalence of immunity must approach 90% to achieve this goal (*6*).

Fortunately, in South America the incidence of yellow fever is lower than in Africa, in part because of higher vaccination coverage and low human population density in the endemic region, but also due to fundamental differences in the natural history of the virus. Jungle yellow fever in South America is characterized by virus transmission in the forest canopy between monkeys and a single species (or, at most, a few species) of *Haemagogus* mosquitoes. Disease occurs in sporadic fashion when humans are exposed to mosquitoes that have previously fed on a viremic monkey, principally as a result of occupational activities (for example, forest-clearing). Absent in tropical South America is the analogue of the African moist savanna populated by extremely high densities of multiple efficient yellow fever vectors and a relatively large human population. Until recently, much of South America was free from the urban vector, *A. aegypti*, whereas this mosquito is prevalent at very high breeding densities in towns and villages throughout Africa.

Figure 2 illustrates the collapse of effective *A. aegypti* control in the Americas. Efforts to control the urban vector were initiated almost immediately after discovery of mosquito transmission in 1900. By the 1930s, great strides had been made, and with the leadership of PAHO, intensified eradication efforts after World War II had reduced the vector to a limited area of the United States, the Caribbean, and to the northern tier of countries in the continent of South America. Of course, there were no effective barriers to reinfestation other than local vector control, which became increasingly difficult to maintain due to resource constraints, expanding urbanization, and increased challenges to sanitation. By the 1970s, *A. aegypti* had returned, and over the next 20 years reoccupied its previous territory. This change, together with increasing human migrations in and out of the endemic zone, greatly increases the risk that urban yellow fever will reappear in South America. Should that occur, there would be a proportional probability for export of viremic humans to distant receptive areas where the urban mosquito vector is also present, such as in parts of the Caribbean, Central and North America, Asia, and Australia.

During the recent increase in jungle yellow fever activity in 1999–2001 in Brazil, epizootic virus activity reached the outskirts of major cities, such as Belo Horizonte, creating opportunities for urbanization. What stopped yellow fever virus from crossing from the jungle cycle to the urban cycle? We don't really know. Are there differences in the *A. aegypti* populations with respect to their ability to transmit the virus? Is the breeding density of *A. aegypti* too low? Does dengue, now prevalent in many areas of South America, provide a degree of cross-protection? Is yellow fever virus relatively inefficient in producing effective viremia levels in humans above the threshold of infection of local *A. aegypti* strains? Or are the multiple factors interacting to form a relative barrier to urbanization?

FIGURE 2. Changes in the distribution of *Aedes aegypti* in the Americas, 1930–2000.



Source: Courtesy of Dr. D.J. Gubler, Division of Vector-borne Infectious Diseases of the Centers for Disease Control and Prevention, Fort Collins, Colorado, U.S.A.

The interaction between yellow fever and dengue in South America is particularly interesting. Dengue appeared in Brazil in the early 1980s and has caused multiple urban epidemics of significant magnitude in all countries of the South American continent. There are two interactions to consider. The first of these is based on dengue adaptive immunity cross-protecting against yellow fever, reducing viremia and mosquito infection, and mitigating the disease syndrome. There are both epidemiological (*7*) and experimental (*8*) lines of evidence supporting cross-protection. Cross-protection can also extend to immunization with the live, attenuated 17D vaccine, since prior dengue immunity has been shown to reduce the effectiveness of yellow fever vaccine (*9*). The second interaction is more hypothetical. Artificial immunity to yellow fever vaccine could potentially enhance dengue virus infection and increase the risk of dengue hemorrhagic fever (DHF). In a recent study of human subjects, prior yellow fever immunity increased the viremic response to a live, attenuated dengue vaccine, suggesting enhancement of dengue replication in vivo (Monath T., unpublished, 2002). At this point in time, there is no evidence that yellow fever vaccine could increase the risk of DHF, but our experience is extremely limited because the natural experiment, where large numbers of yellow fever-immune persons are exposed to dengue virus, is only now unfolding. Moreover, the situation is complicated by an additional covariate—the strain and genotype of dengue virus—which is critically important in determining the risk of DHF (*10*). Prospective studies will be required to dismiss any untoward effects of yellow fever vaccination on the pathogenesis of dengue virus genotypes.

In addition to adaptive immunity, many other host factors, much less well understood, including age, gender, and genetics, influence disease expression of yellow fever virus. Disease severity is highest at both extremes of age, in males, and in Caucasians. The predilection for males is not appreciated in Latin America,

because there is such a strong male occupational risk for exposure to jungle yellow fever, but there is also an excess of male cases in Africa, where there is no clear occupational reason. Moreover, there is a higher reactogenicity of yellow fever vaccines as well as a higher immune response to 17D vaccine in males than in females (*11*). Host genetics appear also to underlie the emergence of a newly recognized, potentially lethal viscerotropic adverse event associated with yellow fever vaccine (*12*), which is discussed at greater length later in this chapter.

Yellow fever is a vaccine-preventable illness, and the continued occurrence of epidemic disease represents a failure of public health. No resident or traveler to an endemic area should suffer this illness. The 17D vaccine was developed by Max Theiler, Hugh Smith, and their colleagues at the Rockefeller Foundation in the 1930s using empirical adaptation by serial passage of the wild-type virus in mouse and chick cells. The 17D vaccine virus differs from its wild-type parent at 31 amino acid mutations, representing a change of about 0.8%. The precise molecular basis for attenuation is not completely understood, but it is clear that it is multigenic. There are seven manufacturers of 17D vaccines worldwide, but only three—in Brazil, France, and Senegal—produce large amounts of vaccine that can be used in the EPI or for emergency mass vaccination. The other producers are mainly focused on local or traveler markets. Roughly 100 million doses of vaccine are produced annually, but the demand is increasing. The EPI is rapidly being implemented in South America and is being extended in some countries to cover populations in receptive (non-endemic) regions. In Africa, implementation of routine yellow fever vaccination and (in particular) catch-up immunization would present a dramatically increased demand for vaccine. If yellow fever were to be introduced into Asia or other receptive areas with large human populations, there would be significant shortfalls in vaccine supply to deal with a large-scale emergency.

Rare Adverse Events Caused by the 17D Vaccine: YEL-AND and YEL-AVD

After more than six decades in use, yellow fever 17D vaccine had attained a reputation as one of the safest and most effective vaccines ever developed. However, beginning in 1996, concern arose about a newly recognized, rare syndrome associated with 17D vaccine, characterized by multiple organ failure (*13*). During a mass vaccination campaign, two such cases were uncovered in Brazil and were carefully studied (*14*). Concern over safety of the vaccine interrupted plans for mass vaccination in São Paulo state and elsewhere in the coastal region of Brazil in 2000–2001.

Yellow Fever Vaccine-associated Neurotropic Adverse Events (YEL-AND, Previously Known as Post-vaccinal Encephalitis)

Yellow fever 17D virus retains a degree of neurovirulence as demonstrated by intracerebral inoculation of mice and monkeys and by the occurrence of rare cases of post-vaccinal encephalitis in humans. These cases have occurred principally, but not exclusively, in very young infants. Fifteen cases occurred during the 1950s, when there was no age restriction on use of the vaccine in infants. Of the 15 cases, 13 (87%) occurred in infants $\leq$ 4 months of age, and all were $\leq$ 7 months of age. Recommendations for restriction of use of 17D vaccine to infants > 6 months of age (*15*) were followed by a reduction in reports of encephalitis. Since 1960, only 11 cases have been reported in the literature, one of which occurred in a 1-month-old infant in France, where the age limitation was not universally practiced at the time. The current recommendation for the minimum age for vaccination in the United States is 9 months (*16*). The incidence of post-vaccinal encephalitis in infants < 9 months of age may be estimated at 0.5–4/1,000 based on two reports that provide denominator data (*17*).

In contrast, the risk of developing YEL-AND in persons > 9 months of age is believed to be very low. Only nine such cases have been published (*17*). In the United States, five cases have been reported between 1965 and 2002, with ages ranging from 3 to 71 years. Recent intensified surveillance of vaccine-associated adverse events has identified cases of self-limited YEL-AND in adults. Four suspect cases in adults were found between June 2001 and August 2002, raising concern about whether neurotropic accidents are being underreported (*18*). Based on the estimated number of vaccine doses sold during that interval to nonmilitary travelers (approximately 200,000 doses), and assuming all cases were identified by surveillance, it may be estimated that the incidence of YEL-AND could be as high as 1:50,000. This rate is in the same range as that reported for aseptic meningitis in North America and Europe for the Urabe strain of mumps vaccine.

The syndrome associated with 17D encephalitis is characterized by onset 7–21 days after immunization, fever, and variable neurological signs including meningismus, convulsions, obtundation, and paresis. The clinical course has typically been brief and recovery generally complete. One 3-year-old patient died, and a 29-year-old had residual mild ataxia 11 months after onset.

Vaccine-associated Viscerotropic Adverse Events (YEL-AVD)

As noted earlier, this syndrome represents a newly recognized and apparently rare complication of 17D vaccines. Cases have been associated with vaccines manufactured in Brazil (17DD substrain), France, the United Kingdom, and the United States (17D-204 substrain). At least 13 cases (11 fatal) have been described of a syndrome closely resembling wild-type yellow fever, and seven case histories have been published (*13*, *14*, *18*, *19*). Of the total of 13 cases, seven occurred in adults immunized for travel, and six were in children and young adults living in an endemic region. Four of the five cases occurring in the United States were in elderly patients; had a diversified and complex clinical presentation labeled "multi-organ failure," reflecting some uncertainty as to the role of YF17D in direct viral injury; and did not have sufficient postmortem evaluation to clarify pathogenesis. In contrast, virological evidence in the cases occurring in Brazil and Australia supported the conclusion that an overwhelming infection with 17D virus was responsible (*14*, *19*). In persons surviving long enough to enable assessment of the immune response, antibody titers to yellow fever were significantly higher than expected (≥ 1:10,240), consistent with an overwhelming infection (although a secondary response in the setting of prior heterologous flavivirus exposure was not ruled out). Similarities of the syndrome to wild-type yellow fever included rapid onset of fever and malaise within 3–5 days of vaccination, jaundice, oliguria, cardiovascular instability, hemorrhage, and midzonal necrosis of the liver at autopsy. Large amounts of yellow fever viral antigen were found in the liver, heart, and other affected organs.

The recognition of viscerotropic adverse events is especially difficult in endemic areas, where the syndrome could be confused with wild-type yellow fever. The recent recognition of YEL-AVD in developed countries may be attributable to improved surveillance for vaccine-associated adverse events. Nevertheless, only 14 serious adverse events (not all of them due to viscerotropic accidents) were reported to the Vaccine Adverse Events Reporting System (VAERS) in the United States between 1990 and 1998, during which time 1,443,686 doses of 17D vaccine were administered, a rate of 0.97/100,000. Based on reports to VAERS in the United States for that period, the incidence of vaccine-associated viscerotropic events was estimated at < 1:400,000 (*13*). However, during the intensified surveillance between June 2001 and August 2002, two cases of YEL-AVD were reported (*18*), for an esti-

mated incidence of 1:100,000. The true incidence will remain unknown until prospective surveillance is applied to large populations undergoing primary vaccination.

Vaccine-associated viscerotropic adverse events are apparently not caused by mutations arising in the virus, but instead appear to be related to individual host susceptibility (*12*). Analyses of the vaccine lots and seed viruses associated with cases revealed no evidence for mutations in the vaccine that could explain the adverse events. The host factors responsible for increased susceptibility are unknown and are not identifiable by laboratory tests or medical history. A genetic basis is likely. Two cases (one confirmed) in a single family have been recorded in Brazil.

Advanced age appears to be a risk factor for serious adverse events, including YEL-AVD, just as it is for severity of wild-type disease. A retrospective analysis of VAERS data revealed a higher incidence of serious adverse events (neurologic or multisystem involvement) to 17D vaccine in elderly persons, with persons > 65 years having a risk 12–32 times higher than adults 25–44 years of age, suggesting that waning immunity with age may play a role (*20*).

What is responsible for the recognition of YEL-AVD in the mid-1990s? In Brazil, the occurrence of two cases during a massive vaccination effort in which 36 million doses (many primary immunizations) were given in a short timeframe, together with improved surveillance for vaccine-related adverse events, may have uncovered a rare syndrome, which may have occurred previously but was ascribed to infection with wild-type virus. However, in Australia, Europe, and the United States, the number of doses of vaccines had not increased dramatically, nor had the sensitivity of adverse event reporting. One interesting hypothesis is that the occurrence of the syndrome coincides with cessation of concurrent administration of yellow fever vaccine and immune serum globulin for the prevention of hepatitis A in travelers. Immune serum globulin preparations contain yellow fever antibodies (*21*), because 5–10% of plasma donations are from persons vaccinated during military service. The concurrent administration of yellow fever antibody and vaccine could protect the brain and visceral organs from blood-borne infection. Absent the passive delivery of antibody, a few individuals appear to have a genetic predisposition for unrestrained 17D infection.

Future Priorities

Where should research on yellow fever be focused in the 21st century? The molecular basis for attenuation of yellow fever virulence is a high research priority, because serious consideration should now be given to development of a rationally designed and safer vaccine. The host genetic factors determining susceptibility to flaviviruses are quite well known in the mouse, but need to be elucidated in humans. Our knowledge of the pathophysiology and pathogenesis of yellow fever is so rudimentary and descriptive that no rational approach to treatment is possible. In addition to clinical research, we need improved rapid diagnostic methods, practical and inexpensive enough to be put into use throughout the endemic area. Finally, the biggest challenge of all is dealing effectively with the specter of re-urbanization of yellow fever in South America and of the disease's spread to other receptive areas of the world. We need to reactivate field research on the dynamics of yellow fever transmission, particularly at the interface of the jungle and urban cycle. It is obvious that we need to expand yellow fever vaccine coverage, particularly in Africa, but we will also need to better understand the newly emerging vaccine-related safety issues and develop sound public health policies based on risks and benefits. Finally, there is an expanding interest in the 17D virus infectious clone as a vector for foreign genes and in the use of these chimeric viruses as novel vaccines (*22*). The latter approach promises important new vaccines against dengue, Japanese encephalitis, and West Nile virus.

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Part II
The Cutting Edge

HAEMOPHILUS INFLUENZAE TYPE B: THE BURDEN IN ASIA

John Clemens[1] *and Paul Kilgore*[2]

Prior to the availability of a vaccine, *Haemophilus influenzae* type B (Hib) meningitis was the most common cause of bacterial meningitis in the United States. An estimated 20,000–25,000 cases of invasive Hib disease occurred annually in the country during this period (*1*). Even with the appropriate use of antibiotics and optimal clinical care, an estimated 5% of cases of Hib meningitis were fatal (*2*), and many children who survived the disease were left with lifelong neurological disabilities (*3*). For this reason, public health officials accorded high priority to the development of safe and effective vaccines against Hib, particularly vaccines that could be administered early in infancy.

The development of potent polysaccharide-protein conjugate vaccines against Hib and the demonstration that these vaccines could confer high-grade protection against invasive Hib infections represented a major landmark for vaccinology during the twentieth century. Moreover, the ability of these vaccines to reduce carriage of Hib organisms allowed the vaccines to confer unexpectedly high levels of herd immunity to Hib in vaccinated populations, which in turn enabled control of invasive Hib disease even with incomplete levels of vaccine coverage (*4*, *5*).

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Another major development in the evolution of these vaccines was the successful incorporation of Hib conjugates into multivalent, combination vaccines with DTP and other routine vaccines for infants (*6*). This meant that delivery of Hib conjugates in routine immunization schedules for this age group could be accomplished without requiring additional injections, a factor of major importance in augmenting provider and parental compliance with, and demand for, Hib vaccines.

The attractiveness of the vaccines led rapidly to their widespread use in Australia, Europe, and later, through the efforts of PAHO, to their introduction in Latin America. Yet, despite the demonstration of the importance of Hib as a major pathogen in certain other areas of the developing world, especially sub-Saharan Africa, movement of these vaccines into public health programs for the poor in Africa and Asia, was, until recently, almost nonexistent.

A major force to remedy this disparity was the recent creation of the Vaccine Fund, provided by the Bill and Melinda Gates Foundation and by the governments of several industrialized countries, for use by the Global Alliance for Vaccines and Immunization (GAVI). This fund currently supports the introduction of Hib conjugate, as well as various other vaccines, into infant immunization programs for the world's poorest countries and provides support for the improvement of pub-

lic health infrastructure for vaccine delivery. It is noteworthy, however, that to date the Vaccine Fund has been used to purchase Hib conjugate vaccines for the developing countries of Africa, but not those of Asia.

While there are many possible reasons why Hib conjugate vaccines have not penetrated public health programs for the poor in Asia, one major contributor to this situation is the widespread perception among clinicians and public health policy professionals in the countries of this region that the burden of invasive Hib disease is low in infants and children. Thus, it remains for policymakers in Asia to be convinced of a high disease burden, since even if Hib conjugate vaccines are made available free of charge in the short run via the Vaccine Fund, it seems likely that procurement of these moderately expensive vaccines will have to be sustained partly by scarce local financial resources in the long run. Therefore, the economic argument for using Hib conjugate vaccines in Asia depends largely on the resources to be saved by the prevention of Hib disease, and the economic justification for the vaccines' use hinges on the existence of a high disease burden.

TABLE 1. Importance of *Haemophilus influenzae* type B (Hib) as a cause of bacterial meningitis in Asian children.

Country	Bacterial meningitis cases due to Hib (%)
Bangladesh	43–47[a]
China (Mainland)	32–52
China (Taiwan)	29–39
China (Hong Kong)	21–29
India	0–51
Indonesia	0–11
Iran	10
Iraq	25
Israel	42
Japan	35–59
Jordan	50
Kuwait	45
Malaysia	16–50
Nepal	65
Pakistan	50
Philippines	5–34
Republic of Korea	6–42
Saudi Arabia	30–66
Singapore	19
Thailand	37–48
United Arab Emirates	63
Vietnam	30–53

[a] Ranges derive from countries with multiple studies.

In support of prevailing perceptions of a low disease burden of Hib in Asia, past population-based studies have found rates of Hib meningitis to vary widely (*7*), in contrast to the consistently high rates observed in the United States during the pre-vaccine era. Yet, as shown in Table 1, recent reviews of case series of bacterial meningitis in infants and children in Asia have regularly found Hib to be a major cause of this syndrome (*8*, *9*).

Several years ago the First International Conference on *Haemophilus influenzae* type b infection in Asia addressed this apparent paradox. The Conference concluded that past studies were too flawed to provide guidance about the true Hib disease burden in Asia, and that prospective, population-based studies using appropriate microbiological techniques were needed (*9*).

To address this issue, during the past three years several prospective, population-based studies of the burden of Hib meningitis in children under the age of 5 have been launched in Asia. One such effort was organized by investigators at the International Vaccine Institute (IVI), in collaboration with scientists at the Center for Vaccine Research at the UCLA School of Medicine. This project set up two-year, prospective surveillance studies that comprehensively tracked meningitis in defined populations of under age 5 in three areas of the Far East: Nanning, China; Jeonbuk, South Korea; and Hanoi, Vietnam. Aggressive efforts were made to establish surveillance at all treatment sites where children in the target populations with meningitis were being seen, as well as to ensure proper collection and laboratory evaluation of diagnostic specimens from patients with suspected cases. Despite these measures, annual rates of culture-confirmed Hib meningitis were found to be below 10 cases per 100,000 children under age 5 in each site (*10*). These rates contrast with the annual rates

of 40–60 cases per 100,000 children under 5 generally observed in the United States prior to the use of modern Hib vaccines (*6*).

For several reasons, however, we believe it is still premature to conclude that Hib is not a problem of sufficient magnitude to warrant introduction of modern Hib conjugate vaccines into public health programs for Asian children. Firstly, Asia is a heterogeneous continent, and there remains the possibility that Hib is an important problem in some areas of Asia, while not in others. One review (*8*), for example, has suggested that the data on Hib disease burden reveal a pattern of a greater burden in the Middle East and in South/Southeast Asia than in East Asia. Secondly, the sites selected for the IVI study were areas in which populations were well served by accessible medical facilities in which appropriate diagnostic tests could be undertaken. Many parts of developing countries in Asia are not as well served as these three study sites, and it is unknown whether the epidemiology of Hib in poorer areas is similar to that for areas that are better served. Thirdly, although descriptive epidemiological studies attempting to quantify the disease burden of Hib meningitis are useful, since this syndrome is amenable to clinical detection and microbiological diagnosis, in some areas of the developing world Hib pneumonia constitutes an even greater share of the invasive Hib disease burden. Unfortunately, because of the difficulty in isolating Hib from routine cultures of normally sterile body fluids in children with Hib pneumonia, the magnitude of the burden of Hib pneumonia is not readily discernable from descriptive epidemiological studies. Since prevention of Hib pneumonia can provide a compelling justification for the use of Hib vaccines, failure to consider the disease burden of Hib pneumonia as well as other Hib invasive syndromes may be a serious omission in disease burden assessments that are undertaken to guide vaccine policy development.

For these reasons, just as cross-sectional case series showing that Hib is a common cause of bacterial meningitis are not sufficient to indicate that the population incidence of this syndrome is high enough to warrant the use of vaccines. The low incidence rates of Hib meningitis observed in recent longitudinal descriptive studies of young children in Asia do not provide sufficient evidence to close the door on the use of Hib vaccines in public health programs for the poor in Asia. A controlled field trial of a Hib-conjugate vaccine in infants in the Gambia demonstrated the utility of using the vaccine-prevented incidence of culture-negative syndromes clinically compatible with invasive Hib to infer the magnitude of the "iceberg" of the culture-negative Hib disease burden (*11*). This has given rise to the concept that Hib vaccines can be used as "probes" to more completely identify the burden of invasive Hib disease, especially Hib pneumonia. One such probe study is currently being undertaken in Lombok, Indonesia. If this important study finds a substantial disease burden attributable to Hib pneumonia, it may motivate additional probe studies elsewhere in Asia to inform judgments about the need for introducing Hib vaccines into public health programs for infants in this region.

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DEVELOPMENT OF A LIVE VARICELLA VACCINE: CURRENT STATUS AND PROSPECTS

Michiaki Takahashi[1]

INTRODUCTION

Varicella is a highly contagious disease in children that causes fever and an average of 250 to 500 vesicles. A varicella patient poses a threat to hospital pediatric wards, necessitating the transfer of other patients to other wards. Complications of varicella in immunocompromised cases are occasionally life-threatening. A live varicella vaccine (Oka strain) was developed in the early 1970s by a classical method: 11 passages in human lung cells at 34°C, then 12 passages in guinea pig embryo cells, followed by propagation in human diploid (MRC-5) cells. Tolerability of the vaccine is excellent in healthy children and it is highly effective, with 85%–87% efficacy against clinical varicella and 97% against severe varicella. Recently, a genetic difference was found between vaccine Oka virus (V-Oka virus) and its parental virus (P-Oka virus). Major base and amino acid substitutions are accumulated in gene 62 (immediately early gene). Evidence suggests that a mutation in gene 62 is related to the attenuation of Oka-varicella-zoster virus (VZV).

A sequela of varicella infection may be the later occurrence of herpes zoster, particularly for the elderly. The incidence of herpes zoster is estimated at approximately 15% among the elderly population worldwide, if average life expectancy is assumed to be 70 years. Postherpetic neuralgia is another sequela that mainly affects the elderly. The pathogenesis of herpes zoster has been elucidated. The main route of VZV to the dorsal ganglia is via the peripheral nerves from vesicles in the skin. In follow-up studies of vaccinated leukemic children, the incidence of herpes zoster is several times higher in the group with rashes after vaccination, as compared with those without rashes after vaccination. Since no or few rashes appear after vaccination of normal children, the incidence of the vaccine virus becoming latent in dorsal ganglia may be far lower than that of natural varicella infection. Thus, most vaccinated children are expected to be free from the risk of herpes zoster in future. For adults and elderly persons with a history of varicella, varicella vaccine has been given in an attempt to boost immunity against VZV. Enhancement of cell-mediated immunity is observed in most of them. Although questions regarding the duration of elevated immunity remain, severe postherpetic neuralgia in the elderly is expected to be prevented by administering varicella vaccine.

Several overviews of a live varicella vaccine (Oka strain) have been published (*1–9*). The following sections discuss the main points regarding the development, clinical use, and prospects of this vaccine.

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Primary Isolation of the Vaccine Virus

Fluid was taken from the vesicles of a 3-year-old boy who had typical chickenpox, but was otherwise healthy. The fluid was stored at –70°C until it was inoculated onto primary cultures of human embryo lung (HEL) cells. Characteristic foci appeared after 7–10 days at 34°C. The virus strain was named Oka, after the boy from whom the vesicular fluid was derived (*10*).

Difficulties in Preparing "Cell-Free" Varicella-Zoster Virus

Since the earliest studies of in vitro propagation of varicella-zoster virus (VZV), it has been recognized that virus produced in cell cultures remains strongly cell associated; the inability to obtain cell-free infectious virus has hampered biological and immunological studies of VZV. Attempts were made to identify a suitable method for isolating cell-free virus from infected cultures and the composition of a suspension medium that would keep the infectivity of the virus as stable as possible.

Because VZV is highly heat-labile, particular caution was required in the selection of a suspension medium that would preserve its infectivity. After comparing several media, simple phosphate-buffered saline (Ca, Mg free) was selected as the most suitable, with sucrose (final concentration, 5%), sodium glutamate (0.1%), and other constituents (*11*).

Rationale for and Design of a Live Varicella Vaccine

VZV spreads from cell to cell, forming distinct foci that are visible by microscopy, even in unstained cell cultures, and that are clearly visible after methylene blue or fluorescent antibody staining. Cell-mediated immunity seems essential, or at least as important as humoral immunity in preventing the spread of VZV in vivo. Since inactivated or subunit viral antigens are usually weak inducers of cell-mediated immunity, it was reasoned that a live vaccine might be the most useful for the prevention of varicella.

It had been very difficult to demonstrate the pathogenicity of VZV in laboratory animals. It was anticipated that attenuation would be proven only by extensive clinical trials, and that testing of only a limited number of candidate strains would be feasible. The classical (empirical) method of attenuation using passage in foreign cells was used. Of the various kinds of nonprimate cultured cells tested for susceptibility to infection with VZV (Oka strain), only guinea pig embryo fibroblasts (GPEF) were found to be somewhat susceptible.

VZV (Oka strain) was passaged 11 times in HEL cells at 34°C and 12 times in GPEF cells at 37°C, and then propagated in human diploid cells (W1-38) (*10*). The virus thus obtained exhibited better capacity for growth in GPEF than the original or other wild-type strains, which suggests that the vaccine virus is a variant with host dependency.

Biological and Biophysical Properties of the Vaccine Virus

The Oka vaccine virus is temperature sensitive and has an enhanced capacity for growth in guinea pig embryo cells (*3*). Oka strain has been differentiated from other wild-type viruses by restriction-endonuclease digestion of extracted purified viral DNA, followed by agarose gel electrophoresis. In a comparison of the vaccine type DNA and wild-type virus DNAs, significantly different cleavage patterns were seen using HpaI, BamHI, BglI, and PstI enzymes (*12–14*). A more practical approach utilizing polymerase chain reaction (PCR) and restriction endonuclease digestion of the resulting DNA fragments was developed. Analysis of five variable regions with repeat elements (termed RI–R5) in the VZV genome—a cutting site of PstI in the PstI siteless region—was described (*14*). Later, we described a novel laboratory method for distinguishing the Oka strain from other isolates by combination analysis with the single strand-conformational polymorphism of repeating re-

gion 2 and with PstI cleavage of the PstI siteless region (*15*). Although Oka strain can be distinguished from other isolates of VZV using the methods described above, vaccine virus cannot be reliably distinguished from its parental virus by those methods.

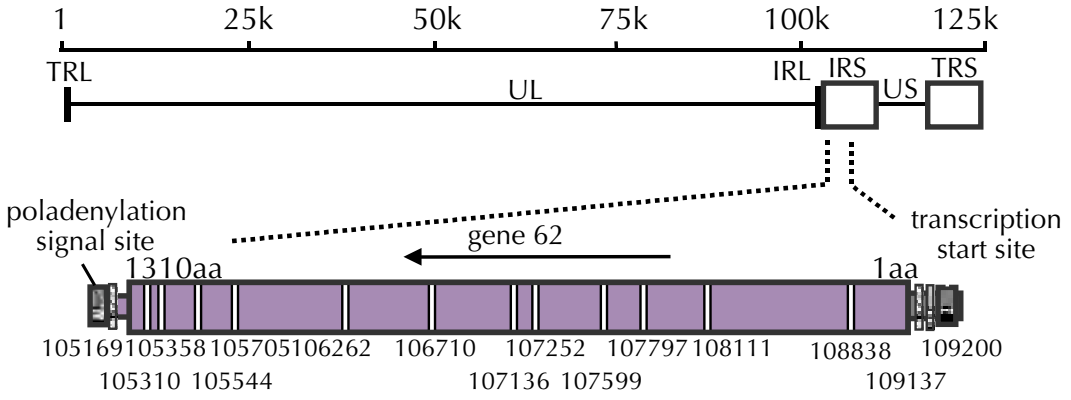
Difference of DNA Sequences of OKA Varicella Vaccine and its Parental Virus

VZV is composed of 71 genes, classified as immediately early (IE), early (E), and late (L), which are known to function in cascading fashion in infected cells. Thus, IE genes have been regarded as the most important genes in initiating VZV growth in infected cells (*16*).

Genes 4, 10, 61, 62, and 63 have been reported as IE genes. When sequences were compared between V-Oka and P-Oka virus, no difference was found in the nucleotide sequences of genes 4, 10, 61, or 63, though as many as 15 nucleotide replacements and eight amino acid changes were identified in gene 62 of the Oka vaccine virus (Figure 1) (*17, 18*). When the entire sequence of gene 62 was amplified by PCR, the reaction products from the vaccine virus were composed of a mixture of at least eight different clones that had a variety of mutations in that gene. On the other hand, the sequence analysis of nine clones derived from the Oka parental virus demonstrated that the parental virus consisted of a single sequence (*18*). It was further demonstrated that 15 base substitutions are specific for V-Oka and are not present in nine clinical isolates: three are from varicella patients around the same period of isolation of P-Oka (1971–1972), three are from varicella patients in the same clinic in 1995–1996, and three are zoster cases in different areas of Japan in 1995–1996 (*19*).

It was also demonstrated that S7-01 virus, a clone vaccine virus which had mutations in all eight amino acids (as found in the vaccine virus in IE62), spread more slowly in HEL cells (*19*). Thus, the substitutions that have accumu-

FIGURE 1. Structure of gene 62 and sequence analysis of the OKA parental and vaccine viruses.



position (100000+)	5169	5310	5356	5544	5705	6262	6710	7136	7252	7599	7797	8111	8838	9137	9200
Oka parental	A -	A L	T I	A V	T A	T R	A A	T A	T S	A V	A L	T P	A M	A -	A -
Oka vaccine	A/G -	A/G L/S	C V	G A	C A	C G	A/G A	C A	C G	A/G V/A	A/G L/P	C P	A/G M/T	A/G -	A/G -

Source: Gomi Y, Imagawa T, Takahashi M, Yamanishi K. Oka varicella vaccine is distinguishable from its parental virus in DNA sequence of open reading frame 62 and its transactivation activity. *J Med Virol* 2000;61:497–503.

lated in gene 62 are likely to be important for the differences in the replication and spreading from infected to uninfected cells. Because V-Oka had been passaged in guinea pig cells and in human fibroblast cells at a low temperature, mutant viruses have been selected and grown under selective pressure. The reason why so many amino acid substitutions were accumulated in gene 62 of V-Oka is still unclear, but it is possible that the mutants IE62 contained in V-Oka may have a competitive advantage over P-Oka IE62 in interacting with some cellular transcription factor in guinea pig cells (*19*).

Early Clinical Trials: Vaccination of Healthy and Hospitalized Children

With the informed consent of the parents, healthy children who were living at home and had no history of varicella received various doses of Oka strain varicella vaccine virus. A dose of 500 PFU elicited seroconversion in 19 of 20 children. Even at a dose of 200 PFU, an antibody response was detected in 11 of 12 children. No symptoms due to vaccination were detected in these children (*10*).

The first clinical trial of the vaccine in hospitalized children was undertaken in an effort to terminate the spread of varicella among children with no history of the disease (*10*). In the hospital where the trial was conducted, chickenpox had frequently spread in the children's ward, with severe cases seen on some occasions. In this protocol, children with no history of varicella were vaccinated immediately after the occurrence of a case of varicella. These children were suffering from conditions including nephritic syndrome, nephritis, purulent meningitis, and hepatitis. Twelve children had been receiving corticosteroid therapy. An antibody response was documented in all of the vaccinated children; within 10–14 days after vaccination, six children developed a mild fever, and two of the six developed a mild rash. It was uncertain whether these reactions were due to vaccination or to naturally acquired infection modified by vaccination. No other clinical reactions or abnormalities of the blood or the urine were detected. Thus, in this ward, the spread of varicella infection was prevented except in one case: a child who was not vaccinated because his mother mistakenly believed that he already had varicella became severely ill. This study offered the first proof that the Oka strain varicella vaccine was well tolerated by patients receiving immunosuppressive therapy and stirred hopes that this vaccine would prove practical for the prevention of varicella.

Protective Efficacy of Vaccination in Early Clinical Trials

In an examination of its protective efficacy, the vaccine was given to susceptible household contacts immediately after exposure to varicella (*20*). Twenty-six contacts (all children) from 21 families were vaccinated, mostly within three days after exposure to the index cases. None of the vaccinated children developed symptoms of varicella. In contrast, all 19 unvaccinated contacts (from 15 families), exhibited typical varicella symptoms 10–20 days after the onset of the index cases. In three families, one sibling contact received the vaccine and the other did not; none of the vaccinated children developed symptoms, whereas all unvaccinated controls exhibited typical symptoms. In general, the antibody titers after clinical varicella were 8–10 times higher than those after immunization. This study clearly demonstrated that vaccination soon after exposure was protective against clinical varicella.

In an institution for children under 2 years old, prompt vaccination had a similar protective effect (*21*). Varicella developed in an 11-month-old infant in a ward for 86 children. A total of 33 children over 11 months of age were not vaccinated, partly because they were expected to still possess maternal antibody. A small viral dose (80 PFU) was used for immunization. Of the vaccinated group, eight developed a mild rash and one of these eight had a mild fever (under 38°C) two to four weeks after vaccination. In contrast, typical varicella

developed in all 43 unvaccinated children during the 10 weeks after the onset of the index case. Symptoms were severe in 16 cases, with confluent vesicles and high fever; after recovery, scars remained in 13 of these 16 cases. These results suggested that vaccination with as little as 80 PFU frequently stopped the spread of varicella among children in close contact with one another.

Isolation of VZV from the Blood of Naturally Infected and Vaccinated Children

VZV could be recovered from blood mononuclear cells of immunocompetent patients for several days before and after onset of the disease (Table 1) (22). In contrast, no VZV could be recovered from a total of 27 children, 4 to 14 days after vaccination at a dose of 5,000 PFU (Table 2). It is generally believed that at the time of primary VZV infection, the virus multiplies in the respiratory mucosa and the regional lymph nodes, and that this multiplication leads to a primary viremia, during which the virus is delivered to the viscera, where further multiplication ensues. A secondary viremia, greater in magnitude than the first, then occurs and delivers virus to the skin, leading to the appearance of a rash. The above results suggest that the magnitude of replication of the vaccine virus in the susceptible viscera is far less than that of wild-type VZV, but sufficient to induce an immune response. Although the route of infection with the virus was not the same, it seems that viremia may be a marker of the virulence of VZV for the host, and the vaccine virus may be attenuated to the degree that it lacks the capacity to cause a viremia, except, possibly, in rare instances.

Vaccination of Children with Malignant Diseases

In the first vaccination trials in children with malignant diseases with virus doses of 200, 500, or 1,500 PFU, chemotherapy was suspended for one week before and one week

TABLE 1. Viral isolation from mononuclear cells and antibody responses after close contact with varicella patients.

	Viral isolation from mononuclear cells		Detectable antibodies[a]	
Day of testing after onset of varicella	Positive subjects/No. tested	%	Positive subjects/No. tested	%
–11	0/3	0	ND[b]	
–7	0/4	0	ND	
–6	0/1	0	0/1	0
–5	1/2	50	ND	
–4	1/3	33	0/2	0
–3	ND		0/1	0
–2	4/4	100	0/4	0
–1	4/5	80	0/5	0
0	4/17	24	0/13	0
1	7/32	22	0/28	0
2	0/14	0	0/12	0
3	0/3	0	4/12	33
4	0/1	0	9/18	50
5	0/3	0	14/14	100

[a] Measure by the assay for fluorescent antibody to membrane antigen.

[b] ND = Not done

Source: Asano Y, Itakura N, Hiroishi Y, Hirose S, Ozaki T, Kuno T, *et al*. Viral replication and immunologic responses in children naturally infected with varicella-zoster virus and in varicella vaccine recipients. *J Infect Dis* 1985;152:863–868.

TABLE 2. Isolation of varicella-zoster virus from children inoculated with live virus vaccine (Oka strain).

	Virus isolation source							
	Mononuclear cells		Throat		Detectable antibodies[a]		Positive skin reaction	
Day of testing after vaccination	Positive vaccinees/ No. tested	%	Positive vaccinees/ No. tested	%	Positive vaccinees/ No. tested	%	Positive vaccinees/ No. tested	%
0	ND[b]		0/3	0	0/28	0	0/22	0
3	0/11	0	0/8	0	0/8	0	0/10	0
4–5	0/14	0	0/13	0	0/11	0	1/11	9
6–7	0/17	0	0/16	0	2/8	25	8/11	73
8–9	0/6	0	0/6	0	2/5	40	4/5	80
10–14	0/11	0	ND		8/8	100	6/7	86
30–60	ND		ND		28/28	100	17/20	85

[a] Measure by the assay for fluorescent antibody to membrane antigen.

[b] ND = Not done

Source: Asano Y, Itakura N, Hiroishi Y, Hirose S, Ozaki T, Kuno T, *et al*. Viral replication and immunologic responses in children naturally infected with varicella-zoster virus and in varicella vaccine recipients. *J Infect Dis* 1985;152:863–868.

after vaccination (*23*). Of 12 immunized children with acute lymphocytic leukemia, 10 had been in remission for six months or less, one for nine months, and one for forty-eight months. Of these children, four had fewer than 3,000 white blood cells/mm^3, but most had positive skin-test reactions with dinitrochlorobenzene, purified protein derivative, or phytohemagglutinin. Three of twelve children developed a mild rash; 13 papulae or incomplete vesicles developed in one of three children who received 1,500 PFU; 30 and 25 papulae, respectively, developed in two of five children who received 200 PFU; four children who received 500 PFU did not develop a rash; and one child had a fever (39°C) for one day about three weeks after vaccination. These results offered hope that a live varicella vaccine could be administered with some precautions to high-risk children (*1*, *7*, *24*).

Clinical Vaccine Trials in the U.S. and Europe and Licensure of the Vaccine

In the U.S., the National Institutes of Health (NIH) Collaborative Study Group was organized, and clinical trials were started with live varicella vaccine (Oka strain) produced by Merck Research Laboratories (West Point, PA, U.S.A.). Many investigations were conducted by that group, including clinical reactogenicity, the frequency of household transmission from vaccinated acute leukemic children with rash, and the persistence of immunity. Other study groups also conducted clinical trials, most of which yielded favorable results. In Europe, clinical trials were conducted with varicella vaccine (Oka strain) prepared by SmithKline RIT (Rixensart, Belgium). In 1983, the Expert Committee was held at the World Health Organization in Geneva to prepare a manuscript entitled "Requirements for the Live Varicella Vaccine." The resulting document was circulated for review by authorities around the world and was finally published in 1985 (*25*, *26*). Meanwhile, in 1984, the live varicella vaccine (Oka strain) produced by SmithKline RIT was licensed for administration to high-risk children in several European countries.

In 1986, live varicella vaccine produced by the Research Foundation for Microbial Diseases of Osaka University (BIKEN) was licensed in Japan for use in high-risk children and for optional use in children at normal risk. In South Korea, live varicella vaccine (Oka strain) was licensed for uses similar to those in Japan. in 1988. In 1995, live varicella vaccines

(Oka strain), produced by Merck Research Laboratories, were licensed for the universal immunization of healthy children in the U.S.

Vaccine Efficacy

Several follow-up studies, conducted after licensing of the vaccine in Japan, indicated that breakthrough cases occur in 15%–20% of the vaccine recipients. However, approximately 60% of such cases are extremely mild (a few vesicles) and 20% are mild (several to 50 vesicles). Thus, it is estimated that clinically significant breakthrough cases are no more than 5% of the varicella vaccine recipients. A quantitative comparison of the severity of symptoms of natural varicella and of breakthrough cases in vaccine recipients found that the symptoms of breakthrough cases are far milder than those of natural varicella (*27*).

In the U.S., several reports of breakthrough cases—approximately 15% of the vaccine recipients—manifested clinical symptoms. Conclusive data appeared in 2001 in the U.S.; a case-control study was conducted from March 1997 through November 2000 for 330 potential cases, of which 243 (74%) were in children who had positive PCR tests for VZV. Of the 202 children with PCR-confirmed VZV and their 389 matched controls, 23% of the former and 61% of the latter had received the vaccine (vaccine effectiveness, 85%). The vaccine was 97% effective against moderately severe and severe disease. Thus, it was concluded that varicella vaccine is highly effective as used in clinical practice (*28*).

Tolerability of the Vaccine

The varicella vaccine (Oka strain) has been shown to be safe and very well tolerated. Adverse clinical reactions (rash, fever, redness, and swelling) due to the vaccine are rare and generally mild, if at all, in normal children (*29*).

The risk of clinical reactions following the administration of Oka strain varicella vaccine was higher among high-risk individuals. A large study of 663 children attending a pediatric clinic during a seven-year period showed that vaccination produced adverse reactions in 32.4% of children with malignant disease when administered with chemotherapy, compared with only 0.3% of those with other conditions, including congenital heart disease, neuromuscular disease, and immunological diseases (*2*). However, all the reactions were mild and resolved spontaneously. Importantly, administration of the Oka strain vaccine had no significant impact on relapse rates in children with acute leukemia (*2*). Likewise, children with other underlying diseases have also been effectively vaccinated with no adverse effect on their medical condition (*2*).

Herpes Zoster and the Live Varicella Vaccine

It has generally been believed that VZV in the skin vesicles travels up the sensory nerves to the posterior ganglia, where it persists; this seems to be the main route of virus migration. A major question about live varicella vaccine had been whether the vaccine virus becomes latent, resulting in the later development of zoster. Since zoster is relatively uncommon in healthy children, long-term follow-up of vaccinated healthy children was required to answer this question definitively. However, children with acute leukemia tend to develop zoster soon after natural infection. Therefore, it was assumed that careful observation of the incidence of zoster in vaccinated children with acute lymphocytic leukemia would yield valuable insight.

A retrospective follow-up study of children with acute leukemia found that zoster occurred far more frequently in the group that developed a rash after vaccination (17.1% or 3.13 cases per 100 person-years; $n = 70$) than in the group without rash (2.4%, or 0.46 cases per 100 person-years; $n = 250$) (*1, 2*). These figures suggested that an absence of rash after vaccination is closely correlated with a low incidence of zoster, indicating that the incidence of zoster would be lower among vaccine recipients than among children who had natural varicella.

Studies by U.S. National Institute of Allergy and Infectious Diseases Collaborative Study Group showed clearly that an absence of rash is correlated with a low incidence of zoster. Of 268 vaccinated children with VZV rashes, 11 (4.1%) had zoster. In contrast, there were only two cases of zoster (0.7%) among the 280 vaccinated children with no VZV rash. The relative risk of zoster in the children who had had a VZV rash was 5.75 (*30*).

Besides the main virus migration route (i.e., via the sensory nerves), there may be a minor hematogenous migration route to the ganglia. However, no viremia could be detected in healthy vaccine recipients, while viremia could be detected in cases of natural varicella for several days before and just after appearance of the rash (*21*). Therefore, whatever the route, it seems far less likely for the vaccine virus than for wild-type virus to become latent in the ganglia and cause subsequent zoster.

Immunization of the Elderly to Enhance Immunity to VZV Assessed by the VZV Skin Test for Cell-mediated Immunity and Humoral antibody

The VZV skin test has been shown to be useful for assessing the susceptibility of individuals to clinical varicella (*31*). The skin test was negative or weakly positive during the early stage of herpes zoster infection, and strongly positive during recovery (*32, 34*). In a small-scale clinical trial, elderly individuals were immunized in order to prevent herpes zoster, and, hopefully, severe postherpetic neuralgia (*35*). Sixty individuals (≥50 years old) were screened for VZV antibodies and were given a VZV skin test for cell-mediated immunity. All were seropositive, but eight were skin-test negative. Thirty-seven individuals, including the eight with negative skin tests, were immunized with varicella vaccine (3.0×10^4 PFU/dose). After five to seven weeks, the skin test reaction showed increased positivity, with a change in score from (−) to (+, ++) in seven of eight subjects, from (+) to (++,+++) in three of five subjects, and from (++) to (+++) in six of ten subjects. Enhancement of the VZV antibody titer (twofold or greater) was observed in all 15 vaccine recipients with a prevaccination titer of ≤1:16, and in 19 of 24 subjects with a prevaccination titer of ≥1:32.

These results indicate that giving live varicella vaccine with a high viral titer can induce a good boost to immunity, particularly cell-mediated immunity, to VZV in the elderly, as assessed by the VZV skin test.

Immunity to VZV in 35 elderly subjects who were vaccinated previously was followed up for four years. All were positive by the VZV skin test after the previous vaccination. After four years, 31 (88.6%) were positive by the skin test, and four were negative and became positive after revaccination (*36*). These results suggest that administering live varicella vaccine to the elderly is effective for enhancing immunity, particularly cell-mediated immunity to VZV, and that enhanced cell-mediated immunity lasts four years in most vaccine recipients.

The duration of immunity enhanced by vaccination is a crucial matter for the application of vaccination to the prevention of zoster, particularly for postherpetic neuralgia. It is expected that vaccination of elderly persons around and older than 60 years of age at four- to five-year intervals will significantly reduce their risk of severe herpes zoster and, particularly, of severe postherpetic neuralgia. A large scale clinical trial is under way in the U.S. for the prevention of herpes zoster, particularly postherpetic neuralgia, by giving live varicella vaccine (produced by Merck Research Laboratories) to elderly subjects.

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HEPATITIS A VACCINES

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INTRODUCTION

Despite the recent successful development and international marketing of inactivated hepatitis A vaccines, hepatitis A remains a common infectious disease in many regions of the world. Transmission occurs largely by the fecal-oral route, although in recent years a rise in parenteral transmission has been noted in economically developed countries where infections have been related to illicit injection drug use. In such nations, point-source outbreaks due to ingestion of contaminated food also continue to occur sporadically, as well as less dramatic outbreaks that are associated with preschool day care centers and maintained via person-to-person transmission. But in less developed countries, infections are much more prevalent. Transmission occurs in the early years of life and is related in general to inadequate water supplies and poor public health sanitation.

Hepatitis A causes significant morbidity, but only rarely leads to death (*1*). The incubation period averages around one month, and onset of the illness may be sudden in nature. Most cases of fulminant hepatitis are reported in older individuals or in the very young. Relapsing hepatitis and cholestatic hepatitis are also recognized complications of infection with hepatitis A virus (HAV), but there are no chronic sequelae of hepatitis A such as those which occur with hepatitis B or hepatitis C. There is no association with cirrhosis, no persistence of the virus (except perhaps rarely, and only for a matter of months, in infected premature infants), and certainly no association with hepatocellular carcinoma.

In the United States, prior to the licensure of inactivated hepatitis A vaccine in 1995, hepatitis A accounted for approximately 50% of the cases of acute hepatitis that precipitate visits to the emergency room or to personal physicians. That picture is not much different today (*2*). The most recent summaries from the U.S. Centers for Disease Control and Prevention (CDC) indicate that there are approximately 30,000 cases of hepatitis A reported to public health authorities annually. The incidence has decreased somewhat since the licensure of the vaccine, but the proportion of cases of hepatitis due to HAV infection is similar to what it was prior to licensure. This reflects, no doubt, the relatively high cost of this vaccine, and the fact that it generally has been administered only to individuals in special, high-risk populations.

Thus, while the vaccine is extremely efficacious in preventing disease in immunized persons, as pointed out below, economic considerations have limited its ability to control the spread of HAV within the U.S. population. Overseas, in regions where hepatitis A is considerably more prevalent than in the United States, the vaccine has had even less impact on public health.

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Inactivated Hepatitis A Vaccines

The chronology of the hepatitis A vaccine begins with the first description of the syndrome of infectious hepatitis as a disease distinct from other causes of infectious jaundice. This occurred early in the last century, at which time the disease was known as "catarrhal jaundice" (*3*). By the end of World Word II, hepatitis A was clearly distinguished both clinically and epidemiologically from hepatitis B. These two infections were shown to be due to agents that were immunologically distinct (*4*), although the alphabetic system for classification of the hepatitis viruses did not follow until several years later. By that time, pooled human immune globulin was known to be protective against infectious hepatitis when administered parenterally, either prior to or as long as two weeks after exposure (*5*). This important finding indicated early on that circulating antibodies are highly protective against symptomatic hepatitis A, and that neither secretory immunity nor cytotoxic T-cell activity is required for protection against the disease.

These early observations were followed by the classic clinical studies of the natural history of hepatitis A that were carried out by Krugman beginning in the 1950s and extending into the 1970s (*6*, *7*). However, the modern era of hepatitis A virology began in 1973, when HAV particles, the causative agent of hepatitis A, were identified in human fecal material by Feinstone, Kapikian, and Purcell working at the National Institutes of Health (*8*). To accomplish this, these investigators used the then relatively new technique of immune electron microscopy, demonstrating the aggregation of viral particles by convalescent sera containing specific antibodies to the virus. These pioneering studies paved the way for development of sensitive and specific serologic tests for hepatitis A, and shortly thereafter, in large part because of these tests, to the recognition of the third major type of viral hepatitis in humans, then called "non-A, non-B hepatitis," and now known as hepatitis C.

The breakthrough that led directly to the hepatitis A vaccines available today was the isolation and propagation of HAV in cultured cells by Provost working with Hilleman at Merck in the latter part of the 1970s (*9*). In 1986, a team led by Binn at Walter Reed Army Medical Center described the successful immunization of small primates with a prototype vaccine produced by formalin-inactivation of virus particles harvested from infected cell cultures (*10*). This seminal work demonstrated that cell culture infections could produce sufficient amounts of viral antigen for vaccine production, and it was followed shortly afterwards by advanced vaccine development efforts within the industry. In 1992, the first demonstration of clinical efficacy in humans was reported by Werzberger and colleagues in a now classic study carried out in Monroe, New York, using an inactivated vaccine (Vaqta) produced by Merck (*11*). Comparable efficacy was subsequently shown to exist for a similar vaccine (Havrix) produced by SmithKline-Beecham (now GlaxoSmithKline, or GSK) in a study carried out in Thailand (*12*). This vaccine was the first to be licensed by the U.S. Food and Drug Administration, receiving approval in 1995. Both the Merck and GSK vaccines are now registered in many countries, and they have been joined on the market by other inactivated hepatitis A vaccines produced in Europe and Japan. These vaccines as a group are marked more by their similarities than by their differences. A more complete description of the Merck and GSK vaccines that are licensed within the United States can be found elsewhere (*13*).

By and large, all of these vaccines have been produced using "old" technologies (*14*). Although in some cases the vaccine antigen is highly purified from accompanying cellular materials prior to inactivation, the basic principles underlying the inactivated hepatitis A vaccines are those employed for production of the Salk inactivated poliovirus vaccine. This is somewhat ironic for an infectious agent that has only been discovered in the past few

decades, but it is consistent with what we know about the infectious agent, which, like the polioviruses, is a member of the family *Picornavidae*. A brief review of HAV virology makes it clear why this type of vaccine is prevalent among hepatitis A vaccines today, although an attenuated vaccine has been used extensively in China.

The Virology of Hepatitis A

The HAV particle contains three large capsid polypeptides (VP1, VP2, and VP3) that contribute to a very tightly assembled, non-enveloped viral capsid that protects the positive-strand viral RNA packaged within from nuclei present in the external environment (*15*). This capsid possesses receptor-binding activities that direct the virus to its cellular site of replication. Sixty copies of each of the capsid polypeptides are presumed to be present in each particle, given what is known about the structure of this and related viruses. They fold in a way that conformationally determines the neutralizing antigenic epitopes of the virus (*16*). Thus, when the capsid proteins are individually expressed from recombinant cDNA, the proteins have very poor immunogenicity and elicit only very low levels of neutralizing antibodies in animals. The generation of a protective antibody response thus requires immunization with the complete viral capsid in its assembled form. While it is possible to assemble such a particle from capsid polypeptides expressed in bacteria (*17*), the production of virus particles in infected cell cultures has thus far proven to be the only practical pathway to vaccine manufacture on a commercial scale.

A second important point concerning the antigenicity of the virus is that there is only a single serotype of HAV (*18*), despite the existence of multiple viral genotypes that are defined by differences in the nucleotide sequence of the RNA genome. Thus, infection (or immunization) with any one strain of HAV confers protection against all other strains of the virus. This cross-strain protection extends even to several simian genotypes, despite the fact that these particular strains of HAV do demonstrate differences in the amino acid sequences of some critical neutralization epitopes. From a practical point of view, the fact that there is only one serotype makes it possible for a single hepatitis A vaccine antigen to protect against the disease anywhere in the world. From a theoretical perspective, the lack of significant antigenic diversity suggests that the capsid antigens may play a critical role in the viral life cycle, perhaps in recognition of the cellular receptor for the virus.

As indicated above, the major scientific advance that made the hepatitis A vaccine possible, given that recombinant approaches proved to be impractical, was the development of cell culture systems allowing the propagation of the virus (*9*). Either primary or continuous African green monkey kidney cells are permissive for replication of the virus and are usually used for primary isolation of the virus. MRC-5 cells generally are used for production of the viral antigen for vaccine manufacture. The infection in both of these cell types is typically noncytopathic. It is also not very robust, with the titer of virus produced at least 10- to 100-fold less than what would be expected with poliovirus. Some variants of the virus that have been highly adapted to growth in cell culture are cytopathic, at least in part through induction of apoptosis in infected cells (*19*, *20*). Such viruses can be used in conventional plaque-reduction neutralization assays. On the other hand, much more has been learned about the neutralizing antibody response to the virus using radioimmunofocus inhibition assays, which depend upon the use of a radiolabelled antibody for detection of cell foci infected with HAV under an agarose overlay (*21*).

Although the hepatitis vaccines that are licensed today are, by and large, cell-culture–propagated, whole virus, inactivated vaccines, a live attenuated vaccine has enjoyed extensive use in China (*22*, *23*). This vaccine utilizes a strain of HAV that has been propagated and adapted to growth in cell culture. Studies done

by Provost and Hilleman and their colleagues at Merck during the late 1970s and early 1980s demonstrated clearly that passage of the virus in cell culture leads to its attenuation for primates, including humans (*24*, *25*). This was subsequently confirmed in studies done with a second viral isolate at the National Institutes of Health (*26*). However, neither of these vaccine development programs led to a virus that had an acceptable balance of attenuation and immunogenicity, and these efforts were eclipsed by the subsequent success of the inactivated vaccine. There is not much in the literature concerning the attenuation properties of the Chinese hepatitis A vaccine, even though it has been used quite extensively in that country.

The licensed inactivated vaccines generally are formulated with an alum adjuvant and used in a two-dose regimen (*13*). They have low reactogenicity and, although they have been associated with a low incidence of anaphylaxis and central nervous system adverse events, they appear to be among the safest vaccines in the infectious disease armamentarium. As mentioned above and described in greater detail below, they have excellent efficacy in the prevention of disease.

Hepatitis A Vaccine Efficacy

Table 1 summarizes the two pivotal efficacy studies that supported the licensure of Vaqta, the Merck vaccine, and Havrix, the vaccine licensed by GSK in the mid-1990s. The Vaqta trial was carried out in Monroe, New York, within an orthodox Jewish community in which most families were large and which had extensive day care arrangements for very young children (*11*). Historically, prior to the vaccine study, this community had experienced high rates of hepatitis A in children and young adults with almost annual summertime epidemics. The vaccine efficacy trial was begun with the intent to deliver a two-dose regimen, but a typical seasonal epidemic of hepatitis A broke out within the community shortly after the study was started, and efficacy was proven before a second dose could be administered. Hence, it was shown that one dose of vaccine was sufficiently immunogenic to provide protective immunity. In fact, no individual developed hepatitis in that trial who had been immunized more than 16 days previously. The overall vaccine efficacy was 100%, with 95% confidence intervals.

The Havrix trial in Thailand was quite different, although its conclusions were not. It involved almost 40,000 children aged 1 to 16 who were immunized with either hepatitis A vaccine or, as a control, a hepatitis B vaccine rather than a placebo (*12*). Although this clinical efficacy trial monitored the ability of the vaccine to prevent endemic rather than epidemic disease, it gave a very similar result (94% protective efficacy), one that is statistically identical to the result obtained with the Merck vaccine in Monroe, New York. Both studies are indicative of nearly complete protection against the disease following immunization.

TABLE 1. Hepatitis A vaccine efficacy.

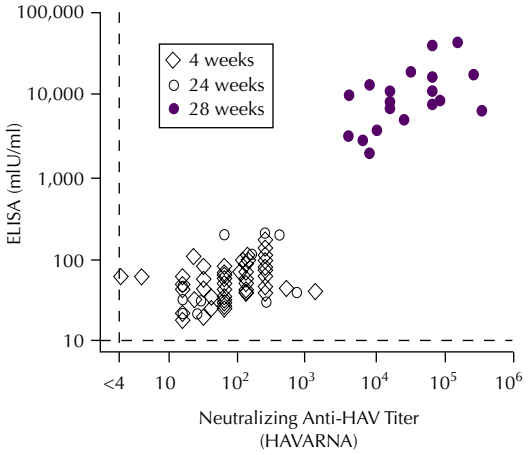
Vaccine	Study site (Subject ages)	No. of subjects	Vaccine efficacy (95% CI)
Vaqta™ (Merck) 1 dose; 25 units	Monroe, N.Y. (2–16 years)	1,037	100% (85%–100%)
HAVRIX® (SKB) 2 doses; 360 EL.U.	Thailand (1–16 years)	38,157	94% (79%–99%)

Sources: Werzberger A, Mensch B, Kuter B, Brown L, Lewis J, Sitrin R, *et al*. A controlled trial of a formalin-inactivated hepatitis A vaccine in healthy children. *N Engl J Med* 1992;327(7): 453–457. Innis BL, Snitbhan R, Kunasol P, Laorakpongse T, Poopatanakool W, Kozik CA, *et al*. Protection against hepatitis A by an inactivated vaccine. *JAMA* 1994;271(17):1328–1334.

Following the completion of the Vaqta efficacy study in Monroe, we had the opportunity to study the anti-HAV antibody response in study participants and to compare it with that present in persons receiving immune serum globulin at a dose known to be protective. We determined antibody titers using the radioimmunofocus inhibition viral neutralization assay alluded to above, as well as a hepatitis A virus antigen reduction neutralization assay, and compared these titers with the level of antibody determined in an ELISA assay (*27*). As shown in Figure 1, there was a very close correlation between the results of these different assays. This indicates that the ELISA assay, which is commonly available in the clinical setting, can be used as a measure of the protective antibody response.

Virtually every immunized individual had antibody within four weeks of receiving the first dose of Vaqta. Most had antibody levels that equaled or exceeded those present seven days after the administration of immune globulin. There was a substantial booster effect when a second dose of vaccine was given six months after the first. The neutralizing antibody titers were substantially elevated in both assays (*27*). This booster effect most likely extends the duration of protection. Prior to the clinical vaccine efficacy trials, it was recognized that the efficacy of these vaccines could be predicted from the measurement of neutralizing antibody levels in the blood (*21*). These studies confirmed that notion, in addition to providing formal proof of vaccine efficacy.

FIGURE 1. Anti-HAV antibody titers in recipients of Vaqta vaccine who participated in the Monroe, New York, efficacy study, and in recipients of immune globulin.

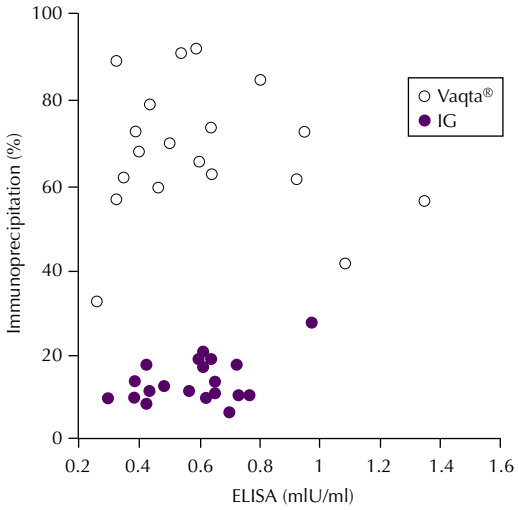


Despite the fact that the neutralizing antibody titer is an excellent correlate of protection, antibodies that are induced within the first few weeks after active immunization are qualitatively dissimilar from those present in immune serum globulin (*27*). Figure 2 shows antibody titers in persons who had received a single dose of the Merck vaccine 24 days previously, plotted along with antibody titers in persons who had received immune globulin one week before being bled. When titers obtained in the ELISA assay were compared with those detected in a viral immunoprecipitation assay (one employing HAV particles that were endogenously labeled during their production in cell culture), the relative activities were strikingly different in the vaccine vs. immune globulin recipients. Although formal measurements of the affinity of these antibodies for HAV have yet to be done, the data suggest that antibody is of low avidity in the early weeks after immunization with vaccine (*27*). In contrast, the recipient of immune globulin, while similarly protected, appears to have a much lower abundance of high avidity antibody. However, these differences, while interesting, are not likely to be of significance clinically, much as borne out by the results of the clinical trials.

Pathogenesis and Mechanisms of Protection

It is very likely that the protection afforded by vaccines is due to the ability of antibody to limit the spread of virus within the liver during the early stages of infection. Current views of the pathogenesis of this infection hold that the virus usually enters via the gastrointestinal tract and establishes a primary infection in epithelial cells within the crypts of the small

FIGURE 2. Antibody titers determined by an enzyme-linked solid-phase immunoassay (ELISA) and by a viral neutralization test (HAVARNA) 4 and 24 weeks after a first dose of vaccine, and 4 weeks after a second, booster dose of vaccine given at 24 weeks.



Note: There is an excellent correlation between the immunoassay and viral neutralization results. Antibody titers were determined by an immunoprecipitation assay employing labeled virus particles and by ELISA approximately four weeks after a first dose of vaccine, or one week after a dose of immune globulin. Sera with similar ELISA reactivities have levels of reactivity in the immunoprecipitation assay that are many-fold higher among the immune globulin recipients compared with the vaccine recipients. Greater reactivity in the immunoprecipitation assay, which employs very small amounts of viral antigen, is likely due to the presence of high affinity antibody. Figures modified from Lemon et al. (27).

Source: Figures modified from Lemon SM, Murphy PC, Provost PJ, Chalikonda I, Davide JP, Schofield TL, et al. Immunoprecipitation and virus neutralization assays demonstrate qualitative differences between protective antibody responses to inactivated hepatitis A vaccine and passive immunization with immune globulin. *J Infect Dis* 1997;176(1):9–19.

intestine (*28*). Whether by release of virus into the intestine and reentry via specialized M cells in the terminal ileum, or by direct invasion of the virus through the epithelial cells of the small intestine, there is spread of the virus via the bloodstream to the liver. The production of virus by infected hepatocytes leads to a secondary viremia of much greater magnitude (*29*, *30*), and this results in the further spread of the virus within the liver, with growing numbers of hepatocytes being infected over a period of several weeks. When this noncytopathic infection of the liver is finally recognized by the immune system, there is a variable degree of collateral damage to the liver that occurs during the process of viral elimination. It is unclear exactly how the immune system accomplishes the elimination of the infection, but it is probably through a combination of innate antiviral host defenses involving the expression of interferons and cytokines, and the induction of an adaptive, cytotoxic T-cell response (*31*).

It seems likely that very small amounts of neutralizing antibody, either from passive administration of immune globulin or from prior immunization, act by limiting both the primary and secondary viremia. This would reduce the number of infected hepatocytes within the liver at the time of recognition by the immune system, resulting in minimal, if any, inflammation and necrosis within the liver as the infection is eliminated. Such a series of events was termed "passive-active immunity" by Krugman and was recognized as leading to long-term protection against HAV following the use of immune globulin in epidemic settings (*7*). With respect to vaccine-induced immunity, events are less well understood. It is possible that small amounts of vaccine-induced antibody may actually prevent the spread of the virus to the liver through the bloodstream and thus may block infection at its earliest stages.

Recommendations for Vaccine Use

Recommendations for the use of hepatitis A vaccines within the United States have largely targeted persons at increased risk of the disease, based on risk factors associated with acquisition of hepatitis A in this country (*2*). These include travel to developing regions where the infection is more prevalent, close association with children under the age of 2 who are attending preschool day care centers, multiple sexual partners (particularly among male homosexuals), and illicit injection drug

use, which is increasingly recognized as a risk factor for parenteral transmission of HAV (32). Although HAV generally has not been considered to be parenterally transmitted, the high titer secondary viremia that marks the prodromal phase of the infection provides an excellent opportunity for transmission by contaminated needles or other drug paraphernalia. Despite the identification of these specific risk factors, however, a source of infection cannot be ascertained in a large proportion of persons presenting with hepatitis A.

As of this writing, there are three categories of individuals for whom this vaccine is recommended in the United States (2). The first consists of individuals who are at increased risk of acquiring hepatitis A, as described above. A second category includes those who are at increased risk of fulminant liver disease if they become infected with HAV, even though they may be at no more risk for infection than the general population. Leading that list are individuals with chronic liver disease due to hepatitis C virus infection. Finally, it has been recommended that children who live in areas with a high historic prevalence of hepatitis A infection be immunized uniformly after the age of 2 years. The vaccine is not approved for use in children under age 2, since there is not enough information available concerning the immune response to the vaccine in this age group to make such a recommendation. Furthermore, maternally-acquired antibodies to HAV can lead to reduced immunogenicity of inactivated hepatitis A vaccines.

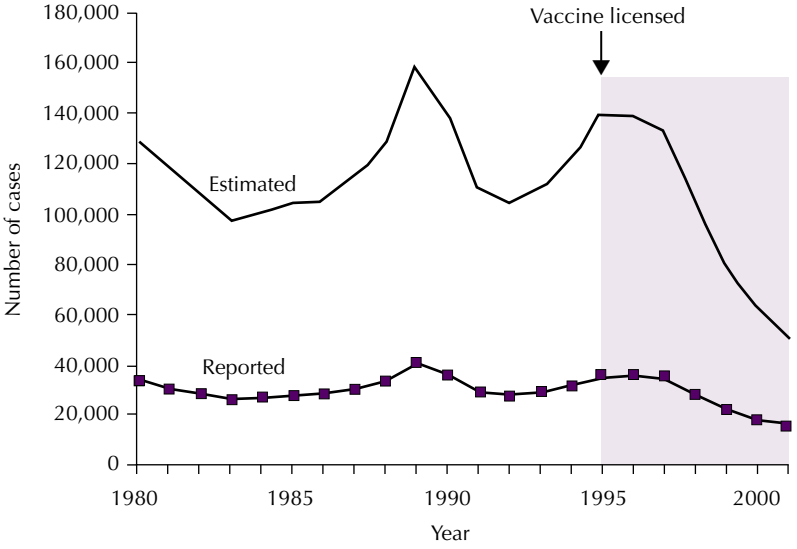
Areas of historically high prevalence are defined in the United States as those in which the incidence of infection exceeds 20 cases per 100,000 persons per year, or about twice the national average (2). The recommendation to immunize children in these regions is based on recognition that children play an important role in the transmission of this virus, given the fact that it is largely spread by the fecal-oral route. Several projects have demonstrated that universal immunization of children will essentially eliminate, if not eradicate, the virus from a community, causing very significant reductions in the number of hepatitis A cases. A case in point is Monroe, New York. Over the six years that followed the vaccine efficacy study, children continued to be immunized against hepatitis A. There have been virtually no cases of hepatitis A recognized in the community, despite a long prior history of annual hepatitis A outbreaks (33). Similar results have been obtained by the CDC in demonstration projects carried out with the GSK vaccine in California.

Vaccine Effectiveness

The single largest remaining difficulty with hepatitis A vaccines is that they remain relatively expensive. In general, their high cost continues to restrict their use and thus their overall benefit within the public health context. Unquestionably, the vaccine has prevented morbidity in individuals who have been immunized. However, it is difficult—outside of the context of particular situations in certain communities—to say with assurance that the vaccine has reduced the overall public health burden related to hepatitis A. It is interesting to look at the reported incidence of hepatitis A since the vaccine's introduction in 1995. As shown in Figure 3, incidence has been declining generally over the past several decades in the United States and no longer shows the large cyclic swings that occurred up to the middle of the last century. This almost certainly reflects disruption of prior, long-standing transmission patterns through improved public health sanitation. There has been an acceleration of the rates of decline in disease incidence since 1995, but it is difficult to know whether this is related to the vaccine and its availability, or to continued nationwide improvements in living conditions and sanitation infrastructure.

Historically, the United States is a country with at most a low or intermediate incidence and prevalence of HAV infection. However, it is important to note that hepatitis A vaccines have had essentially no impact on the global disease burden due to hepatitis A. Outside

FIGURE 3. Reported and estimated incidence of hepatitis A cases, United States, prior to and following the introduction of the hepatitis A vaccine into clinical practice in 1995.



Source: Figure modified from United States of America, Department of Health and Human Services, Centers for Disease Control and Prevention. Prevention of hepatitis A through active or passive immunization: Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 1999;48(RR-12):1–37.

of the few economically developed countries that have been able to afford them, the global effectiveness of these vaccines has been negligible.

In 2003, the cost of the vaccine at public tender within the United States was approximately US$ 11 for a pediatric dose and US$ 18 for an adult dose. It is very clear that a vaccine of this price is not going to be available in those regions of the world where it is most needed; i.e., developing areas with improving sanitation in which hepatitis A is becoming more apparent as infection is increasingly delayed from early childhood to adolescence and beyond, when disease accompanies infection more regularly. Public health policy-makers must consider the vaccine-preventable morbidity and mortality of hepatitis A within the context of other preventable diseases that are prevalent in their regions. They must reach a decision regarding where to commit very limited public health resources. It is unlikely that the answer for many would be the hepatitis A vaccination.

Summary

Hepatitis A vaccines have proven highly successful from a scientific point of view. They are exceptionally efficacious when given to individuals prior to exposure to HAV and may even provide some protection if given a week or more after exposure. They probably provide very long-term protection and are relatively safe. However, despite these very positive and desirable attributes, these vaccines have had relatively little impact on the health of the public outside of the relatively few populations residing in highly developed areas of the world.

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CONJUGATE MENINGOCOCCAL VACCINES FOR AFRICA

F. Marc LaForce[1]

INTRODUCTION

Over the last 100 years, sub-Saharan Africa has suffered repeated epidemics of meningococcal meningitis. The human toll has been enormous; the 1996–1997 outbreak resulted in more than 188,000 reported cases and over 20,000 deaths. The first part of this paper, therefore, will provide background information on epidemic meningitis in sub-Saharan Africa. The second part will describe the general characteristics of meningococcal polysaccharide (PS) vaccines, which have been traditionally employed to control epidemics in this corner of the world; and meningococcal conjugate vaccines, whose development and widespread use offer an attractive alternative, principally due to their greater potency, among other factors. The final section highlights the activities of the Meningitis Vaccine Project, a partnership created in 2001 between the World Health Organization (WHO) and the Program for Appropriate Technology for Health (PATH) with the goal of eliminating epidemic meningitis as a public health problem in sub-Saharan Africa.

EPIDEMIC MENINGITIS IN AFRICA

Epidemic meningitis in Africa has been a significant problem for at least 100 years (*1*). Figure 1 shows the cases of meningitis between 1950 and 1996 in Africa's infamous meningitis belt that was first well characterized by Lapeysonnie (*2*).[2] Over the last 10 years, the belt has extended southward, and epidemic meningococcal meningitis has been reported in Angola, Democratic Republic of Congo, Rwanda, and Uganda. Approximately every 10–12 years, sizeable epidemics of meningitis occur, and over the last 10–15 years baseline rates of meningitis have been increasing as well. During 1996–1997, Africa suffered a massive outbreak of Group A meningococcal meningitis that was responsible for close to 200,000 reported cases and 20,000 deaths. Because these numbers reflect only those cases which were officially reported to health authorities, the true magnitude of the problem is most likely underestimated. The year 2002, the latest for which figures are available, was considered a "non-epidemic" year, yet more than 44,000 cases and 3,000 deaths were reported from African countries.

Disease burden of this magnitude should be considered an unacceptable public health menace everywhere in the world. Nonetheless, these data do not adequately capture the chaos, confusion, and often misinformation that result whenever an outbreak of meningo-

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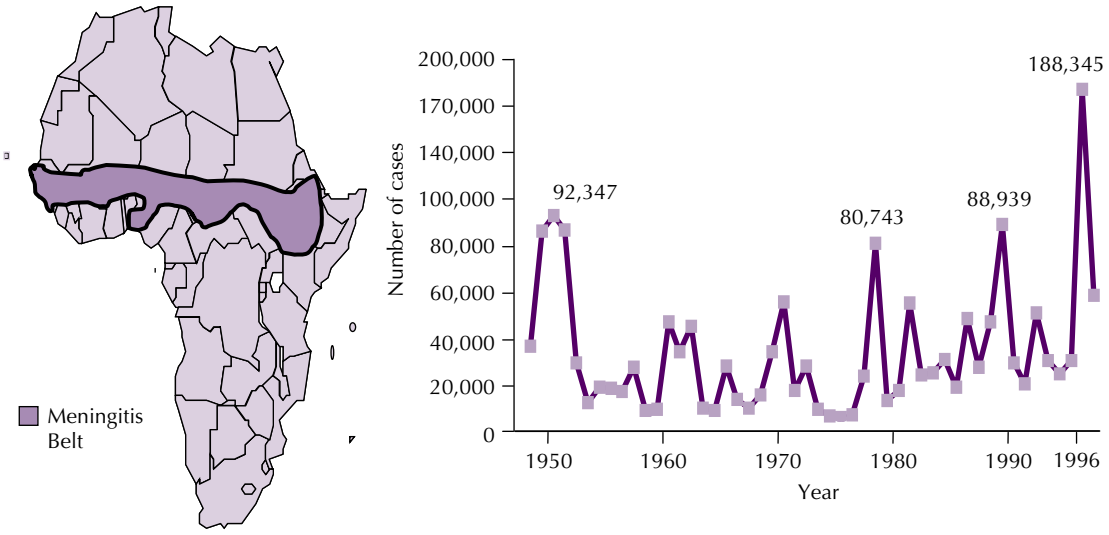
[2] Guinea Bissau, the Gambia, and portions of Guinea, Mali, Burkina Fasso, Benin, Nigeria, Niger, Chad, the Central African Republic, Sudan, Eritrea, Ethiopia, and Kenya.

FIGURE 1. Epidemic meningitis in Africa's meningitis belt, 1950–1966.



coccal meningitis occurs. Often, routine public health services such as childhood immunizations cease, and public health authorities and clinicians become overwhelmed attempting to respond to the clinical and preventive challenges these outbreaks pose. However, the epidemics themselves are very circumscribed temporally. They begin during the dry season, usually in December or January, and promptly cease with the first rains in May. Persons of age 6 months to 29 years make up >95% of cases. The highest rates of disease are in infants, but because of the wide age distribution, most cases occur in individuals >5 years of age (*3*).

Meningococcal Vaccines

Table 1 shows the general characteristics of polysaccharide (PS) and conjugate meningococcal vaccines. Control of epidemic meningococcal meningitis has largely depended upon use of the A/C polysaccharide vaccine. PS vaccines have been available for more than 30 years, and these vaccines are quite effective in individuals >age 2. However, PS vaccines are not reliably immunogenic in children 2 years of age and under, do not induce memory, and have had little effect on colonization in community-based studies. However, when polysaccharide antigens are linked to proteins such as diphtheria and tetanus toxoids, their immunogenic properties are dramatically increased. Conjugate vaccines stimulate T helper cells and provide good humoral antibody response and memory (*4*).

TABLE 1. Properties of polysaccharide and conjugate meningococcal vaccines.

Property	Polysaccharide vaccines	Conjugate vaccines
Immunogenicity:		
in 5-year-olds to adults	High	High
in young children	Poor	High
Response to booster	Poor	High
Quality of antibody in children		
Avidity	Low	High
Bacterial activity	Low	High
Induction of memory	+/–	Yes
Effect on colonization	+/–	Yes

Given the dramatic success of conjugate Hib vaccine in eliminating *Haemophilus influenzae* meningitis, and the equally impressive data

from the United Kingdom after the introduction of a Group C conjugate meningococcal vaccine, there has been considerable interest in the development of conjugate meningococcal vaccines to combat African meningococcal outbreaks (*5*, *6*). In fact, conjugate A/C meningococcal vaccines were tested in the Gambia and Niger in the early and mid-1990s, but the projects were discontinued because these vaccines were not considered commercially viable.

Development of the Meningitis Vaccine Project

After the devastating 1996–1997 epidemic there was renewed interest in the development of conjugate meningococcal vaccines at WHO. The Epidemic Vaccines for Africa Project was created by the Organization, and a series of in-depth discussions with vaccine manufacturers were held in 1999 and 2000 to explore their interest in developing these vaccines. In addition, with the help of a dedicated group of consultants, a costing model for the vaccines' development was done. A collaboration aimed at exploring the possibility of developing conjugate meningococcal vaccines gradually evolved between WHO and the Children's Vaccine Project at PATH. A series of expert panels were convened during 2000 and 2001, and these groups concluded that the development of the vaccines held potentially important public health advantages. They cited the previously mentioned successes that followed the introduction of conjugate Hib and meningococcal C vaccines. A proposal was prepared and sent to the Bill and Melinda Gates Foundation, and in June 2001 the Meningitis Vaccine Project (MVP) was created with a US$ 70 million grant. The project is a 10-year partnership between WHO and PATH with the goal of eliminating epidemic meningitis as a public health problem in sub-Saharan Africa through the development, testing, licensure, and widespread use of conjugate meningococcal vaccines.

Soon after the project was funded a series of discussions were held with African public health officials that focused on understanding the limitations of introducing new vaccines in sub-Saharan Africa. Three overarching considerations emerged from these meetings: first, vaccine cost was cited as the most important limiting factor to the introduction of new vaccines; second, the African meningitis belt countries are among the poorest in the world; and third, wide use of a conjugate meningococcal vaccine would not be possible unless the vaccine were priced at less than $0.50 per dose. These discussions were key in the sense that they forced the project partners to make affordability—i.e., a vaccine priced at less than $0.50 per dose—an important criterion for the product's development.

Extensive discussions took place throughout the fall of 2001 about the makeup of the conjugate vaccines being developed by MVP. The project was committed to the testing of a polyvalent Expanded Program on Immunization (EPI) vaccine (DTPw, Hib, HepB, Men A/C) being developed by Glaxo Smith Kline (GSK). This product was being developed by GSK for markets outside of Africa but there was interest on the part of various African health ministries in having the product tested in this region because of the major simplification in their logistics with the availability of a polyvalent EPI vaccine with a conjugate A/C meningococcal component. Discussions were held between GSK, MVP, and the Ministry of Health of Ghana, and plans have been formulated to begin clinical trials of this polyvalent product in December 2003. The vaccine would be proposed for use in selected meningitis belt countries as a replacement for a pentavalent product (DTPw, Hib, HepB) that was being introduced as an EPI vaccine in several African countries as part of the Global Alliance for Vaccines and Immunization initiative.

For epidemiological and logistical reasons, a decision was also made to develop a monovalent A meningococcal conjugate vaccine. Historically, the majority of meningococcal isolates from Africa have been Group A, and developing a conjugate monovalent A vaccine offered the advantages of simplicity, less risk, affordability, and the potential for a solid pub-

lic health impact. The monovalent A conjugate vaccine was developed to be used as a single dose for mass vaccination campaigns throughout the meningitis belt for persons ages 1–29 years. In addition, the vaccine would be tested as an EPI antigen in infants <1 year old, so that it would be available as an EPI vaccine for those countries unable or unwilling to purchase the heptavalent (DTPw, Hib, HepB, Men A/C) product previously described.

Throughout the fall of 2001 and the spring of 2002, MVP negotiated with major vaccine manufacturers, but no satisfactory agreement could be reached. Consequently, beginning in February and March of 2002, discussions were initiated with a consortium of manufacturers to develop a conjugate A vaccine. This partnership evolved into a group of three companies. SynCo Bio Partners, an Amsterdam-based Dutch contract manufacturer, agreed to produce vaccine grade A PS. BiosYnth, a discovery company in Siena, Italy, agreed to develop a conjugation method for the product. Lastly, the Serum Institute of India, based in Pune, agreed to manufacture the A conjugate vaccine at a target price of $0.40 per dose.

Clinical lots of the monovalent A conjugate vaccine will be available by the second quarter of 2004. Phase 1 studies in India could begin as early as the first quarter of 2004, and phase 2 studies could start in Africa in the second or third quarter of 2004. The project wishes to conduct a large demonstration study in 1–29-year-olds in one of four meningitis belt countries classified as hyper-endemic for meningococcal disease (Burkina Faso, Chad, Mali, and Niger). The vaccine could be licensed in India as early as 2007.

An Innovative Approach to Vaccines Development

The model that has been described for the introduction of conjugate meningococcal vaccines is quite different from the one commonly used to develop most licensed vaccines. In the traditional scenario, major vaccine companies choose the products to be developed and assume the financial risk associated with the development phase. For obvious reasons, vaccine manufacturers are most interested in products that are likely to bring financial return to that particular company. Vaccines for diseases that are almost exclusively seen in developing countries, such as Group A *Neisseria meningitidis,* are largely ignored unless the size of the travel market warrants the product's development. Group A *N. meningitidis* falls in this category of "not likely to be developed," because meningococcal polysaccharide vaccines currently service the travel market, and African countries are usually unable to purchase a conjugate meningococcal vaccine at a price that is attractive enough to interest major vaccine manufacturers.

BOX 1. Challenges and opportunities inherent in the vaccine development model being pursued by the Meningoccocal Vaccine Project.

Challenges:
- Higher risks.
- Technical and managerial complexity dealing with technology transfer and with clinical and regulatory issues.

Opportunities:
- Low cost of vacine (target price, US$ 0.40).
- Acceptable timelines (2006–2007).
- No opportunity costs.
- Tailor-made for Africa.
- Developing country vaccine capability is strengthened.
- Can serve as a model for other orphan vaccines.

Box 1 shows the challenges and opportunities in the model being developed by the Meningoccocal Vaccine Project. The model carries higher risk for several reasons. There is greater technical and managerial complexity, and technology transfer must occur smoothly if timelines are to be met. Supporters and critics have all predicted that technology transfer of the conjugation method from BiosYnth to Serum Institute of India will be difficult. In addition, there are the regulatory hurdles of li-

censing the vaccine in India for use in Africa. On the other hand, there are important opportunities. A low-cost conjugate vaccine that is effective against a major African public health problem is of great interest to the region's ministries of health and of finance. The ability to use grant funds to cover development costs and thus minimize risk to the partners allows for the development of a vaccine that otherwise might not have been developed. Lastly, the model, if it is successful, might well prove to be a useful paradigm for the introduction of other vaccines (*7*).

Over the project's first year and a half, a number of important lessons have emerged. The first is that price is important. Second, altruism is not enough to get a needed vaccine produced. Third, developing a vaccine must make economic sense to all of the project's partners. Fourth, project members' travels over the past 18 months have enabled them to come into contact with a group of excellent vaccine manufacturers in developing countries—the so-called "emerging suppliers." Fifth, working with these manufacturers might offer a useful model for providing additional needed vaccines in the future that today have only limited market potential as defined by major vaccine manufacturers.

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London School of Hygiene and Tropical Medicine, London, England
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THE EFFICACY AND EFFECTIVENESS OF PNEUMOCOCCAL CONJUGATE VACCINES

Keith P. Klugman[1]

INTRODUCTION

Acute respiratory infections remain the leading cause of death in children and are also the leading infectious cause of death in adults (*1*). As *Streptococcus pneumoniae* (the pneumococcus) is the leading bacterial cause of these infections, the development of a conjugate vaccine has been an important public health goal, though this has been frustrated by the large number of vaccine serotypes of pneumococci causing invasive disease. The development of *Haemophilus influenzae* type b conjugate vaccine laid the groundwork for the development of multivalent pneumococcal conjugate vaccines. Two important experiences with *Haemophilus* conjugate vaccines led to the conclusion that pneumococcal vaccines may have efficacy beyond direct protection of immunized children from invasive pneumococcal disease. The first is the demonstration that communities in which children received *Haemophilus* conjugate vaccine experienced reductions in invasive disease greater than those expected by the level of immunization coverage in the community. One such example was the Navajo community in the United States of America, where the burden of invasive disease was reduced by 57% and 73% respectively, in communities with vaccine coverage of only 22%–40% and 40%–60%, respectively (*2*). Furthermore, a study conducted in the Gambia showed that in addition to the significant impact on invasive *Haemophilus influenzae* type b disease, the vaccine reduced pneumonia—defined by consolidation on X-ray—by more than 20% (*3*).

SEROTYPES IN THE VACCINE

Although the distribution of the leading pneumococcal serotypes causing invasive disease in children is similar in most countries, there is some global diversity with serotypes 1 and 5, which are common in South America and in developing countries, but not in the U.S. (*4*, *5*). The first vaccine to reach phase 3 clinical trial and licensure, however, has been designed to cover the seven leading serotypes causing invasive disease in children in the U.S. (*6*). This pneumococcal conjugate vaccine contains oligosaccharide or polysaccharide capsular material of serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F, conjugated to the diphtheria cross-reacting molecule CRM_{197}. The vaccine was developed by Wyeth-Lederle, in Pearl River, New York. A vaccine consisting of the same serotypes but conjugated to meningococcal outer membrane proteins, developed by Merck, in Philadelphia, Pennsylvania, was studied for its

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efficacy against otitis media in a population of Finnish children, but the development of that vaccine has not proceeded to an application for licensure (*7*). A nine-valent conjugate vaccine using the CRM_{197} conjugate has recently been tested in a large phase 3 clinical trial in Africa (*8*). The same vaccine is under investigation in the Gambia. Trials are ongoing of 11-valent vaccines with the addition of serotypes 3 and 7 for the reduction of invasive disease in the Philippines (conjugated to tetanus and diphtheria toxoids, developed by Aventis Pasteur in Lyon, France) and for the reduction of otitis media in the Czech and Slovak Republics (conjugated to *Haemophilus* D protein, developed by GSK Biologicals in Brussels, Belgium).

Efficacy Against Invasive Disease

To date, three large clinical trials have documented the efficacy of pneumococcal conjugate vaccines against invasive pneumococcal disease. In the first, a study conducted in the Kaiser Permanente Health Maintenance Organization in northern California (U.S.A.), the vaccine efficacy was 97% (*9*). The same vaccine in the Navajo nation in the U.S. had an efficacy of 86% in the intent-to-treat analysis (*10*), and the nine-valent vaccine in South Africa had an intent-to-treat efficacy of 83% against vaccine serotypes (*8*). These studies were underpowered to detect an increase in the number of nonvaccine serotypes causing invasive disease. The South African study also reveals efficacy against the cross-reacting serotype 6A, but not against serotype 19A (*8*). While most of the invasive disease in the U.S. studies was pneumococcal bacteremia without a source of infection, most of the pneumococcal disease prevented in the South African trial was due to pneumonia and meningitis.

Invasive Disease in HIV-infected Children

The global HIV pandemic has had a major impact on the burden of pneumococcal disease in children (*11*). It is therefore essential to the success of a vaccination strategy in countries where HIV is endemic that the pneumococcal conjugate vaccine reduce invasive pneumococcal disease among HIV-infected children. This issue was addressed in the South African study, and the nine-valent conjugate vaccine was shown to reduce invasive pneumococcal disease in HIV-infected children by 65% in the intent-to-treat analysis (*8*).

Effectiveness Studies in Invasive Disease

The U.S. is the only country to date to introduce pneumococcal conjugate vaccine into its routine immunization program. Two studies in that country on the effectiveness of the vaccine after its introduction have been reported. The first demonstrated significant reductions in vaccine serotypes and vaccine-related serotypes in children in northern California (*12*). The larger effectiveness study conducted in seven states by the U.S. Centers for Disease Control (CDC) revealed significant reductions in 2001 (after vaccine introduction) from 1998–1999 (prior to introduction), for each of the seven vaccine types, from 63% for type 9V to 83% for types 4, 14, and 19F (*13*). Vaccine effectiveness against all vaccine serotypes was 78%, and there was a 50% reduction against vaccine-related serotypes (significantly so for serotypes 6A and 9A). Protection against serotype 19A was not significant, although there was a reduction of 40%, which tended to significance ($p = 0.09$). It is important to note that while vaccine serotypes were reduced from an average of 156 cases per 100,000 in 1998 and 1999 to 34 cases per 100,000 in 2001, nonvaccine serotypes increased from 12 to 16 per 100,000 over the same period. This increase in nonvaccine serotypes was not significant, but there was a trend in that direction ($p = 0.014$). These data suggest that the vaccine has had a major effect on invasive disease due to vaccine serotypes and vaccine-related serotypes in children under 2 years of age, and that serotype replacement is likely to occur, but that the amount of replacement may be small compared to the

scale of the reduction of invasive disease due to vaccine serotypes. An important observation from the CDC effectiveness study was that there were significant reductions in invasive disease caused by vaccine serotypes among adults. It has been known for some time that children in the household, particularly those in day care, represent a risk for invasive pneumococcal disease in adults (*14*), and it has also been demonstrated that the proportion of invasive pneumococcal disease in adults in the U.S. due to pediatric serotypes has increased in recent years (*15*). These data suggest that there has been a significant herd immunity effect since the introduction of the pneumococcal conjugate vaccine and that the cost-effectiveness of this vaccine may be greatly enhanced by it. The CDC surveillance has also documented the effectiveness of the seven-valent vaccine in reducing pneumococcal meningitis by 59% (*13*).

Vaccine Efficacy Against Otitis Media

Vaccine efficacy against otitis media has been investigated in two large clinical trials. In the first, in Finland (*16*), vaccine efficacy against specific serotypes could be documented by the performance of tympanocentesis among vaccinated children with otitis media. The seven-valent CRM_{197} conjugated vaccine reduced otitis media due to vaccine serotypes by 57% and all confirmed pneumococcal otitis media by 34%. There was a non-significant overall reduction in otitis media of only 6%, as the proportion of nonvaccine-type pneumococci increased by 33%. Similar results have been presented for the seven-valent vaccine conjugated to meningococcal outer membrane proteins (*7*). An analysis of otitis media episodes in vaccinated children in the Kaiser Permanente study (*9*) revealed a 7.8% reduction in otitis media visits in the intent-to-treat analysis, with increasing protection of up to 12.3% in children who had frequent otitis (defined as five episodes in six months or six episodes in a year). The vaccine also prevented 20% of ventilatory tube placement. Pneumococcal conjugate vaccines therefore have been shown to significantly reduce otitis media when the infection is caused by vaccine serotypes, but the overall impact of the vaccine on otitis media has been reduced by the phenomenon of replacement by nonvaccine serotypes.

Vaccine Impact on Carriage

A number of studies have shown that children who have received pneumococcal conjugate vaccines have had about a 50% reduction in carriage of vaccine serotypes, but that serotype replacement occurs. The reduction in carriage of vaccine serotypes appears to be a vaccine-mediated inhibition of acquisition of carriage, rather than direct eradication of existing carried strains. The impact of the vaccine on carriage was recently reviewed (*17*).

Vaccine Impact on Pneumonia

The seven- and nine-valent conjugate vaccines have been evaluated for their impact on pneumonia. In the Kaiser Permanente study (*9*), there was a reduction in pneumonia (with a positive chest radiograph) of 20.5% in fully immunized children and 17.7% in the intent-to-treat analysis. The nine-valent conjugate vaccine has been shown in the South African trial to have a similar level of efficacy in the prevention of first episodes of radiographically defined pneumonia. The reduction in first episodes among fully immunized children was 25% (*8*). In both studies, the Hib conjugate vaccine was given to both vaccinees and controls, so there is a reasonable inference that the combination of these vaccines may reduce radiologically confirmed pneumonia by approximately half. The efficacy of these vaccines in the prevention of pneumonia is possibly the most important public health aspect of their efficacy, and it will be important to monitor the effectiveness of these vaccines in preventing pneumonia when they are introduced in developing countries.

Prevention of Antibiotic Resistance

The nine-valent conjugate vaccine has been shown to reduce invasive pneumococcal disease due to penicillin-resistant strains by 67% (*8*). These data, as well as data documenting the impact of the vaccine on the carriage of antibiotic-resistant pneumococci (*18*, *19*), support the observation that the introduction of the vaccine has been associated with a decrease in antibiotic-resistant invasive disease in the U.S. (*13*).

Safety

While there have been no significant associations of severe adverse events with the introduction of the conjugate vaccine in the U.S., an association of vaccination with an increased incidence of asthma was found in the South African study (*8*). This association has not been found in other studies, but the introduction of conjugate vaccines should be accompanied by careful surveillance for any unanticipated adverse events.

Conclusions

The introduction of pneumococcal conjugate vaccine has been associated with a dramatic reduction in invasive disease due to vaccine serotypes and significant reductions in pneumonia and meningitis. The impact on otitis media has been reduced by the phenomenon of serotype replacement. Herd immunity induced by the vaccine has led to significant reductions in invasive disease in adults in the U.S. The vaccine has also reduced the burden of antibiotic-resistant pneumococcal disease and has reduced disease in HIV-infected children. These data suggest that this vaccine may be a very valuable public health intervention in developing countries. However, the largest factor limiting vaccine introduction is cost. A consortium of scientists, governments, nongovernmental organizations, and industry is being developed under the auspices of the World Health Organization to design strategies that may enable the rapid deployment of these effective vaccines.

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Part III
The Future

ROTAVIRUS VACCINES

Roger Glass,[1] *Umesh Parashar,*[1] *Joseph Bresee,*[1] *Jon Gentsch,*[1] *Reina Turcios,*[1] *and Baoming Jiang*[1]

INTRODUCTION

Rotavirus vaccines are very advanced in their development, and two vaccines currently in clinical trials by Merck and GlaxoSmithKline (GSK) could be licensed and available for use within two to three years. It is important to recognize the Pan American Health Organization's (PAHO) leadership and its contribution to the immunization of children in the Americas and to the control of vaccine-preventable childhood diseases. In considering the future introduction of rotavirus vaccines, the role and leadership of PAHO could be key in global efforts to prevent this most common cause of severe diarrhea in children.

Why is a rotavirus vaccine necessary? Rotavirus is the most common cause of severe gastroenteritis in children worldwide. This virus was discovered by Ruth Bishop in 1973 and has a natural history that is quite simple: 1) all children are infected in their first years of life, 2) primary infections after the first few months of life cause diarrhea that can be severe and sometimes fatal, and 3) natural immunity occurs following the initial infection, and children rarely get severe rotavirus diarrhea more than once. Rotavirus has been called a "democratic virus" because it infects all children—rich and poor—and knows no geographic boundaries. Consequently, improvements in water or sanitation that might reduce the incidence of other enteric infections will not change the incidence of rotavirus diarrhea. Vaccines provide the most realistic approach toward prevention. While oral rehydration therapy (ORT) is effective in treating all acute watery diarrheas, including rotavirus diarrhea, access to ORT is limited in many areas of the world. Furthermore, despite programs that have intensely promoted ORT worldwide for 20 years, more than 2 million children still die each year from diarrhea.

THE DISEASE BURDEN

The global burden of rotavirus disease is enormous. Rotavirus infections cause an estimated 450,000 to 550,000 deaths each year; these deaths are concentrated in the poorest countries of Asia, Africa, and the Americas (*1*) (Figure 1). All children are infected with rotavirus in their first few years of life, and most episodes of the disease are mild: only 10%–20% of children will have diarrhea severe enough to require medical attention, between 1 in 30 and 1 in 80 children will develop severe dehydration that may require hospitalization and intravenous rehydration, and about 1 in 250–300 children in developing countries will die from their first infection. Worldwide, rotavirus is associated with about 5% of deaths in children under 5 years of age. Rotavirus can be detected in fecal specimens from 20%–60% of children hospitalized for diarrhea in both developed and developing countries. A vaccine would

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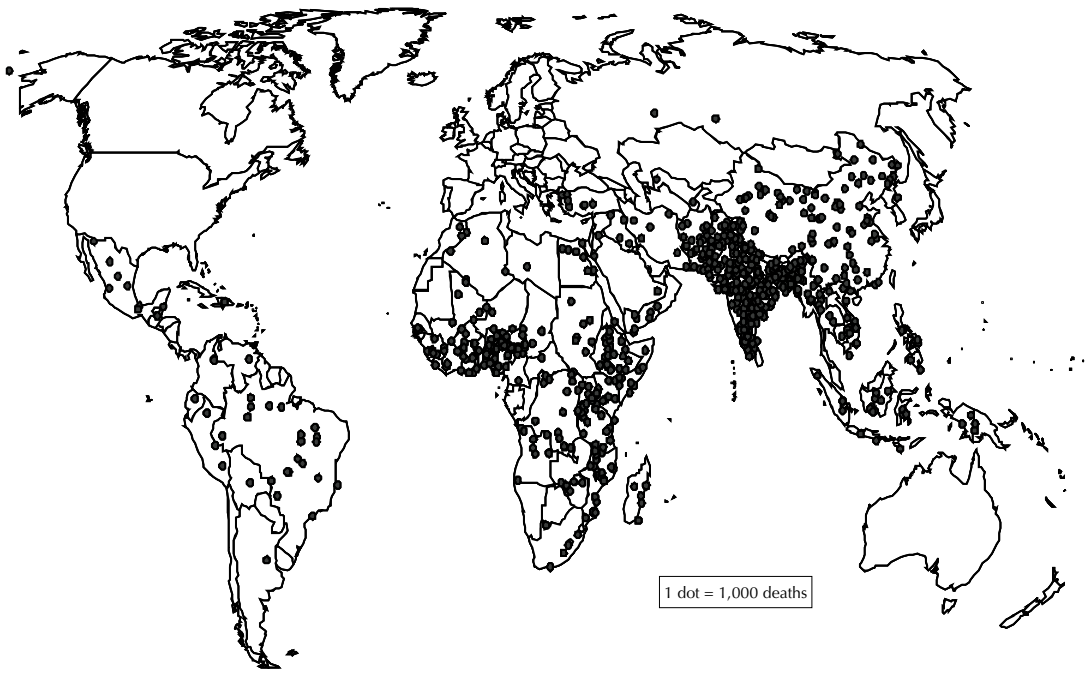
FIGURE 1. Estimated global distribution of the 450,000 annual deaths caused by rotavirus.



Source: Parashar UD, Hummelman EG, Bresee JS, Miller MA, Glass RI. Global illness and deaths caused by rotavirus disease in children. *Emerg Infect Dis* 2003;9:565–572.

save many lives of children in the developing world, while preventing hospitalizations and milder illness among all vaccinated children.

Over the last 20 years, the development of a rotavirus vaccine has been a priority for all international health agencies—the World Health Organization, the United States Institute of Medicine, the former Children's Vaccine Initiative, the Children's Vaccine Program at the Program for Appropriate Technology for Health, and the Global Alliance for Vaccines and Immunization (GAVI). The global health community is at last giving some recognition to this problem and putting some emphasis on advancing the cause of the prevention of rotavirus through the development and use of vaccines.

A Brief History of Rotavirus Vaccines

The United States of America was the first country to license and recommend rotavirus vaccination for all children as part of the routine program of childhood immunizations. This 1998 decision was based in part on national estimates of the burden of disease and on the licensure of the first rotavirus vaccine. Studies of children hospitalized for diarrhea in the U.S. demonstrated a peak of winter admissions among those under 5 years of age that was due to rotavirus. Diarrhea is coded on the discharge record of 9% to 12% of all children under 5 years of age admitted to hospitals each year, and rotavirus is responsible for 30% to

50% of these admissions—or 3% to 6% of all hospitalizations—for approximately 70,000 rotavirus admissions per year. The impact of the vaccine should be to flatten down winter peaks of diarrhea hospitalizations, an impact that could be seen within two years of the introduction of a new vaccine.

The first vaccine against rotavirus was developed by Kapikian and his colleagues at the U.S. National Institutes of Health (NIH) (*2*) and manufactured by Wyeth Pharmaceuticals. The vaccine, RotaShield, was based on a strain of rotavirus derived from a rhesus monkey, which was combined with three additional strains developed by reassortment. The reassortant strains combined genes from the rhesus rotavirus parent virus with a single neutralization gene from three common serotypes so that each reassortant maintained the attenuation and growth properties of the original virus but had the neutralization characteristics of a common human serotype.

Rotaviruses are composed of 11 segments of double-stranded RNA, which each encode a protein. The outer capsid proteins, VP4 and VP7, represent the antigen against which neutralization antibodies are directed. These outer capsid proteins also define the serotypes. There are four major serotypes in the world: G1, G2, G3, and G4, and the first vaccine was developed to target these four serotypes. Further epidemiologic studies have identified other novel strains, including some formed by genetic reassortment between human and animal strains. These are clearly less important but represent ways that the virus can evolve in the future.

There are a number of important differences between the epidemiology of rotavirus in developed and developing countries, and these differences can also affect the way a live oral vaccine might work. For instance, rotavirus is a winter disease in temperate climates but a year-round disease in the tropics; therefore, children in developing countries are exposed to rotavirus throughout the year, but children in temperate climates are first exposed during the winter. Thus, many children in the tropics are exposed to rotavirus at a younger age than are children in temperate climates. This observation underscores the importance of immunizing children early in the first year of life. The rotavirus strains circulating in the U.S. are primarily the four common serotypes, whereas in some developing countries, other unusual strains are more abundant, such as G5 strains in Brazil, G9 strains in India, and G8 strains in Africa. We will need to learn more about the importance of these strain differences on the efficacy of vaccines as more vaccines are tested in these settings. Case fatality rates for rotavirus disease are clearly much higher in developing countries for reasons that are likely complex. In addition, mixed infections are a problem in developing countries and may suggest that the vehicles of transmission are different and the inoculum size is larger. Live oral vaccines for rotavirus, unlike live oral vaccines for polio and cholera, may face a greater challenge and perform less well in these settings. It is clear that any new live oral vaccine that comes forward will have to be tested in developing countries.

RotaShield, the first vaccine against rotavirus, was licensed in the U.S. in August 1998 to be administered in three doses to children at 2, 4, and 6 months of age (*3*). Preliminary tests of the vaccine demonstrated an efficacy of 70% against mild rotavirus diarrhea and nearly 100% against severe disease. The vaccine caused mild fever on days 3 to 5 following vaccination but no other severe adverse effects. In a study by Perez-Schael (*4*), the vaccine was also protective in children in a poor neighborhood of Venezuela, suggesting that this vaccine could also have prevented childhood deaths from rotavirus in developing countries. Over the following nine months, some 800,000 children were immunized with over a million doses of the vaccine. The national immunization program in the U.S. came to an abrupt halt in July 1999, when the National Immunization Program (NIP) of the Centers for Disease Control and Prevention (CDC) reported 15 cases of

intussusception linked to the vaccine (*5*). An investigation of this association demonstrated a significant risk of intussusception in the two weeks following administration of the first dose of the vaccine (*6*). About six other studies used different epidemiologic methods to assess the risk of intussusception following vaccination and established an attack rate for the vaccine ranging from 1 excess case in 2,500 vaccinees (as originally reported by NIP) (*6*) to 1 excess case in 28,000 vaccinees (as reported in an ecological study by Simonsen et al. (*7*) at NIH). A consultative group convened by CDC's National Vaccine Program Office put the risk at 1 intussusception per 11,000 vaccinees, recognizing that full and accurate data might never be available. For a disease that causes little mortality in the U.S., this risk seemed unacceptable to American pediatricians. Nonetheless, for the developing world, the risk was clearly minimal compared with the risk of death from rotavirus itself. The vaccine was subsequently withdrawn by the manufacturers and, while it remains licensed by the U.S. Food and Drug Administration, it is no longer available for use.

One feature identified in the epidemiologic studies was that intussusception spared children in their first 3 months of life and that the risk of natural disease rises sharply (nearly eightfold) between 3 and 7 months of age (*8*). We do not know why very young infants have such a low incidence of intussusception, but whatever protects these children from natural disease might also protect them against intussusception associated with the vaccine. Clearly, future live oral vaccines should be tested in younger children to take advantage of this natural protection.

We were soon left without a rotavirus immunization program, but with many lessons learned from this wrenching experience. The first lesson was that live oral vaccines are effective against rotavirus and an immunization program can lead to rapid introduction of a vaccine if authorities provide a global recommendation for its use. This is very important since it provides clear direction for the next generation of vaccines. Clearly, the scientific principles for establishing a vaccine have been well established. Second, we learned not to put all our eggs in one basket. With RotaShield, we had only one vaccine moving forward with little competition. It will take five to eight years until the next group of vaccines becomes ready for licensure, a delay which will witness some 2.5 to 4 million rotavirus-related deaths that might have been prevented. Developing countries were upset because the rhesus vaccine might have prevented deaths there, and its rapid withdrawal meant that they could not completely count on the provision of a large supply of affordable vaccine by multinational suppliers. Last, the company producing the vaccine, Wyeth Pharmaceuticals, did not have a global market plan or an adequate supply of vaccine to meet international demand. If the company had thought globally and tested its vaccine in many countries, some countries might have accepted this risk, given the obvious benefit of the vaccine in settings where the disease is more often fatal. If that had occurred, we might still have a vaccine today.

However, the withdrawal of RotaShield also had a number of positive effects and provided some new opportunities. First, other vaccine manufacturers—GSK and Merck—were slowly developing products before the lead vaccine was withdrawn, but these have since been fast-tracked. Hopefully, we will now have more vaccines sooner and in larger quantities than might have been available before. These new vaccines can now be tested in the U.S., something that would have been ethically difficult to do if a recommendation for routine rotavirus immunization remained in place. Because the risk of intussusception was small with the rhesus vaccine, future vaccines will require immunization of more than 60,000 children to ensure that the risk of intussusception is less than that observed for RotaShield. This number requires that new vaccines be tested in many countries, and some such studies are currently being conducted in developing countries. Also, some emerging manufacturers in developing countries have stepped

forward to consider preparing rotavirus vaccines. China has already licensed a vaccine, and companies in India and Indonesia are considering vaccine development. The need for a rotavirus vaccine has clearly matured in the international community and is now a priority. Despite the many problems caused by the removal of the rhesus vaccine, there have been many positive developments that give reason to be optimistic for the field.

Future Directions

Where would we like to be with rotavirus vaccines in 10 years? How can we make vaccines available to all children in the world within a decade? What can be done to speed up vaccine development and introduction? How can we optimize efficacy of the vaccine for children in developing countries? How can we assure an adequate supply of rotavirus vaccine at a reasonable cost? The ultimate goal of an international rotavirus vaccine program is to immunize 60% to 80% of the world's children in about 10 years, and a 50% to 60% decline in the current number of rotavirus-associated deaths and hospitalizations in children worldwide. These goals have become the target of GAVI in a program currently being developed. In 10 years, we could anticipate having several live oral vaccines licensed, manufactured, and introduced into routine use in many countries; however, this goal will require surveillance for rotavirus disease now so that we can measure the disease burden in countries that might consider the introduction of vaccines in the near future. The same surveillance system could also be used to monitor the impact of vaccine introduction as future plans progress. To achieve these goals, there is clearly a need for enhanced advocacy for rotavirus vaccine use and for financing. If these vaccines are to be used, they need to be affordable at a sustainable price for purchase by developing countries and by the donor community, such as the PAHO Revolving Fund for Vaccine Procurement, United Nations Children's Fund (UNICEF), or the Vaccine Fund.

A number of candidate vaccines are currently in development (Table 1); two candidate vaccines have already been abandoned. Discarded vaccines include one based on the RIT4237 strain, an attenuated rotavirus strain developed by SmithKlineRixensart in the

TABLE 1. Live attenuated oral rotavirus vaccines currently in development or human trials.

Product	Company	Concept	Status
LLR	Lanzhou Institute of Biological Products (China)	Monovalent lamb strain (P[12]G10)	Licensed (China) 2000
Rotateq	Merck (USA)	WC-3-based multivalent human-bovine reassortant	Phase 3
Rotarix (89-12)	GlaxoSmithKline (Belgium)	Monovalent human strain (P[8]G1)	Phase 3
UK-reassortant vaccine	Wyeth Ayerst/NIH (USA)	UK-based multivalent human-bovine reassortant	Phase 2
RV3	University of Melbourne (Australia)	Neonatal human strain (P[6]G3)	Phase 2
116E	Bharat Biotech (India)	Neonatal natural bovine-human reassortant strain (P[11]G9)	Phase 1
I321	Bharat Biotech (India)	Neonatal natural bovine-human reassortant strain (P[11]G10)	Phase 1

1980s. This vaccine was highly effective in initial trials but was withdrawn from development after studies demonstrated low efficacy among children in developing countries (*9*). The RotaShield vaccine was licensed in 1998 and widely used for nine months, but it was withdrawn in the U.S. following identification of intussusception as a rare adverse event. The Chinese vaccine, LLR, which is based on a lamb strain of rotavirus, has been licensed in China since 2000, but concerns about its quality and efficacy remain. New vaccines that are in clinical trials today include the pentavalent vaccine based upon a bovine rotavirus strain being prepared by Merck and an attenuated monovalent human serotype 1 strain under development by GSK. Three candidate neonatal strains are also in early-stage development: an Australian strain, RV3, discovered by Bishop and her colleagues (*10*), and two Indian strains—116E and I321—being developed by Bharat Biotech in India. Finally, a reassortant vaccine based upon the UK strain of bovine rotavirus (similar to the Merck vaccine) has undergone preliminary testing by NIH.

Preliminary data on the Merck and GSK vaccines have been presented recently. Perez-Schael reported that the GSK trials in Latin American children demonstrated a vaccine efficacy of 65% against any rotavirus and 79% against severe disease requiring hospitalization (*11*). This is similar to the results of the rhesus vaccine, suggesting that such a candidate can make a major impact on the prevention of hospitalizations for rotavirus in the Americas. This vaccine also protects against G9 strains, confirming heterologous protection against non-G1 serotypes.

Preliminary results have also suggested a similar high level of efficacy for the Merck vaccine. Heaton reported an efficacy of 75% against mild disease and greater efficacy against severe disease on the basis of results for several hundred children with an early formulation of the vaccine's components (*12*). These results are preliminary, but they suggest that the strategy of using live oral vaccines can be effective, and they underscore concern that the ultimate success of these vaccines will depend heavily on a high level of safety against intussusception and, ultimately, on cost. If either of these vaccine trials were to identify an excess number of vaccinees suffering from intussusception in the two weeks following immunization, vaccine development would again come to an immediate halt.

The parallel development and testing of several rotavirus vaccines has been recommended as a means to ensure the success of future rotavirus development efforts (*13*). Our past experience with individual candidate vaccines has led to long periods of delay between the withdrawal of one candidate and its replacement by the next. Developing multiple vaccines will help ensure a larger supply of product and more competitive prices, which will be needed to expedite use of these vaccines in developing countries (*9*). It is worth noting that these processes to manufacture a rotavirus vaccine are well established and traditional, as they are based on simple tissue culture techniques. Many companies that make other live oral vaccines could potentially make rotavirus vaccine as well. The key issues for success involve maximizing titer, conducting large-scale efficacy trials, ensuring vaccine quality through the many regulatory requirements now in place, and ensuring a high level of safety against severe adverse events.

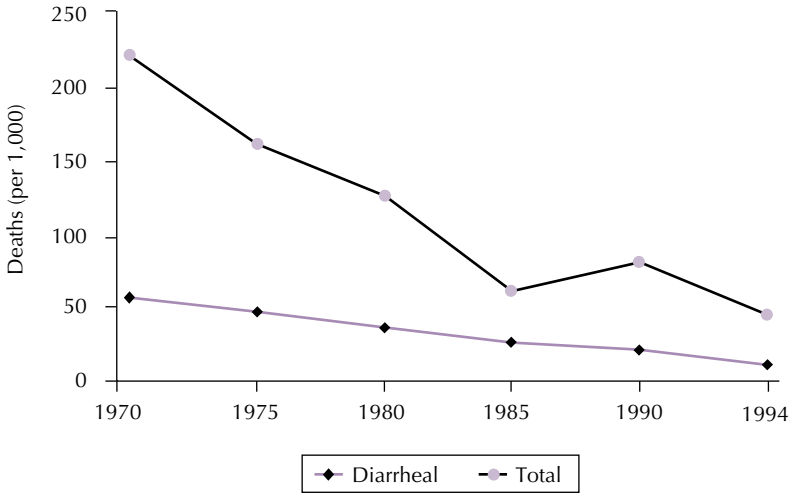
Numerous obstacles remain to making rotavirus vaccine. Many people still do not know what rotavirus is, either in the U.S. or in countries where this infection is a killer. We have asked health ministers about rotavirus disease in their countries, and many have commented that they do not have the disease at all or are working to improve water and sanitation in order to prevent rotavirus diarrhea. These responses highlight the lack of understanding of rotavirus disease, because children in every country contract rotavirus and they do so despite the quality of the water or the level of sanitation. Clearly, a major educational effort will be needed to move the cause of rotavirus vaccines forward. A substantial hurdle to vaccine development is a lack of a good immune proxy

for protection; thus time-consuming, large clinical trials are required to demonstrate efficacy. Even larger trials are needed to demonstrate low rates of intussusception. Nonetheless, the true target of these vaccines will need to be developing countries, where mortality from rotavirus remains a major problem. Multinational vaccine manufacturers need to recognize the global importance of rotavirus vaccines so they can plan to participate in this important future vaccine initiative. Regional surveillance activities are being set up so countries can assess the disease burden of rotavirus in their own settings and recognize the value that a vaccine could provide. A regional network of rotavirus surveillance is now in place and operating in nine countries of Asia and has demonstrated the impact that sentinel hospital surveillance can provide in creating awareness of the disease (*14*). Preliminary data from the first year of surveillance indicates that within these nine countries, between 29% and 60% of all children under 5 years of age who are hospitalized for diarrhea have rotavirus as the responsible pathogen. In two of these countries—Thailand and Malaysia—the predominant strain is G9, which has not been included in the polyvalent reassortant vaccines. A similar surveillance network is being organized in the Americas.

Summary

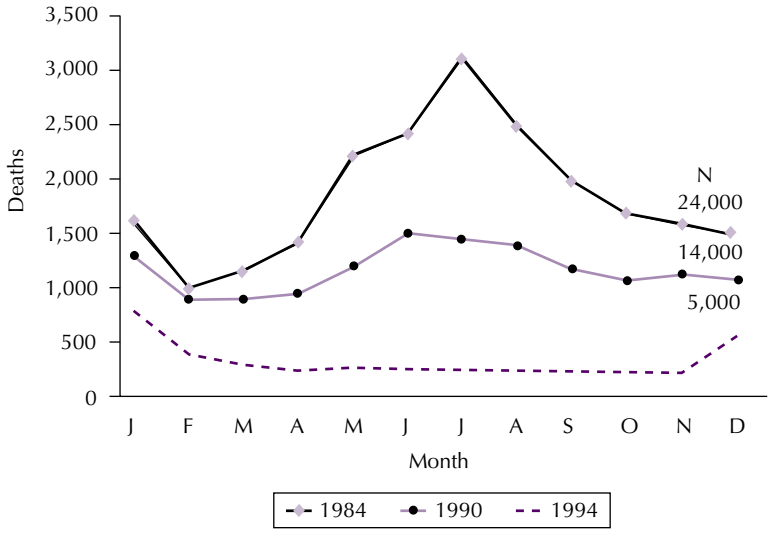
What would be the impact of a rotavirus vaccine in Latin America? One case study of the history of diarrheal disease mortality in Mexico can put this in perspective (*15*). In the past, diarrhea has been a major cause of childhood mortality in Mexico, but over the past 30 years, many interventions have led to major improvements that have resulted in a decline in diarrhea-related deaths from more than 50,000 in 1970 to fewer than 5,000 in 1995 (Figure 2). This decline has been accompanied by an interesting shift in the seasonality of the deaths. Before and during the early days of the interventions, there was a major peak in diarrhea-associated deaths in the summer months, a

FIGURE 2. Diarrheal mortality in children under 5 years of age, Mexico, 1970–1994.



Source: Gutiérrez G, Tapia-Conyer R, Guiscafré H, Reyes H, Martínez H, Kumate J. Impact of oral rehydration and selected public health interventions on reduction of mortality from childhood diarrhoeal diseases in Mexico. *Bull World Health Organ* 1996;74(2):189–197.

FIGURE 3. Seasonality of diarrheal deaths in children, Mexico, 1984–1994.



Source: Villa S, Guiscafré H, Martínez H, Muñoz O, Gutiérrez G. Seasonal diarrhoeal mortality among Mexican children. *Bull World Health Organ* 1999;77:375–380.

time when bacterial diarrheas were predominant; however, the current residual peak of diarrhea-related deaths occurs in the winter and is largely due to rotavirus (Figure 3). The next strategy for Mexico to further decrease the impact of diarrheal diseases would be to prevent morbidity and mortality due to rotavirus.

Rotavirus represents the "lowest hanging fruit" for new vaccine development. The disease burden is large, the principles to develop a vaccine have been worked out and well established, and we have extensive experience in conducting clinical trials with new candidate vaccines. This is a challenge that can be met in the next decade. Furthermore, there should be great incentive for health ministries to introduce a rotavirus vaccine in the future as it could represent a quick fix to a serious problem: within one year, participating countries could see a measurable decline in hospitalizations and deaths from diarrhea.

We should recognize and applaud the important role of the Pan American Health Organization as a leader in global efforts to control vaccine-preventable diseases. As new rotavirus vaccines become available, we hope that PAHO's continued efforts in the area of childhood immunizations will help Latin America be the first region of the world where this disease can be prevented by the regional introduction of a new generation of rotavirus vaccines.

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TYPHOID FEVER AND CHOLERA VACCINES

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TYPHOID

Typhoid fever, the generalized infection of the reticuloendothelial system, gut-associated lymphoid tissue, and gall bladder, is caused by the highly human host-restricted pathogen *Salmonella enterica* serovar Typhi. The disease exhibited a case fatality rate of circa 15% before the discovery by Woodward and colleagues (*1*) that treatment with chloramphenicol could drop the case fatality to < 1%. Following this discovery, the treatment became a practical cost-effective control measure to diminish typhoid mortality in developing countries. However, the tenuous nature of this control measure became apparent first with the appearance of chloramphenicol-resistant strains (*2*) and, more recently, with the emergence of *S.* Typhi strains carrying R factor plasmids encoding resistance to chloramphenicol, trimethoprim/sulfamethoxazole, and amoxicillin (*3*). These events have rekindled interest in the possible use of typhoid vaccines to control typhoid in affected developing countries.

The Pan American Health Organization has played a pivotal role in the development and evaluation of the efficacy and practicality of typhoid vaccines. The heat-inactivated phenol-preserved whole cell typhoid vaccine, independently developed by Almoth Wright in England and by Richard Pfeiffer in Germany at the end of the nineteenth century, was shown in field trials to provide a moderate level of protection. However, it was highly reactogenic (fever, malaise), making its widespread use unpopular among health authorities. In the 1960s, the World Health Organization sponsored randomized, controlled field trials of the heat-inactivated phenol-preserved and the acetone-inactivated whole cell parenteral typhoid vaccines in several countries, including Guyana (*4–6*). The trial in South America was unique in that surveillance was maintained for seven years (*6*). Two spaced doses of the heat-inactivated phenolized vaccine conferred 65% efficacy, and the acetone-inactivated vaccine conferred 89% efficacy in preventing bacteriologically confirmed typhoid fever over seven years of follow-up. The extensive outbreaks of chloramphenicol-resistant typhoid in Mexico (1972), Vietnam (1973), and Peru (1980) stimulated the development of a new generation of typhoid vaccines, including attenuated strain Ty21a used as a live oral vaccine and purified Vi polysaccharide used as a parenteral vaccine.

Ty21a

This vaccine strain was derived in the early 1970s by Germanier and Furer (*7*) from wild type strain Ty2 (known from volunteer challenge studies to be pathogenic in humans) by nonspecific chemical mutagenesis and selec-

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tion of a stable mutant that had inactivation of *galE* and did not produce Vi capsular polysaccharide. Controlled phase 1 and 2 trials in adult volunteers in North America demonstrated the safety of Ty21a and provided preliminary evidence that it could prevent typhoid fever (*8*). Thereafter, a field trial was carried out in Alexandria, Egypt, in 32,388 schoolchildren between the ages of 6 and 7 years with an initial formulation of Ty21a consisting of bicarbonate pretreatment followed several minutes later by ingestion of a liquid cocktail containing reconstituted lyophilized vaccine. Three doses (10^9 colony forming units each) were administered at an every-other-day interval. Over three years of follow-up an efficacy of 96% was demonstrated (*9*).

Unfortunately, in view of the highly encouraging efficacy results, the formulation used in Egypt was not amenable to large-scale manufacture. Accordingly, four large-scale field trials of Ty21a were carried out in Santiago, Chile (*10–13*), that examined the safety and efficacy of other formulations of Ty21a, two of which were subsequently licensed and used in many countries. These formulations included enteric-coated capsules containing lyophilized vaccine (*10*) and an improved "liquid" formulation consisting of a sachet with buffer powder and a sachet with lyophilized vaccine that were co-mixed with 100 ml of water to produce a vaccine "cocktail" (*12*); with both formulations, each individual dose of vaccine contained 10^9 cfu.

The placebo-controlled studies in Santiago, Chile, unequivocally established the safety of Ty21a and its efficacy in preventing typhoid fever, as well as the practicality of the logistics required for mass vaccination of schoolchildren. In total, more than 534,000 schoolchildren 5–19 years of age at the time of enrollment participated in the trials and received at least one dose of vaccine or placebo control. The smallest trial involved 91,000 subjects, and the largest included 216,692 subjects. The minimum period of follow-up in any trial was three years. These trials represented a collaboration among the Ministry of Health of Chile, the Center for Vaccine Development of the University of Maryland School of Medicine, the World Health Organization, and the Pan American Health Organization.

Over three years of follow-up, the enteric-coated capsule formulation of Ty21a conferred 67% efficacy in a field trial in Área Norte, in the city's northern sector (*10*), whereas the "liquid" formulation provided 79% efficacy over three years in a field trial carried out mainly in Área Suroriente, in the southeast (*12*). One of the most important features of the Santiago field trials was the maintenance of follow-up of two trials for five and seven years, respectively. This allowed an assessment of the duration of efficacy conferred by Ty21a. Thus, in the Área Norte trial, the enteric-coated capsule formulation conferred 62% protection over seven years, and in Área Suroriente the liquid formulation conferred 78% protection over five years of follow-up (*14*). Knowing the duration of protection conferred by a vaccine subsequently allowed public health authorities to assess the need for booster vaccinations and also facilitated assessments of the cost-effectiveness of vaccination programs.

A large-scale effectiveness trial was also carried out in Área Sur, in Santiago's southern sector, that compared the practicality of administering between two and four doses of vaccine over an eight-day period and studied the incidence of typhoid fever in each group (*13*). Recipients of the four-dose regimen had a significantly lower incidence of typhoid fever than those who were given three doses (*13*). Based on those data, a four-dose schedule was eventually recommended in North America, while a three-dose schedule was adopted in the rest of the world (*15*).

During the decade that field trials of Ty21a were carried out in Chile, epidemiologic evidence suggested that large-scale vaccination with Ty21a (consequent to the field trials) was resulting in herd immunity (*16*). Indeed, the incidence rate of typhoid fever in the placebo control group in the first field trials in Área Norte was observed to fall whenever field tri-

als were undertaken in other administrative areas of the city (*16*).

In association with the field trials of efficacy of Ty21a in Chile, the measurement of two immunologic responses were identified that correlated with protection, including serum IgG ELISA O antibody (*16*) and the enumeration of IgA O antibody-producing cells (ASCs) detected among peripheral blood mononuclear cells (*17*). Levine and colleagues (*16*) found that Ty21a regimens that elicited stronger IgG O antibody responses showed higher protection. Kantele (*17*) immunized young Finnish adults with the identical formulations, lots, and immunization schedules as used in the Chilean field trials and found that those that elicited the strongest IgA O ASC responses were the formulations and regimens that had given the highest protection in the field trials. As expected (since it does not express Vi), Ty21a does not elicit a Vi antibody response.

Vi Polysaccharide

In the 1950s, attempts were made to purify Vi polysaccharide and use it as a parenteral vaccine (*18*). However, the purification methods available at the time inadvertently denatured the antigen. Subsequently, it was discovered that the detergent hexadecyltriethylammonium bromide allowed purification of nondenatured Vi from *S.* Typhi and *Citrobacter freundii* (*19*, *20*). Following initial phase 1 studies in adults in the United States and France (*21*), the first clinical evaluation of a Vi vaccine in a developing country population was carried out in Chile, where a phase 2 clinical trial showed the safety and immunogenicity of Vi vaccine (*21*). Thereafter, two large-scale field trials of Vi vaccine (single dose, 25 μg) were carried out in 6,438 schoolchildren and adults in Nepal ages 5–44 years (*22*) and 11,384 schoolchildren in South Africa (99% were 5–16 years of age) (*23*), establishing the efficacy of Vi vaccine. During 17 months of follow-up in Nepal, an efficacy of 72% (*22*) was observed for culture-confirmed typhoid fever, and in the South African field trial 64% protection (*23*) was recorded over 21 months of follow-up. During three years of follow-up in South Africa, an efficacy of 55% was observed (*24*).

The protection elicited by Vi vaccine is mediated by serum Vi antibodies. As with several other polysaccharide vaccines that are T-independent antigens, Vi is poorly immunogenic in infants (*25*).

New Generation Typhoid Vaccines

Ty21a and Vi polysaccharide vaccines are both well tolerated, and each confers a moderate level of protection against typhoid fever. Thus, they represent an important advance over the old highly reactogenic killed whole cell parenteral vaccine. Nevertheless, Ty21a and Vi each suffer from certain drawbacks. For example, Ty21a requires 3–4 doses to elicit substantive protection, and Vi is poorly immunogenic in infants and toddlers. For these reasons, new generation typhoid vaccines are under development. Several groups have engineered new attenuated *S.* Typhi vaccine candidates in the quest to develop a strain that is as well tolerated as Ty21a but sufficiently more immunogenic so as to serve as single-dose live oral vaccine. Candidate live oral vaccine strains that have completed phase 1 trials with encouraging results include CVD 908-*htrA* (*26*), Ty800 (*27*), X4073 (*28*), and ZH9 (*29*). CVD 908-*htrA* has also been evaluated in a phase 2 trial with highly encouraging results (*30*).

Based on the success of polysaccharide-protein conjugate vaccines in converting *Haemophilus influenzae* type b, and pneumococcal and meningococcal polysaccharides to T-dependent antigens that are immunogenic for young infants and that elicit immunologic memory, Szu and colleagues (*31*) prepared candidate Vi conjugate vaccines and demonstrated their safety and immunogenicity in eliciting serum IgG Vi antibodies (*32*). The efficacy of administering two doses, spaced six weeks apart, of a vaccine consisting of Vi conjugated to *Pseudomonas aeruginosa* exotoxin A was evaluated in a large-scale, randomized controlled field trial in Vietnam in 11,091 children 2–5 years of age (*33*).

During 27 months of follow-up, active surveillance (detection of cases of typhoid through weekly household visits) revealed an efficacy of 91.5% (95% CI, 77.1–96.6%) (*33*).

Summary on Typhoid Vaccines

It is anticipated that over the next few years the Vi conjugate vaccine and at least one engineered attenuated strain live oral vaccine will become licensed products.

Cholera

Three extraordinary epidemiological events occurring at the end of the twentieth century exemplify why cholera harnesses the attention of public health authorities today:

- the return of cholera to Latin America in 1991, after a century of absence, and its rapid dissemination, leading to more than one million cases by 1994 (*34*);
- the explosive outbreak of El Tor cholera among Rwandan refugees in Goma, Zaire, in 1994, resulting in some 70,000 cases and 12,000 deaths (*35*); and
- the appearance in 1992–93 of epidemic cholera in the Indian subcontinent caused for the first time by a *V. cholerae* serogroup other than O1, so-called O139 Bengal (*36*).

Theoretically, potential target populations for cholera vaccines include individuals of all ages who live in high-risk areas during cholera epidemics, children (and perhaps adults) living in areas of very high endemicity, and travelers from industrialized countries who visit areas of the developing world where cholera is endemic or epidemic.

Oral Cholera Vaccines

Two modern oral cholera vaccines have been licensed by regulatory authorities in many countries. One is a nonliving vaccine consisting of inactivated *V. cholerae* O1 administered in combination with B subunit of cholera toxin (BS-WCV) (*37*). The other vaccine is a genetically engineered attenuated strain of *V. cholerae* O1, CVD 103-HgR, used as a single-dose live oral vaccine (*38*). Latin America and Asia have been the sites of important clinical and field trials for these vaccines.

BS-WCV

This oral vaccine contains 10^{11} heat-inactivated and formalin-inactivated *V. cholerae* O1 (a mixture of classical Inaba, classical Ogawa, El Tor Inaba, and El Tor Ogawa organisms), coadministered with 1.0 mg of B subunit, along with buffer. Three spaced doses of an early formulation of the vaccine (distinct from the current commercial formulation) conferred 85% protection during the initial six months of surveillance, and 50% protection over three years of follow-up in a large-scale, randomized field trial conducted in Bangladesh during the mid-1980s (*39*). The occurrence shortly after completion of the vaccination of one of the largest seasonal cholera epidemics ever recorded in the Matlab Bazar field allowed a definitive assessment of the efficacy of that early formulation of the BS-WCV.

The current commercial formulation, rBS-WCV, utilizes a recombinant BS (to help diminish production costs) (*40*) and is manufactured by SBL Vaccin AB in Stockholm, Sweden. It is marketed under the names Dukoral® or Colorvac® and is well tolerated by adults and children when administered as a two-dose immunization regimen, 10–14 days apart.

Three randomized, double-blind, controlled trials were undertaken in Latin America to assess the efficacy of the two-dose regimen of the commercial formulation; one of these field trials also provides data on the protective efficacy of an unusual three-dose regimen (with an additional booster dose given 12 months after the first two doses). In the first, relatively small, trial, two doses of the rBS-WC vaccine given two weeks apart conferred upon a group of Peruvian soldiers (710 vaccinees, 714 placebo controls) a high degree of short-term protection (86% protective efficacy) against epidemic

cholera in the face of exposure to a common source vehicle of transmission (encountered shortly after vaccination) that resulted in a high rate of cholera among placebo recipients (*41*). In contrast, in the second, much larger, placebo-controlled field trial of efficacy in Lima, Peru, which included children as well as adults, two doses of the rBS-WC vaccine given two weeks apart did not provide significant protection against either hospitalized cases (detected by passive surveillance) or field cases (detected by active surveillance) during a 12-month follow-up (*42*). However, after administration of the third dose of vaccine one year later, significant (61%) protection was observed over the next year of observation, including against hospitalized cases (82% efficacy) and field cases (49% efficacy).

Another large-scale field trial of the two-dose regimen of the commercial formulation of rBS-WCV was initiated in Arequipa, Peru, a city that had experienced high rates of cholera in the previous three years prior to the initiation of the field trial. During the first two years of follow-up after vaccination, almost no cases of cholera were observed, precluding any assessment of vaccine efficacy. Cholera then returned to Arequipa with several dozen cases of the disease occurring in the field trial participants in the third year after vaccination. Analysis of the cases showed no evidence of vaccine efficacy in this situation (C. Lanata, personal communication) (*43*).

CVD 103-HgR

Single-dose recombinant live oral cholera vaccine CVD 103-HgR was engineered by deleting from wild type *Vibrio cholerae* O1 classical Inaba strain 569B 94% of the gene encoding the A subunit of cholera toxin and by inserting into the hemolysin A locus a gene-encoding resistance to mercury ions (*44*, *45*). CVD 103-HgR is manufactured by Berna Biotech and is commercially available under the names Mutacol® and Orochol®.

The safety and immunogenicity of a single oral dose of this vaccine in subjects as young as 3 months (*46*) and as old as 65 years of age, including HIV-positive subjects (*47*), have been established in a large number of randomized, placebo-controlled, double-blind clinical trials with active surveillance (involving more than 7,000 subjects). These clinical trials were carried out in Africa, Asia, Europe, Latin America, and North America (*46–56*). CVD 103-HgR was licensed based on evidence of efficacy from experimental cholera challenge studies in adult volunteers in North America. A single dose of CVD 103-HgR confers on adult volunteers significant protection against experimental challenge with pathogenic *V. cholerae* O1 of either biotype or serotype (*45*, *57–59*). Notably, almost complete protection (> 95%) was conferred against moderate and severe diarrhea caused by either El Tor or classical biotype. In these experimental challenge studies, protection (against wild type *V. cholerae* O1 of either El Tor or classical biotype) was evident as early as eight days after vaccination and lasted for at least six months (the shortest and longest intervals tested). The single-dose efficacy and rapid onset of protection are attractive characteristics of CVD 103-HgR.

Heretofore, only one large-scale, randomized, placebo-controlled, double-blind field trial has been carried out in a developing country to assess the efficacy of a single dose of CVD 103-HgR in preventing cholera under natural challenge conditions in an endemic area. In that trial, in North Jakarta, Indonesia, 67,508 pediatric and adult subjects received a single dose of vaccine or identically appearing placebo (*60*). Overall, during four years of follow-up, the vaccine did not confer significant long-term protection in this venue (13.5% vaccine efficacy overall). Unfortunately, too few cases occurred during the first four months of follow-up after vaccination to allow a valid comparison with the experimental challenge studies (7 total; 5 in controls, 2 in vaccinees, with 60% vaccine efficacy) (*43*). The disparity is that all but one of the experimental challenge studies had been carried out less than four months after vaccination. Some evidence of long-term efficacy in the Jakarta trial

was seen in an analysis in relation to blood group. In an "intent to vaccinate" analysis assessing vaccine efficacy in relation to blood group, persons of blood group O (an important host risk factor for developing severe cholera) were modestly protected by vaccine ($p = 0.06$, vaccine efficacy = 45%) (*60*).

In the course of an extensive cholera outbreak in a Pacific island archipelago where logistics limited the use of vaccine to a single-dose regimen, the World Health Organization undertook a post-licensure evaluation of the practicality and effectiveness of cholera control using a single oral dose of CVD 103-HgR in persons above 2 years of age (*61*). They calculated a vaccine effectiveness of 79% (CI, 72–85%) for use of CVD 103-HgR in this situation.

It is unclear why a single dose of CVD 103-HgR did not confer long-term protection in the Jakarta trial when a high level of protection lasting at least six months was observed in North American volunteers participating in experimental challenge studies. An important clue may reside in the lower vibriocidal antibody responses that have been observed in subjects vaccinated with CVD 103-HgR in developing countries versus subjects vaccinated in industrialized countries (*50*, *51*, *56*). Three factors have been shown to modulate the vibriocidal antibody response. The first is blood group O (*62*); persons of this blood group (an important host risk factor for the development of cholera gravis) (*62*, *63*) mount a stronger response, especially among immunologically naive persons lacking prior contact with *V. cholerae* O1 (*64*). The second factor is prior exposure to *V. cholerae* O1; subjects with high baseline titers usually do not undergo boosts in titer (*50*, *51*, *53*, *56*, *65*). Thirdly, socioeconomic level is an underlying determinant; populations living in underprivileged conditions manifest lower geometric mean titers, independent of blood group or prior contact with *V. cholerae* O1 (*56*, *65*).

Several live oral viral vaccines, including Sabin polio and RIT bovine rotavirus vaccine, were found to be less immunogenic when given to young children living in low socioeconomic conditions in less-developed countries than children in industrialized countries (*66–68*). Possible explanations for this diminished immunogenicity include interference from enteroviruses, SIgA antibodies in breast milk, and an unreliable cold chain resulting in loss of vaccine potency. This phenomenon was also observed in phase II studies of live oral cholera vaccine CVD 103-HgR in adults and children living in underprivileged conditions in Asia and Latin America (*50*, *51*, *56*, *65*). In order to achieve high seroconversion rates of vibriocidal antibody in Indonesian children and Peruvian adults living in underprivileged conditions, it was necessary to give a tenfold higher dose (5×10^9 CFU) of CVD 103-HgR (*50*, *56*) than the dose (5×10^8 CFU) that is consistently immunogenic in North Americans and Europeans (*48*, *49*). Thus, there exists a poorly understood "barrier" to successful intestinal immunization of children in less-developed countries by live oral vaccines.

Two factors that can contribute to this barrier include small bowel bacterial overgrowth (SBBO) and heavy intestinal helminth infection. Persons living in poverty in developing countries endure fecally contaminated environments. Young children often develop SBBO and "environmental enteropathy" (*69–71*). The relationship between SBBO and vibriocidal response to CVD 103-HgR was investigated in Chilean schoolchildren ages 5–9 years who had lactulose breath H_2 tests to detect proximal SBBO one day before ingesting CVD 103-HgR (*72*). An inverse relationship was found between H_2 production in the small bowel and vibriocidal antibody seroconversion (*72*).

Short chain fatty acids elaborated by SBBO flora may inhibit *V. cholerae* O1 (*73*) and blunt the vibriocidal response to CVD 103-HgR. Alternatively, the abnormal intestinal architecture and increased cellularity observed in the mucosa of children with SBBO (*69*, *70*) may act to mute immune responses.

Cooper and colleagues (*74*) showed that heavy infestation with intestinal helminths diminishes vibriocidal antibody response observed in persons living in underprivileged

conditions in less-developed countries. Ecuadoran childen of non-O blood group treated with the anti-helminthic albendazole exhibited a significantly higher vibriocidal antibody response than children pretreated with placebo (*74*).

Other Cholera Vaccines under Development

A nonliving cholera vaccine containing *V. cholerae* O1 O139 and O1, in combination with BS, has been evaluated in phase 1 and 2 clinical trials (*75*). A nonliving cholera vaccine that contains *V. cholerae* O1 but does not have BS has been locally manufactured and tested in Vietnam (*76*).

Several attenuated *V. cholerae* O1 strains have been tested in phase 1 or 2 studies as live oral vaccines, including Peru 15 (*77*, *78*), CVD 111 (*79*, *80*), and 638 (*81*). Attenuated O139 strains, such as CVD 112 (*82*) and Bengal 15 (*83*), have also undergone phase 1 clinical trials with promising results.

Summary on Cholera Vaccines

In practice, use of the oral cholera vaccines has been limited to immunization of travelers and vaccination of high-risk populations in the face of cholera epidemics. In these situations, the BS-WCV and CVD 103-HgR have proven valuable. Two fundamental questions raised in the course of clinical trials are why oral cholera vaccines are less immunogenic in poor developing country populations than in industrialized populations and how immunogenicity might be increased without administering additional doses of vaccine and without increasing reactogenicity. Finding answers to these questions will be a focus of research efforts in the coming years.

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PROGRESS IN *SHIGELLA* VACCINE DEVELOPMENT

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INTRODUCTION

The family of bacteria *Shigella* is the classic cause of bacillary dysentery, a syndrome of fever, abdominal cramps, diarrhea, scant bloody stools, and tenesmus. Among this microbe's protean manifestations are the hemolytic-uremic syndrome caused by Shiga-toxin-producing strains of *S. dysenteriae* type 1 (*1*) and post-infectious reactive arthritis (*2*). Furthermore, this epithelial-cell invasive bacterium is prone to causing enduring intestinal injury that leads to protein-losing enteropathy, persistent diarrhea, and malnutrition (*3*).

DISEASE BURDEN

Based on an analysis of published studies, it has been estimated that there are 160 million cases of shigellosis in the world each year, resulting in about 1.5 million deaths (*4*). Most of these cases and deaths occur in children younger than five years of age living in developing countries. Several factors contribute to the ability of this organism to produce such a large disease burden. For one, *Shigella* is highly contagious—as few as ten organisms can produce infection (*5*)—readily spreading from person to person by fecal-oral transmission in settings with suboptimal hygiene and sanitation (*6*). Second, one serotype, *S. dysenteriae* type 1, causes true pandemics with high attack rates and case fatality in all age groups (*7*). Third, *Shigella*'s predilection to acquire resistance to multiple antibiotics has limited the availability of effective antibiotics in some locales (*8*). Last is the tendency for *Shigella* to be hyperendemic in areas where HIV is prevalent and the organism has access to a pool of highly susceptible hosts (*9*). Compounding the true burden of disease is the threat that *Shigella* could be used as a biological weapon because of these various microbial, clinical, and epidemiologic properties (*10*, *11*).

FEASIBILITY OF DEVELOPING A VACCINE

Observations in several venues have shown that natural or experimental exposure to *Shigella* antigens induces clinical immunity, and this points to the feasibility of developing an effective *Shigella* vaccine as a way of controlling this disease. For example, monkeys experimentally infected with *S. flexneri* 2a were completely protected when rechallenged with the same strain, whereas all monkeys rechallenged with *S. sonnei* (*12*) or *S. flexneri* 6 (*13*) became ill. In humans, homotypic immunity has been demonstrated in the volunteer challenge model using *S. sonnei* (*14*) and *S. flexneri*

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TABLE 1. Protective efficacy of monovalent and bivalent streptomycin-dependent *Shigella* vaccines.

Parent strain	No. subjects	Age group	Dose (CFU)	No. doses	Efficacy[a] (%)
S. flexneri 2a (*17, 18*)	675	Adults	2–4 × 1010	5	84–100
S. flexneri 2a & 3 (*18*)	278	Adults	4–6 × 1010	5	85
S. flexneri 1 & 2a (*19*)	3,624	Children 2–8 years	2–4 × 1010	3–4	91
S. flexneri 3 & *S. sonnei* (*19*)	3,663	Children 2–8 years	2–4 × 1010	3–4	82

[a]Efficacy against *Shigella* serotypes contained in the vaccine.

2a (*15*) as test strains. Moreover, a cohort study of children living in a *Shigella*-endemic area in Chile showed that an initial infection with *S. sonnei* provided significant (72%) protection against illness following reinfection with *S. sonnei* (*16*). The sentinel studies conducted by Mel and colleagues in Yugoslavia in the 1970s illustrate the potential capabilities of *Shigella* vaccines (Table 1). These investigators showed that various formulations of streptomycin-dependent, non-invasive, live oral *Shigella* vaccines were highly protective against clinical illness when given in multiple doses (3 to 5) at high inocula (more than 10^{10} CFU) (*17–20*), and followed by a yearly booster (*20*).

In the examples just cited, immunity was serotype-specific, which has led to the recognition that the O-moiety of lipopolysaccaride (LPS) is a critical antigen for inclusion in a *Shigella* vaccine. Further support for the notion that the O-polysaccharide is associated with protection comes from observations of Israeli soldiers who were deployed to a field area. Soldiers with pre-existing serum anti-LPS antibody were significantly less likely to become ill upon exposure to the homologous *Shigella* serotype than were seronegative soldiers (*21*). A review of recent volunteer studies similarly suggests a correlation between anti-LPS responses (in particular IgA antibody secreting cell (ASC) levels) after oral inoculation with *S. flexneri* 2a antigens (either via vaccination or wild type challenge) and protection against illness following experimental challenge with the homologous serotype (Table 2) (*22*, *23*). ASC assays are performed using ELISPOT and measure the number of antigen-specific, antibody-secreting peripheral blood mononuclear cells (PBMC) circulating in the bloodstream. Responses (which peak seven days after inoculation) are considered to be an indication that intestinal priming has occurred.

A notable exception which suggests that *Shigella* immunity may not be completely ex-

TABLE 2. Immune responses following oral inoculation with *S. flexneri* 2a 2457T wild type or vaccine strains and protective immunity following challenge with the homologous wild type strain in volunteers.

		Subjects with anti-LPS response (%)		
Immunogen	Inoculum (CFU)	IgA ASC/106 PBMC (geometric mean peak)	Serum IgG antibody	Protective efficacy
S. flexneri 2a 2457T (*15*)	103	92 (239)	50	70
EcSf2a-2 (*59*)	109	100 (59)	53	48
SC602 (*23*)	104	58 (26)[a]	10	50
EcSf2a-2 (*22*)	108	100 (16)	19	27

[a]The geometric mean peak ASC response after vaccination was 43 cells/106 PBMC in those challenged.

Note: CFU, colony forming units; LPS, lipopolysaccharide; ASC, antibody secreting cells; PBMC, peripheral blood mononuclear cells.

plained by the O-antigen is the experience with the T_{32}-Istrati vaccine in large scale field trials in Romania (*24*) and China (*25*). T_{32}-Istrati is a live, oral, non-invasive *S. flexneri* 2a vaccine that, as are streptomycin-dependent strains, is administered at high inocula in multiple doses. It has been reported that T_{32}-Istrati confers 80% to 85% protection against *S. flexneri* 2a disease, and 63% to 89% protection against heterologous *Shigella* strains including *S. sonnei* (*25*, *26*). Similar findings were reported with the FS bivalent vaccine, constructed at the Lanzhou Institute of Biological Products from T_{32}-Istrati and bearing genes for expression of the O-polysaccharide from both *S. flexneri* 2a and *S. sonnei* (*26*). During the 1990s, two double-blind, placebo-controlled efficacy trials of the FS vaccine were conducted in Changge City, China. During six months of surveillance, protective efficacy was 61% to 65% against *S. flexneri* 2a and 50% to 72% against *S. sonnei*. Efficacy against heterologous *Shigella* spp. was 48% to 52% (*27*). The immunological basis for this heterologous protection is unclear. Additional investigations of the T_{32}-Istrati and FS vaccines are warranted to elucidate these field observations.

Approaches to Vaccine Development

This chapter will cover three approaches to *Shigella* vaccine development that are under active investigation: 1) parenteral O-specific polysaccharide conjugate vaccines; 2) nasal proteosomes delivering *Shigella* LPS; and 3) live, attenuated invasive *Shigella* deletion mutants that are administered orally.

Parenteral Conjugate Vaccines

Robbins, Schneerson, and colleagues at the National Institutes of Health have developed a series of vaccines in which the O antigen of *S. sonnei*, *S. flexneri* 2a, or *S. dysenteriae* type 1 is conjugated to *Pseudomonas aeroginosa* recombinant exoprotein A (rEPA). These vaccines elicit robust immune responses in both adults and children. In one trial, 90% of Israeli adults injected intramuscularly with *S. sonnei*-rEPA vaccine mounted a fourfold rise in IgG anti-LPS after a single dose, as did 73% of adults inoculated with the *S. flexneri* 2a-rEPA conjugate (*28*). A second inoculation at six weeks did not boost the response. In comparison, more than 95% of children seroconverted after receiving these vaccines (*29*). Children mounted a booster response to the *S. flexneri* 2a, but not to the *S. sonnei* vaccine. A Phase 3 efficacy field trial conducted among Israeli soldiers demonstrated that a single intramuscular injection of *S. sonnei*-rEPA conferred 74% efficacy against shigellosis (95% confidence interval 28 to 100%, $p = 0.006$) during short-term (2.5 to 7 months) follow-up (*30*). Efficacy was 43% within 17 days after vaccination. These investigators are now trying to optimize the immunogenicity of the polysaccharide conjugate vaccines by succinylating the carrier protein (*31*) and by using synthetic saccharide conjugates (*32*).

Nasal Proteosome Vaccines

Another interesting approach that has been pursued in recent years is delivery of *Shigella* LPS in proteosomes. Proteosomes are purified meningococcal outer membrane proteins that form a multimolecular vesicular structure into which the antigen is non-covalently complexed by hydrophobic interactions. In addition to their carrier function, proteosomes are thought to engender mucosal adjuvanticity. In clinical studies, intranasal immunization appears more immunogenic than oral immunization. A phase 1 trial was conducted in which volunteers were inoculated with proteosome-*S. flexneri* 2a LPS vaccine by intranasal spray on days 0 and 14 (*33*). Four dose levels (by protein content) were assessed. A dose response was apparent whereby at the lowest dose (0.1 mg), 40% of subjects reported transient rhinorrhea for less than one day, while at the highest dose (1.5 mg), 90% of subjects developed rhinorrhea for a median duration of three days. The geometric mean anti-LPS IgA ASC count was 4.8 cells per 10^6 PBMC at the lowest dose and no subject developed a fourfold rise in

serum anti-LPS IgG. At the highest dose, the geometric mean IgA anti-LPS ASC level was 26.4 cells per 10^6 PBMC, and 20% of subjects mounted a fourfold rise in serum anti-LPS IgG. Four to six weeks after vaccination, subjects were challenged with 500 CFU of *S. flexneri* 2a wild type strain 2457T. Whereas no efficacy was apparent against the primary endpoint (diarrhea, dysentery, fever, and early treatment), there was some suggestion that vaccination diminished the severity of illness (*34*).

Live Attenuated Oral Vaccines

The final approach to be covered is construction of vaccines by creating live oral attenuated deletion mutants of *Shigella*. As summarized in Table 3, all of the strains to be described bear the O antigen and express the invasiveness phenotype in an attempt to maximize the magnitude and duration of immune responses. Two strategies for attenuation have been used. One approach is to cripple the bacterium by creating deletions in genes that govern vital metabolic processes, such as the chromosomal genes that regulate biosynthesis of essential aromatic amino acids (*aro*) (*35*), purines (*gua*) (*36*), or aerobactin (*iuc*) (*37*), which allows the organism to scavenge for iron. The second strategy is to disarm *Shigella* by mutating genes that encode specific virulence factors, e.g., *virG* (also known as *icsA*), a plasmid gene that regulates cell-to-cell spread of *Shigella* (*38*, *39*); *set*, a chromosomal gene encoding *Shigella* enterotoxin 1 (ShET1) (*40*), present only in *S. flexneri* 2a (*41*); and *sen*, a plasmid gene responsible for synthesis of ShET2 (*42*), present in nearly all *Shigella* serotypes. The *Shigella* enterotoxins have been identified in rabbit ileal loop and Ussing chamber studies as possible mediators of the watery diarrhea often seen in shigellosis (*40*, *42*). Attenuated *S. dysenteriae* type 1 vaccine strains must contain deletions in the *stx* gene to prevent elaboration of physiologically active Shiga toxin. Strains with mutations in *msbB*, which regulates the acylation of lipid A, are under construction in the laboratory of Sansonetti and colleagues at the Pasteur Institute, and they represent an interesting approach toward eliminating the adverse effects of *Shigella* infection that are related to endotoxin production (*43*).

Vaccines with a Fundamental Mutation in virG

SC602 (Δ*virG*, Δ*iuc S. flexneri* 2a strain 454). SC602 is a vaccine that was constructed by Sansonetti and colleagues from *S. flexneri* 2a strain 454 by creating deletions in *virG* and *iuc* (*44*). Phase 1 dose-response studies of SC602 were conducted at Walter Reed Army Institute of Research and an inoculum of 10^4 CFU was

TABLE 3. Examples of live, oral, attenuated *Shigella* vaccines evaluated in phase 1 trials.

Vaccine	Parent strain	Mutations	Some inocula tested (CFU)	% Subjects with reactogenicity[a]	Geometric mean peak anti-LPS IgA ASC/106 PBMC[b]
WRSS1 (*48*)	*S. sonnei* Mosley	Δ*virG*	103–106	14–33	99–233
SC602 (*23*)	*S. flexneri* 2a 454	Δ*virG*, Δ*iuc*	104–105	20–60	26–154[c]
CVD 1203 (*51*)	*S. flexneri* 2a 2457T	Δ*aroA*, Δ*virG*	106–109	0–80	13–175
CVD 1207 (*53*)	*S. flexneri* 2a 2457T	Δ*guaBA*, Δ*virG*, Δ*sen*, Δ*set*	106–1010	0–20	0.1–35
CVD 12044	*S. flexneri* 2a 2457T	Δ*guaBA*	107–109	Pending	Pending
CVD 12084	*S. flexneri* 2a 2457T	Δ*guaBA*, Δ*sen*, Δ*set*	107–109	Pending	Pending

[a] Generally defined as fever, diarrhea, or dysentery.
[b] Measured seven days following vaccination.
[c] Protective efficacy was 50% among recipients of 104 CFU who were challenged with the wild type parent strain.
Note: CFU, colony forming units; LPS, lipopolysaccharide; ASC, antibody secreting cells; PBMC, peripheral blood mononuclear cells.

considered acceptable for further development (*23*). This inoculum has some reactogenicity, causing mild fever and diarrhea in about 20% of subjects, but elicits notable immune responses, with geometric mean anti-LPS IgA ASC counts of 26 per 10^6 PBMC. When challenged with the wild type parent strain, recipients of 10^4 CFU of SC602 (whose geometric mean anti-LPS IgA ASC count was 43 per 10^6 PBMC) experienced a 50% reduction in the rate of shigellosis. Intestinal colonization with these strains was robust in volunteers, who shed in stool for a mean of 10 days, with 5% of subjects shedding for more than four weeks.

Buoyed by the promising results from clinical trials in North American adults, these investigators initiated studies with SC602 in Bangladeshi children (*45*). The vaccine was well-tolerated when administered to descending age groups, including 1- to 3-year-olds. Unfortunately, neither fecal shedding nor immune responses were detected in these young children. The reasons for the impaired "take" of this vaccine among children from a developing country remain unexplained, but a similar phenomenon has been observed with other mucosal vaccines (*46*). Nonetheless, in view of the promising response to SC602 among North American adults, phase 2 trials are planned to evaluate this vaccine candidate in Israeli soldiers.

WRSS1 (Δ*virG S. sonnei* strain Mosley). Based on observations in animal models that the addition of Δ*iuc* to Δ*virG* provided little supplementary attenuation of *S. flexneri* 2a beyond that provided by Δ*virG* alone, investigators at Walter Reed created a series of *Shigella* vaccine candidates that contain a single deletion mutation in *virG*. The Δ*virG S. sonnei* construct, designated WRSS1 and developed by Hartman, Venkatesan, and colleagues (*47*), was tested in phase 1 trials at the Center for Vaccine Development, and showed a clinical profile similar to that of SC602, with mild reactogenicity and very good IgA ASC anti-LPS responses (*48*). Adverse reactions were noted in 14%, 0%, 30%, and 33% of recipients of 10^3 CFU, 10^4 CFU, 10^5 CFU, and 10^6 CFU, respectively. The corresponding geometric mean anti-LPS IgA ASC counts were 99, 39, 278, and 233 per 10^6 PBMC, respectively. In the near future, this strain will undergo phase 2 testing in Israel and possibly an efficacy challenge trial in the United States. Walter Reed investigators will soon initiate phase 1 trials with an *S. dysenteriae* type 1 vaccine strain bearing deletions in *virG* and in the entire *stx* gene (*49*).

A Vaccine Attenuated on the Basis of Aromatic Auxotrophy and a Mutation in virG

CVD 1203 (Δ*aroA*, Δ*virG S. flexneri* 2a strain 2457T). The remainder of this discussion will focus on the *Shigella* vaccine candidates that were constructed at the Center for Vaccine Development from the wild type *S. flexneri* 2a strain 2457T. CVD 1203 was developed by Noriega and colleagues and contains mutations in *aroA* and *virG* (*50*). This strain was well-tolerated in phase 1 studies when administered at a dose of 10^6 CFU, attesting to the attenuating effects of Δ*aro* and Δ*virG* (*51*); by contrast, 10^3 CFU of wild type *S. flexneri* 2a strain 2457T induces shigellosis in about 90% of subjects (*15*). However, the post-vaccination geometric mean IgA anti-LPS ASC count (13 per 10^6 PBMC) was lower than desired. Unfortunately, at higher doses (10^8 and 10^9 CFU), where the ASC responses were vigorous, CVD 1203 induced unacceptable reactogenicity. In response, further attenuated strains were constructed that were expected to be better tolerated at higher inocula.

Vaccines with Fundamental Mutations in guaBA

CVD 1207 (Δ*guaBA*, Δ*virG*, Δ*sen*, Δ*set S. flexneri* 2a strain 2457T). The next construct of the Center for Vaccine Development to be tested was CVD 1207, which contains deletions in *virG*, *guaBA*, and in the enterotoxin genes *sen* and *set*. In preclinical studies, Δ*guaBA* is more attenuating for *S. flexneri* 2a than Δ*aroA* (*52*). When administered to volunteers, CVD 1207

was remarkably well-tolerated at inocula ranging from 10^6 to 10^{10} CFU; reactogenicity was limited to mild diarrhea in one subject at each of the two highest doses (*53*). The anti-LPS IgA ASC responses increased in magnitude with inoculum size, but achieved a geometric mean of only 35 ASC per 10^6 PBMC at the highest dose (10^{10} CFU). These results indicate that CVD 1207 is the most attenuated derivative of 2457T that we or others have thus far been able to prepare. In fact, CVD 1207 is possibly overattenuated, requiring 10^{10} CFU to elicit a modest ASC response. We therefore hypothesized that a more satisfactory balance between clinical acceptability and immunogenicity might be achieved with CVD 1204 or CVD 1208.

CVD 1204 (Δ*guaBA*) and CVD 1208 (Δ*guaBA*, Δ*sen*, Δ*set*) (*S. flexneri* 2a strain 2457T). CVD 1204 and CVD 1208 are isogenic strains with a fundamental attenuating deletion in *guaBA*. CVD 1204 has a single deletion *guaBA* (*52*) and CVD 1208 has deletions in *guaBA*, as well as the enterotoxin genes *sen* and *set* (unpublished observations). A comparative phase 1 trial was recently completed in which in-patient volunteers were randomized to receive (in double-blind fashion) CVD 1204, CVD 1208, or placebo. This comparison allowed us to ascertain the degree of attenuation attributable to the *guaBA* mutation alone (CVD 1204), the additional attenuation conferred by deletions in the genes encoding ShET1 and ShET2 (CVD 1208), and the relative immunogenicity of these two constructs. Sequential groups of volunteers received higher dosage levels of the vaccine strains, i.e., 10^7 CFU, 10^8 CFU, and 10^9 CFU.

Preliminary results show that CVD 1204 is clearly attenuated compared to its wild type parent (by retrospective comparison), but was nevertheless insufficiently attenuated to serve as a live oral vaccine in humans (unpublished observations). Thus, the *guaBA* mutation alone is insufficient to create a clinically acceptable live oral vaccine strain. Crippling the ability to produce ShET1 and ShET2 significantly further attenuates *Shigella* over what can be achieved with Δ*guaBA* alone, however. Indeed, in the full dose range, CVD 1208 was well-tolerated and further development of this vaccine is planned.

Future Challenges

A number of challenges remain in our pursuit of a safe and effective *Shigella* vaccine that induces lasting immunity. The initial trial demonstrating the short-term efficacy of parenteral O-polysaccharide *S. sonnei* vaccine is encouraging, and we look forward to the results of additional clinical investigation. Whereas the tenuous balance between safety and imunogenicity has beset efforts to develop oral attenuated *Shigella* vaccines for many years, there is hope that a satisfactory equilibrium has been achieved with recent strains such as WRSS1 and CVD 1208. Nonetheless, to reach the goal of developing a vaccine that can be used in both industrialized and developing countries, delivery systems must be optimized, especially for infants and young children living in endemic areas. In addition to enhancing immunogenicity, methods for administering live oral bacterial vaccines must be developed which infants and young children find palatable (*54*). The need for vaccines that prevent shigellosis, a disease with global impact, must be communicated to the private sector to attract the industry support that will enable commercial development of promising candidates. To guide vaccine development and implementation, additional data are needed to measure the cost-benefit of *Shigella* vaccines and to understand the serotype distribution in more detail.

A major challenge in vaccine development is how to provide coverage for the numerous serotypes of *Shigella* that appear epidemiologically important. Most experts agree that for a *Shigella* vaccine to make an impact globally, it must protect against *S. dysenteriae* type 1 (the cause of epidemic and pandemic Shiga dysentery), *S. sonnei* (the main serogroup found in industrialized countries), and all 15 classical *S. flexneri* serotypes (the main cause of endemic disease in developing countries). Although

S. flexneri 2a is the most common *S. flexneri* serotype that causes disease worldwide, the remaining *S. flexneri* serotypes are all also important. Which of the other *S. flexneri* serotypes predominate varies in different geographic areas (*4*).

It would be highly impractical if the live vaccine had to include all *S. flexneri* serotypes. Accordingly, we have shown that a composite of three *S. flexneri* serotypes, including *S. flexneri* 2a, 3a, and 6, provides cross protection against the remaining 12 *S. flexneri* serotypes (*55*). The immunological rationale is that among these three serotypes there is a type or group-specific antigen shared by every one of the 15 *S. flexneri* serotypes. The functional activity of these serological cross reactions was shown in cross protection studies involving Sereny test challenge of mucosally immunized guinea pigs (*55*).

The ultimate plan is to construct a pentavalent vaccine containing five *Shigella* serotypes: *S. sonnei*, *S. dysenteriae* type 1, and *S. flexneri* 2a, 3a, and 6. Our recent experience suggests that the array of mutations used to attenuate CVD 1208, i.e., Δ*guaBA*, Δ*sen*, and Δ*set* (for *S. flexneri* 2a), are well-suited for constructing the remaining four serotypes of *Shigella* that will be contained within our multivalent vaccine (our *S. dysenteriae* 1 strain will have an additional deletion mutation in *stxA*). The clinical response to CVD 1208 also suggests that the five attenuated *Shigella* strains that will comprise the pentavalent vaccine show promise for use as live vectors. The CVD is actively pursuing a program for orally delivering antigens of enterotoxigenic *E. coli* (ETEC) to humans using *Shigella* strains bearing ETEC colonization factors and nontoxigenic heat-labile toxin (*56–58*). Many exciting possibilities are on the horizon for prevention of shigellosis and we are hopeful that safe and effective vaccines will become a reality in the near future.

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HUMAN PAPILLOMAVIRUS

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This chapter briefly discusses cervical cancer and its natural history, and how human papillomavirus (HPV) is involved in its development. It then addresses vaccines that might be used to prevent HPV infection and those that might be used to treat existing infection.

Research has shown that HPV is involved in producing gynecological cancer. Since approximately a quarter million women die each year of cervical cancer, this is a major public health problem. The World Health Organization (WHO) now accepts that cervical cancer is essentially produced entirely by infection with human papillomavirus; indeed, persistent human papillomavirus infection conveys the risk of cancer. The incidence of cervical cancer is not uniformly distributed worldwide. The problem is greatest in Africa and Asia, and affects Europe and North America to a lesser extent. This difference is due in part to the developed world's very good screening programs to recognize cervical precancer. As a consequence, the major burden of cervical cancer mortality falls on the developing world. This situation contrasts with many other cancers, for which there is little difference or for which the burden is higher in the developed world. In the developing world, cervical cancer is the most common cause of cancer mortality. Many different sources of papillomavirus have been found in cervical cancers; type 16 is the dominant type in cervical cancers in almost every country of the world. When 12 different types of papillomavirus are considered, almost 95% of the cases of cervical cancer are HPV positive. This is a significant consideration when developing vaccines to prevent papillomavirus infection, and thus cervical cancer.

Papillomaviruses are not cytolytic and do not kill the cells they infect, rather they produce proliferation. Papillomaviruses produce warts, but they also produce premalignant transformations of epithelial surfaces. Presently, there is no vaccine to prevent papillomavirus infection, as this virus type cannot be grown in cell culture, which is the basis for producing most vaccine viruses. There are four major groups of papillomaviruses; one group in particular is associated with gynecological cancer: HPV-16 and HPV-18 are prototypes of this group. It is important to note that each of the genotypes is also a serotype, and thus is immunologically distinct and will be seen differently by the immune system.

Unfortunately, it is impossible to predict which carrier of HPV will develop cervical cancer. However, how HPV promotes cancer development is clearly understood—the persistence of a high-risk type is required for cancer development. On the other hand, an average of about 15 years pass between acquisition of the infection and the development of cancer. Many people who acquire even higher risk HPV types will not develop long-term infection or be at risk for cancer. Therefore, the goal is to prevent the virus infection that causes

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cancer, even though the vast majority of people who are infected with the virus will never develop the disease.

The risk of developing cervical cancer can be clearly determined by the age of first intercourse: the earlier the age of first intercourse, the higher the probability the infection will develop into papillomavirus-associated cancer. Other factors that have been associated with increased risk include smoking and the use of oral contraceptive pills. Some particular variance of HPV-16 is also associated with increased risk. However, each of these factors only slightly increases the overall risk of acquiring HPV-16 infection, and a very significant element of chance is involved.

How can we intervene with vaccines to prevent cervical cancer? First, we can try to prevent papillomavirus infection through immunization with a prophylactic vaccine. We might also promote other means of controlling the spread of sexually transmitted infections, since the papillomaviruses that cause cancer are sexually transmitted. There is little evidence that such controls will be effective, given this virus's varying infectivity and the number of sexual partners needed to have a very high probability of acquiring it. Second, we can consider that some people have already been infected with the virus and have not yet progressed to the stage where it is causing problems in the epithelium (i.e., premalignant changes). At this point, screening for and treating either the papillomavirus infection or the premalignant changes themselves—the current basis of cervical cancer screening—should allow the cancer to be treated and prevented. Pap smears can identify abnormal cells. Tests to detect papillomavirus can also be performed. Alternatively, where Pap smear screening programs are not available, visual inspection of the cervix is another means of diagnosing HPV infection, with cautionary treatment of abnormal lesions. At this point, we could intervene with an immunotherapeutic vaccine in order to eliminate the infection in patients already infected by the virus. It must also be borne in mind, regardless of the intervention plan, that most papillomavirus infections resolve spontaneously, and that whatever intervention is undertaken at this stage should not compromise the patient's health.

Prophylactic Vaccines

The first generation of prophylactic papillomavirus vaccines are based on virus-like particles. These are particles that are assembled using recombinant DNA technology, with the L1 protein, the major protein of the virus. This protein spontaneously assembles into this virus, like particles expressed into the eukaryotic expression systems from the appropriate ATG initiation codon. These are very conventional vaccines because they induce neutralizing antibodies, which is how all vaccines currently in use are designed to work. They were originally expressed using vaccinia virus but a number of other expression systems may also be used. The virus-like particles resemble the virus both physically and immunologically. The immune system sees them the same way as it would the natural virus infection. The virus particles used in vaccines are currently produced in yeast systems or in insect cells using a vacuolar virus vector. These particles could be used for serology, but only about half of those who become infected with papillomavirus become seropositive after natural infection. Therefore, screening for papillomavirus infection based on virus-like particles is unlikely to be helpful in controlling papillomavirus-associated cancer. The particles protect animal models against challenge with live virus. Several animals can be infected with papillomavirus, including rabbits, dogs, and cows, and papillomavirus vaccine based on virus-like particles has been successful in each of these models. The protection it affords is very much virus-type–specific in the animal models and does not provide any cross-protection against any other virus types, as would be predicted from the serology in humans. Protection requires antibody against the conformational determinants on the virus capsid: if the structure of the virus particle is destroyed, the

denatured capsid proteins no longer work as a vaccine. Antibodies transferred from a protected animal after vaccination to an unprotected animal confer protection, signifying that these are very conventional vaccines.

Vaccine Efficacy

A phase 2 trial of an HPV-16 virus-like–particle vaccine carried out by Koutsky *et al*. seeks to ascertain if a vaccine for the type of papillomavirus most commonly associated with cervical cancer will protect against infection and precancer (*1*). The women included in the primary efficacy analysis had no evidence of HPV infection and were randomly assigned to receive the HPV-16 vaccine or a placebo, delivered three times over six months. A large number (2,392) of young women aged 16 to 23 years were recruited for this study and approximately two-thirds were evaluated in the primary analysis. A number of women were excluded either because they were HPV-16 positive at some point from the time of enrollment through month 7 of the study or because they were lost to follow up. The women that continued were followed for about 17 months after completing the vaccination regimen. Almost all of the women immunized had a strong antibody response, as occurred in the phase 1 clinical trials. Thus, these vaccines are very immunogenic in producing neutralizing antibodies in humans and animals.

Of the 768 women who received the vaccine, there were no cases of persistent HPV-16 infection, as compared to the 41 cases of persistent HPV infection in the 765 women in the placebo group, including five cases of cervical intraepithelial neoplasia grade 1 (CIN 1) and four of CIN 2, which is the immediate precancerous lesion that normally requires treatment. Therefore, using persistent infection as the primary end point, the vaccine was 100% efficacious. Only six cases of transient HPV-16 infection at one visit were detected in the immunized group, as compared to 27 in the placebo group, for an overall efficacy of 91% by this criterion. In summary, if persistent infection is the outcome of interest, the vaccine was 100% efficacious; if any infection—transient or persistent—is the outcome of interest, the vaccine was 91% efficacious. These data are very encouraging. The study participants are still being followed and the study is still ongoing.

Several phase 3 trials of multivalent vaccines are also under way, including vaccines for virus types 16 and 18. It will be very important to determine the duration of protection, and how such vaccines might be used to prevent cervical cancer, both in the developed world (where screening programs are in place) and in the developing world as well. The Bill and Melinda Gates Foundation has expressed interest in these vaccines and their deployment in developing countries.

Second-generation vaccines should also be considered, as they might be easier to use in the developing world either because they are easier or cheaper to manufacture, distribute, or deliver, or because they have a broader or more relevant spectrum of coverage for the virus types found in any particular country. The combination of prophylactic and therapeutic vaccines might be considered as well. Ongoing clinical trials of HPV vaccines are mainly being conducted in the Americas, but the major disease burden is in Asia and Africa. As such, WHO is very anxious to see these vaccines introduced in the developing world as quickly as possible. It has a number of aims it might wish to see achieved if the introduction of these vaccines in the developing world is to be facilitated, with one of the most important ones being understanding the HPV situation of the individual countries where those vaccines might be used. This, in turn, might lead to controlled trials in developing countries in which HPV prevalence is high to determine local safety and immunogenicity.

Therapeutic Vaccines

Although prophylactic vaccines will be good for preventing infections, they will not be useful in treating existing infections. Instead, therapeutic HPV vaccines would be used to treat

existing infections, which is important, given the very large number—an estimated 5 million—of women already infected with papillomavirus who would likely develop cervical cancer if not treated. Even if a prophylactic vaccine were available right now, a therapeutic vaccine is highly desirable, as the shorter lag between the introduction of the vaccine and the reduction in the rate of cervical cancer would make its public health benefits apparent more quickly.

A number of therapeutic vaccines are currently in early-phase clinical trials. Once it is determined which bits of the virus should be put into a therapeutic vaccine a range of delivery systems could be used. The target population for the vaccine, as well as methods for testing its efficacy, must be identified, bearing in mind that although every one of these vaccine systems is being shown to work in at least one animal model, none has been shown to work in humans yet.

The vaccine we tested in a clinical trial is based on two of the papillomavirus nonstructural proteins, expressed in infected cells, but not present in the virus. It is given together with an adjuvant, ISCOMATRIX®, which induces the cytotoxic T cell response needed to cure existing virus infection. This study was a dose-ranging placebo-controlled trial. Vaccine was given to women who already had CIN 3, a precancerous cervical lesion. This vaccine proved to be highly immunogenic. We used skin testing for delayed type hypersensitivity as one measure of cell-mediated immunity. Prior to immunization, the skin test was negative and after immunization, the subjects acquired delayed type hypersensitivity to the E6 and E7 proteins of HPV-16. For trials of therapeutic efficacy, a surrogate marker of efficacy is needed, as we obviously cannot propose to study whether CIN 3, present when subjects are recruited, will progress to cervical cancer. For this study, the surrogate marker that we chose to use was a change in papillomavirus viral load in the cervix. This is a way of measuring the amount of virus present in each cell in the cervix. We observed a reduction in viral load in each of the patients given the active vaccine, and about half of them lost all detectable virus. There was, however, also loss of virus in some of the placebo-controlled patients in the trials, making interpretation of this finding somewhat difficult. Over the 12 weeks that our ethics committee allowed us to watch these women before we had to treat them, no change was observed in the colposcopic appearance of the cervix or in the cervical biopsy histology. This highlights a significant issue for designing future studies of therapeutic HPV vaccines: how long you can observe subjects—without intervention—to determine vaccine efficacy.

In conclusion, evidence from trials of prophylactic HPV vaccines based on virus-like particles suggests that they will be able to prevent HPV infection, probably quite effectively. Therefore, we should be looking at how to plan for the rational use of these vaccines in the future. Early-phase trials of a number of therapeutic vaccines that might be used to treat existing HPV infections are yielding encouraging results, and there are many other potential vaccines that will be subjected to clinical trials. However, the desired health outcome of a therapeutic vaccine has not yet been determined, nor is it known which vaccine, if any, would prove efficacious.

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SUCCESS IN VACCINATING AGAINST *HELICOBACTER PYLORI*

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Just over 20 years ago, in 1982, Barry Marshall and Robin Warren discovered *Helicobacter pylori* in Perth, Australia (*1, 2*). The organism is a gram-negative spiral that lives in the gastric mucosa overlaying the gastric epithelium and produces large amounts of the enzyme urease, which neutralizes gastric acid, and enhances the viability of the organism in the human stomach (*3, 4*).

H. pylori is believed to be one of the most common bacterial infections in humans. When present in the human stomach, it always produces gastritis and, in a subset of infected individuals, plays an important role in the pathogenesis of peptic ulcer disease and gastric cancer. There is evidence to suggest that *H. pylori* has been present in man for at least 2,000 years. The oldest known "patient" is a 1,700-year-old South American mummy—stool samples obtained from the mummy contained *Helicobacter* antigens. Clearly, not only has *H. pylori* been present in South America for thousands of years, but there is genetic evidence suggesting that this organism has been in human stomachs for hundreds of thousands of years. Today, *H. pylori* is a common infection in children in many countries of the Americas. In Brazil and Costa Rica, for example, virtually the entire population of children are infected and have significant gastritis and inflammation in their stomachs. In the United States, about 30% of children carry the infection (*5*) (Figure 1).

Most *H. pylori* infections are acquired in childhood and become lifelong infections if untreated. The risk factors for acquiring this infection appear to be related to overcrowding, such as among children in daycare centers, institutions, orphanages, and foster homes; poor hygienic conditions; and/or low socioeconomic status during childhood (*6*). In addition, in the United States studies suggest that African-Americans and Hispanics appear to be at higher risk for this infection, even when socioeconomic status was similar in all study groups.

Currently, the only known reservoir for this infection is the human stomach. There have been some attempts to identify environmental sources such as water, cats, sheep, and even houseflies for this infection, but the human stomach is the only definitive reservoir for this pathogen (*7*). Transmission of this infection appears to be from person-to-person, and it is oral/oral, fecal/oral, or gastro-oral (*8*).

Gastric and duodenal ulcers have been clearly linked to *H. pylori* infection, and if the infection is prevented or eradicated, these ulcers disappear or do not appear (*9*). Gastric carcinoma also has been associated with *H. pylori*. The data linking *H. pylori* with gastric

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FIGURE 1. Age-specific *H. pylori* prevalence rates in 10-to-20 year olds.

Source: Torres J et al. A comprehensive review of the natural history of *Helicobacter pylori* infection in children. *Arch Med Res* 2000;31:431–469.

carcinoma comes from several sources (*10–12*). An interesting observation is that gastric cancer rates parallel *H. pylori* rates in many countries. Several epidemiologic studies also have demonstrated that there is a three- to eightfold increased risk of developing gastric cancer among *H. pylori*-infected individuals. Finally, the World Health Organization reviewed all these data a number of years ago and classified *Helicobacter pylori* as a type 1 carcinogen, a true cause of cancer.

More recently a study from Japan looked at three different populations of patients (*13*). Uemura and colleagues evaluated 1,246 individuals with documented *H. pylori* infection, and 36 of these individuals developed gastric cancer during the study period. The control group of 280 patients who were *H. pylori* negative did not develop gastric cancer. Of particular interest is the last group of 253 patients that were *H. pylori* positive and were successfully treated with triple antimicrobial therapy—none of them developed gastric cancer. This study suggests that preventing infection, or eradicating a chronic long-standing infection even after many years, can prevent 70% of all gastric cancers, which continue to be a major cancer worldwide (Table 1).

Several other disease manifestations also have been associated with *H. pylori*. Iron-deficiency anemia has now been shown categorically to be associated with *H. pylori* infection. And studies in Alaska and in South Korea have demonstrated that individuals with iron-deficiency anemia resistant to iron therapy can be cured of their anemia simply by eradicating *H. pylori* (*14*). Chronic diarrhea is somewhat controversial, but there is data to suggest that

TABLE 1. Results of a study examining the relationship between *Helicobacter pylori* and gastric cancer, and the prevention of gastric cancer by treating *H. pylori*.

H. pylori status	Sample size	Gastric cancer cases
H. pylori positive	1,246	36
H. pylori negative	280	0
H. pylori successfully treated	253	0

Source: Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, Sasaki N, and Schlemper RJ. *Helicobacter pylori* infection and the development of gastric cancer. *N Engl J Med* 2001;345(11):784–789.

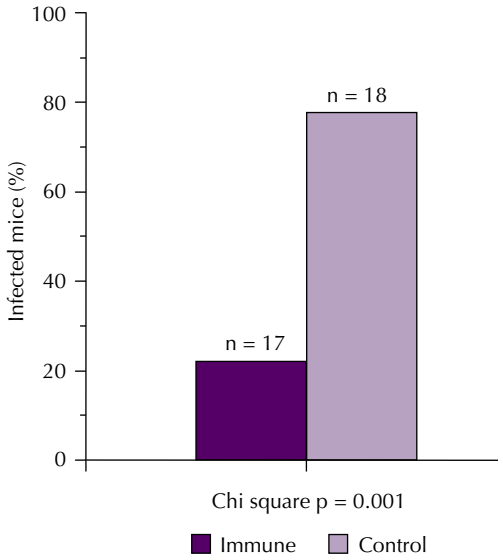
during the early acquisition of *H. pylori*, which occurs in childhood, there is a transient period where the stomach does not make gastric acid allowing other enteric pathogens to infect children and cause diarrheal disease.

The current therapy for eradicating *H. pylori* requires three medications taken for two to four weeks to get acceptable levels of cure (*15*). Unfortunately, there are potential complications with this therapy, the primary one being drug resistance. Not only does *H. pylori* become resistant to the antibiotics, but if a large percentage of the world's population were to be treated with antimicrobial agents in an effort to eradicate *H. pylori*, other pathogens would also become more resistant to these drugs. Finally, eradication of *H. pylori* with antimicrobial agents does not result in long-lasting immunity and such individuals are at risk for reinfection if they live in an endemic region.

Vaccines against *H. pylori* have only been a serious consideration since 1993; therefore most of the studies looking at vaccination against *H. pylori* have been done using animal models, primarily mice. Initial vaccination attempts were oral (mucosal) immunizations in an effort to promote a localized mucosal immune response in the stomach. These were very successful using a simple oral immunization consisting of *H. pylori* bacterial lysate and cholera toxin as a mucosal adjuvant (*16*) (Figure 2).

Over the past ten years several improvements have been made to this approach. There

FIGURE 2. Protection with oral immunization with *H. felis* Sonicate + CT.



Source: Czinn SJ, Cai A, Nedrud JG. Protection of germ-free mice from infection by *Helicobacter felis* after active oral or passive IgA immunization. *Vaccine* 1993;11(6):637–642.

are now a number of purified or recombinant *H. pylori* candidate vaccine antigens that have been used successfully to prevent or cure chronic *H. pylori* infection in animal models (*17*). Newer mucosal adjuvants that have been used also have reduced toxicity in humans. In terms of delivery systems, intranasal and rectal delivery systems have been successfully used, dramatically decreasing the amount of purified antigen that is required compared to oral immunization (*17*).

In order to move these studies to humans, it is important to understand the mechanism of vaccine-induced protection from infection. Initially it was reasonable to assume that mucosal IgA antibodies were responsible for protection with these vaccines. Oral immunization does induce gastric *Helicobacter*-specific IgA and IgG antibodies. In addition, passive immunization accomplished by administering large amounts of monoclonal antibodies into the stomach of infected animals also can prevent

TABLE 2. Role of antibodies in vaccine-induced *Helicobacter* immunity.

Pro	Con
Oral immunization induces gastric *Helicobacter*-specific IgA and IgG.	Antibody titers do not correlate well with protection and only reach significant levels *after* challenge.
Passive gastric or "backpack" administration of *Helicobacter*-specific monoclonal antibodies can prevent infection.[a]	Therapeutic immunization argues against preexisting antibodies blocking infection.[c]
Qualitative changes in antibody specificity are observed after protective immunization.[b]	Protection from infection after immunization of gene-targeted, antibody-deficient mice.[d]

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this infection (*16*). Despite such studies that suggest a protective role for antibodies, immunization of mice that are totally deficient of any antibody production results in excellent protection. Therefore, antibodies do not appear to be required to mediate protection from *H. pylori* infection following immunization (*18*) (Table 2). Although *H. pylori* is not an invasive organism, cell mediated immunity appears to play a key role in protection following immunization. In an early experiment, the transfer of T-cells from a vaccinated animal to one infected with *H. pylori* dramatically reduced the magnitude of infection. In addition, adoptive transfer of TH2 cell lines into infected mice decreased the magnitude of infection and inflammation (*19*, *20*). These studies suggest that protection following immunization is being mediated by the cellular immune system, rather than by the humoral immune system. If, in fact, protection is mediated by the cellular immune system, it may be possible to devise a systemic vaccine to prevent and/or cure *H. pylori* infection. Parenteral immunization using the adjuvant Alum in association with *Helicobacter* antigens primarily results in a TH2 cellular immune response and significant protection from infection, demonstrating that it is

possible to use a systemic immunization to get protection (*21*, *22*). Careful analysis of immunized and challenged animals over time suggests that immunization (oral, intranasal, or systemic) is a viable approach for protecting the host from chronic *H. pylori* infection (*23*).

In summary, over the last 20 years we have learned that protection of mice from *Helicobacter* infection can occur independently of antibodies. CD4 positive T cells are required for protection and CD8 are not required. Vaccination may be used not only to prevent infection but also can be used to eradicate or cure chronically infected individuals. Therefore, based on currently available studies, a systemic (intramuscular) immunization utilizing Alum and *H. pylori* antigens may be an inexpensive and effective method to protect children from *H. pylori* infection and cure adults already chronically infected with *H. pylori*.

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HEPATITIS C

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INTRODUCTION

The hepatitis C virus (HCV) is classified within the *Flaviviridae* family as the *Hepacivirus* genus. Other members of the family include the *Flavivirus* genus and the *Pestivirus* genus. HCV contains a positive-stranded RNA genome of approximately 9,600 nucleotides that encodes a large polyprotein precursor which is cleaved co- and post-translationally to yield individual structural and nonstructural proteins. So far, at least six basic genotypes have been identified phylogenetically, with more than 100 subtypes. This heterogeneity reflects the considerable diversity of the HCV genome, which presents some obvious challenges for vaccine development. For example, within the two envelope glycoprotein genes gpE1 and gpE2, nucleotide diversity is as great as 50% among the different genotypes.

The virus occurs globally, with an estimated 170 million carriers worldwide (*1*). Some of the highest prevalence rates exist in Mongolia and northern and central Africa. Some countries in South America also have high prevalence rates, such as Brazil, which has a seroprevalence of 2.5%–5%. In the United States of America, the prevalence of HCV infection is approximately 1.3%. Genotypes vary according to country. For example, in the U.S., genotypes 1a and 1b predominate, while in North Africa, genotype 4 is prevalent. In China, genotype 1b predominates (Figure 1).

EPIDEMIOLOGY

In developed countries, intravenous drug use is the predominant risk factor for acquiring HCV infection. The U.S. Centers for Disease Control and Prevention has demonstrated that in the U.S., approximately 70% of infections are associated with the sharing of injection needles and syringes (*2*). Other reported risk factors include multiple sexual partners, low socioeconomic status, employment as a healthcare worker (*2*), babies born to mothers with a high viral load or HIV co-infection (*3*), medical procedures involving exposure to infected blood or blood products (*4*), receipt of organs from an infected donor (*5*), and in general, any parenteral exposure to infected blood (potentially, this could include body tattooing with nonsterile equipment, nonsterile ear piercing, and, possibly, the sharing of straws used for snorting cocaine, etc.) (*6*). In developing countries, HCV infection is associated with the same risk factors as in developed countries but in addition, blood transfusion involving donors that are not screened for HCV can be a large risk factor. In contrast, the introduction of HCV-specific immunodiagnostics and nucleic acid testing in the blood banks of developed countries has virtually eliminated the problem of posttransfusion hepatitis C.

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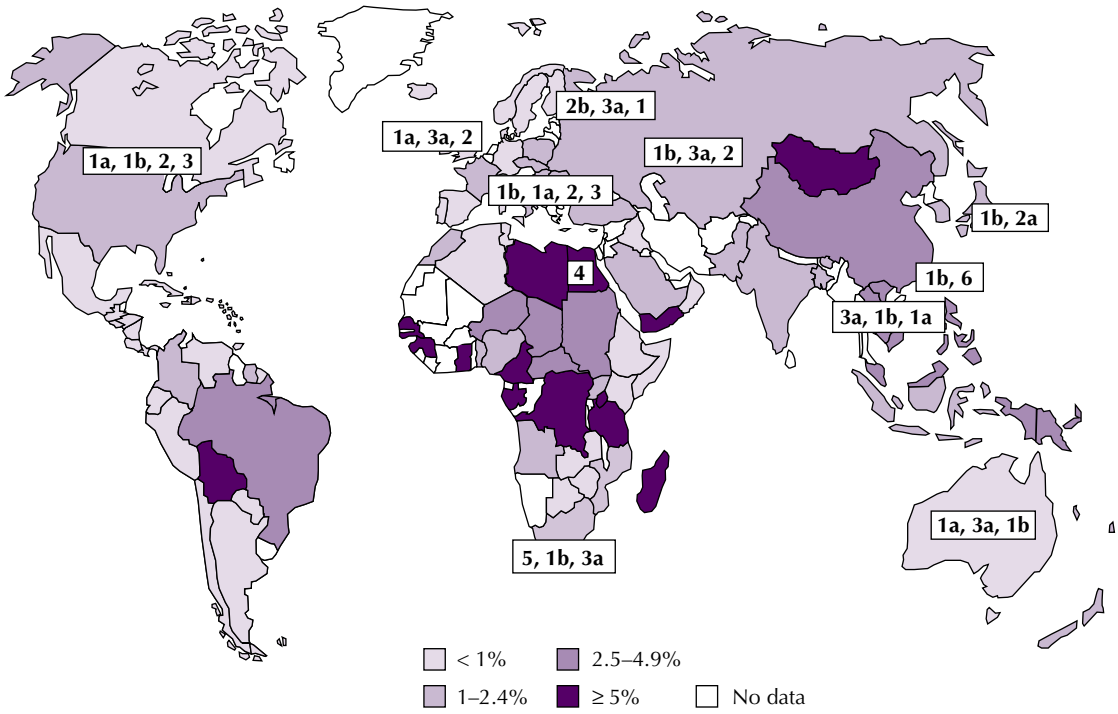
FIGURE 1. Approximate HCV prevalence and genotype distribution.



Source: Ebeling F. Epidemiology of the hepatitis C virus. *Vox Sang* 1998;74(Suppl 2):143–146.

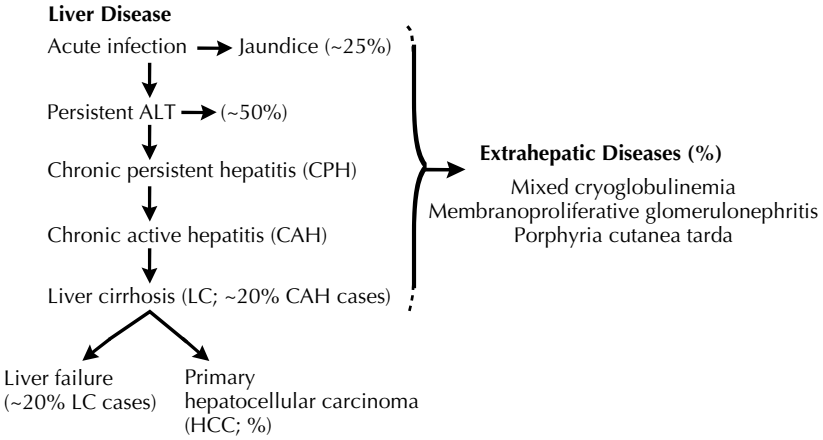
Unfortunately, in many countries, the act of taking blood from volunteers has often led to infection of the donor through the lack of use of sterile equipment. Also, a national campaign in Egypt to control schistosomiasis by mass injections has led to a huge number of HCV infections, again due to the historical use of nonsterile needles and other equipment (*7*).

Natural History

Figure 2 shows the various liver and extrahepatic diseases associated with HCV infection. Acute, recent infection is usually asymptomatic, but in at least 50% of cases, the virus persists for life unless successfully treated under current care guidelines (a combination of pegylated alpha-interferon and ribavirin). It is such persistent HCV infection that can eventually lead to chronic liver diseases such as chronic hepatitis, cirrhosis of the liver, and hepatocellular carcinoma. Although many infected individuals remain symptom-free, a minority can progress—typically after many years or decades of infection—to various forms of chronic liver disease.

It has been estimated that up to 20% of individuals with chronic hepatitis C may progress to some form of liver cirrhosis (*8*). What controls this disease progression is not fully known, although it is clear that alcohol consumption, co-infection with other hepatotropic viruses, and HIV co-infection are all very important cofactors of liver disease (*9, 10*).

FIGURE 2. Summary of potential clinical sequelae of HCV infection.



Source: Houghton M. Hepatitis C viruses. In: Fields BN, Knipe DM, Howley PM, *et al.,* eds. *Fields' Virology.* 3rd ed. Philadelphia: Lippincott-Raven; 1996:1035–1058.

Extrahepatic diseases, such as mixed cryoglobulinemia, glomerular nephritis, and porphyria cutanea tarda, are also closely linked to chronic HCV infection (*11*).

Natural Immunity

Although initial studies in the chimpanzee model of HCV infection indicated a lack of protective immunity to this virus (*12*), very recent studies in both chimpanzees and humans offer strong evidence for a substantive level of protective immunity against HCV. First, several investigators have now shown that chimpanzees that recovered from an experimental HCV challenge were then immune to re-challenge. Importantly, this immunity extended across certain viral subtypes and genotypes (*13–15*). While sterilizing immunity was not developed after the first infection, re-challenged animals showed a clear amelioration of the re-infection, usually leading to an abortive, transient infection (*13–15*). Similarly in man, it has been shown that intravenous drug users that resolved a first HCV infection were 12 times less likely to develop chronic infection following re-exposure than intravenous drug users experiencing their first infections (*16*). Compared with the first infection, re-exposed intravenous drug users experienced reduced viremia and hepatitis indicative of protective, recall immunity. It is noteworthy, however, that as in the case of re-challenged chimpanzees, not all reinfected individuals were able to clear the virus, demonstrating that natural immunity to HCV is certainly not as strong as to the hepatitis A and B viruses. However, this work does indicate the existence of a substantial level of natural immunity to HCV infection, which is therefore supportive of vaccine development.

While there are no well-established correlates of immunity to HCV infection, it has been widely observed that resolution of acute infections in man is associated with early and broad CD4+ and CD8+ T-cell responses to multiple HCV proteins (*17–21*). Anti-envelope antibody titers (as measured in enzyme-linked immunosorbent assay formats) do not correlate with resolution of acute infection (*22*, *23*). However, antibodies to gpE2 that are capable of blocking the binding of HCV to the proposed HCV receptor, CD81 (*24*, *25*), did correlate with rare cases of spontaneous recovery from chronic HCV infection. It should be emphasized that HCV cannot be propagated *in vitro*, and so there is no conventional assay for

viral neutralizing antibodies. Definition of the role of neutralizing antibodies in recovery from HCV infection awaits the development of such an assay. However, various human immunoglobulin preparations containing anti-HCV antibodies have been shown to prevent chronic HCV infection (*26–28*).

Vaccine Approaches

Many years ago, we initiated a vaccine program based on the use of recombinant envelope glycoproteins gpE1 and gpE2 derived from mammalian cells. When co-expressed, the two glycoproteins are translocated into the lumen of the endoplasmic reticulum, where they form a non-disulphide-linked heterodimer tightly anchored to the membrane (*29*). This heterodimer is considered to be a native conformation of the pre-virion envelope (*30*). Following extraction in non-ionic detergent and purification, the envelope glycoproteins—along with oil/water adjuvant compositions—were used to immunize naïve chimpanzees. Various immunization schedules were tested, but typically, vaccinations were administered on months 0, 1, and 7, after which the animals were challenged intravenously two to three weeks later with homologous HCV-1. Five of seven vaccinees were apparently sterilized against this challenge virus, since viral RNA could not be detected at any time in the blood, peripheral blood mononuclear cells, or liver post challenge, using sensitive reverse transcription-polymerase chain reaction methods (*31*). In contrast, all control animals that were similarly challenged became viremic. The two remaining vaccinees became viremic, but only transiently. Resolution of the infection and clearance of virus occurred within a few months. In contrast, seven of 10 control animals became persistent HCV carriers after similar HCV-1 challenges (*31*). The two transiently infected vaccinees elicited lower anti-gpE1/gpE2 titers in response to the vaccine than the five that were completely protected against challenge (in which no virus was ever detected). Complete protection was not related either to anti-gpE1 titers or to antibody titers to the N-terminal, hypervariable region of gpE2, which has been shown to contain virus neutralization epitopes (*32*). However, complete protection did correlate directly with anti-gpE2 titers that blocked the binding of gpE2 to the candidate HCV receptor, CD81 (*25*, *33*).

Five additional vaccinees were also challenged with HCV-1, but all were low responders. Three experienced abortive, transient infections, while the remaining two became chronic carriers of the virus. However, one of the two carriers was the lowest responder of the entire vaccine group, while the other received just one vaccine dose following priming with live recombinant vaccinia virus expressing gpE1/gpE2. In conclusion, out of a total of 12 vaccinees, only two became chronic carriers, in contrast to seven of 10 of the controls ($P = 0.027$), indicating vaccine efficacy.

Further work has now been performed with this vaccine in which chimpanzee vaccinees were challenged with a heterologous virus of the 1a subtype, which is predominant in the U.S. (the vaccine was made from HCV-1 which is also a 1a subtype). In summary, though none of 10 vaccinees challenged with heterologous HCV-H were sterilized against challenge, only one became a chronic carrier of the virus. All the others underwent only transient viremia of approximately one to four months duration. The single carrier also experienced an amelioration of the acute infection in that viral loads and hepatitis were reduced as compared with controls. In contrast, seven of nine control animals receiving a similar heterologous HCV-H challenge became chronic carriers ($P = 0.005$; unpublished data). Since pathogenicity of HCV infection is associated with clinical sequelae of chronic, persistent infection, these data indicate the potential effectiveness of this vaccine in preventing HCV-associated chronic liver disease. Clinical trials with this gpE1/gpE2 vaccine antigen are now under way. Further chimpanzee and human studies are required to establish the extent of cross-protection afforded by this vaccine against other HCV genotypes. Potentially, a cocktail of gpE1/

gpE2 antigens from different genotypes may be required for regional/global protection.

Other vaccine approaches include the use of non-envelope antigens in order to prime a protective cellular immune response. This is a relevant goal considering that recovery from acute infection of humans and chimpanzees has been associated with broad CD4+ T-helper and CD8+ cytotoxic T-cell responses to the virus (*17–21*). Historically, it has proven difficult to prime CD8+ T cells using adjuvanted protein antigens. However, by using the immune-stimulating complex (ISCOM) adjuvant (*34*), we have been able to prime strong CD4+ and CD8+ T-cell responses to recombinant HCV core antigen in rhesus macaques (*35*). This adjuvant is comprised of particles containing cholesterol, phospholipids, and naturally-occurring saponins. When complexed with full-length HCV core derived from *E. coli*, it was possible to elicit specific T-helper and cytotoxic lymphocytes after immunizing macaques on months 0, 1, 2, and 6 with 25–50 μg of core antigen (*35*). The elicited CD8+ responses were longer-lived than responses elicited in other animals using recombinant vaccinia virus expressing HCV core. Currently, attempts to produce a broad T-cell response in chimpanzees by vaccinating with an ISCOM formulation containing more non-envelope HCV protein domains are ongoing. These vaccinated chimpanzees will be challenged with heterologous virus in order to determine prophylactic efficacy. If successful, this type of formulation may be an effective prophylactic on its own or in combination with the above gpE1/gpE2 antigens. Such formulations may also be of therapeutic value for chronically infected patients who typically have very weak cellular immune responses to the virus (*36*).

Conclusions

There is reason to be cautiously optimistic about the development of at least partially effective vaccines against the hepatitis C virus based on evidence for a significant level of natural immunity to HCV infection and the ability to protect vaccinated chimpanzees. The majority of animals vaccinated with recombinant gpE1/gpE2 were protected against the development of chronic infection following experimental challenge with either homologous or heterologous HCV-1a subtypes. Clinical trials are under way with a gpE1/gpE2 vaccine formulation. Future questions to be resolved include the level and durability of protection in man and the degree of cross-protection against other genotypes in this heterogeneous virus genus. A cocktail of envelope antigens, derived from different genotypes, may be required for broad protection. ISCOM-adjuvanted polypeptide vaccine formulations may also be of value both for prophylaxis and for immunotherapy of patients.

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ADVANCES IN INFLUENZA VACCINE DEVELOPMENT

John Treanor[1]

The outlook for influenza vaccine is very good. In fact, the future for influenza vaccine is in the present, for we are on the verge of licensure in the United States and perhaps elsewhere for the first really new vaccine for influenza in the last 50 years.

The influenza virus was identified as the cause of influenza in 1933, and it was shown shortly afterwards that subcutaneous injection of an inactivated form of the virus could induce neutralizing antibodies. By the early 1940s, it had been demonstrated that influenza could be prevented in humans by giving inactivated preparations of virus intramuscularly (*1*), and aside from improvements in production and formulation, our main approach to prevention has not changed substantially since then.

One reason for this is that the vaccine is highly efficacious, especially in healthy adults who can respond well to the vaccine, especially when the match between the vaccine and the circulating epidemic strain is a good one (*2*). There are lower levels of efficacy in some high-risk groups, particularly the elderly, but the vaccine has been shown to prevent complications in those groups as well. Effectiveness has been shown for many outcomes, from death to missed work days (*3, 4*). Therefore, this is a vaccine which clearly works well, is very well tolerated, and whose use should be encouraged as much as possible in a variety of groups.

A recent change is that the list of people for whom the vaccine is recommended has been expanding. Vaccine target groups are persons at high risk of developing complications. The main strategy for use of the vaccine revolves around preventing the influenza complications by targeting the vaccine to high-risk groups. These groups have been identified over a number of years, using epidemiologic studies. They include people 65 years old or older, persons who live in institutional settings where there is a high risk of transmission, and so-called high-risk individuals, such as those with chronic diseases that increase the likelihood that an influenza episode will lead to hospitalization or death.

In addition, the vaccine is used to try to prevent transmission among high-risk individuals, predominantly focusing on their very close contacts—health care workers and family members or other close household contacts.

Several new groups have been added to the list of those targeted for vaccination (*5*). In the United States, it recently has been recommended that influenza vaccine be administered routinely to those aged 50 years or older. This recommendation has been made not because reaching 50 puts a person at higher risk for influenza, but because among those between 50 and 65 years old, there are many high-risk individuals who are not vaccinated

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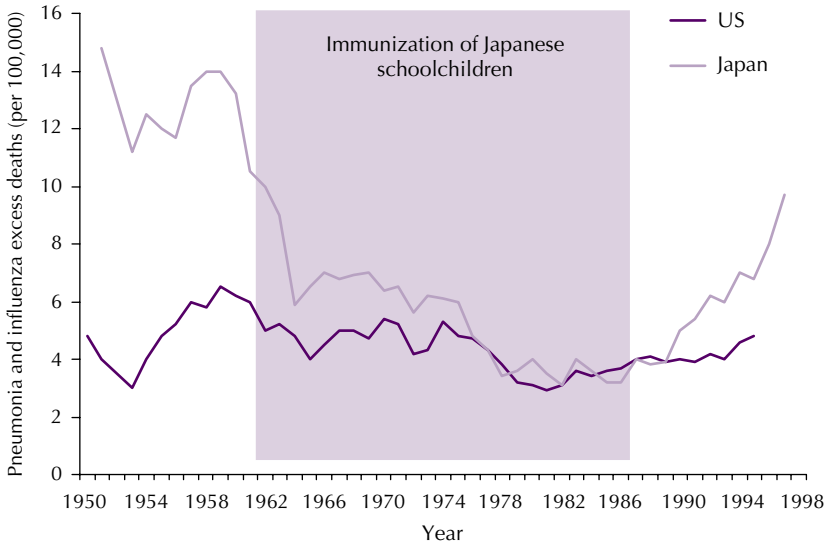
very effectively now. HIV-positive persons have been clearly shown to be at an increased risk for influenza-related complications, and there are studies that show that, if they can respond to the vaccine, the vaccine is both safe and effective in preventing influenza in this group (*6*). Women in their second or third trimester of pregnancy have been identified as being at a much higher risk for influenza-related cardiopulmonary hospitalization. According to studies conducted by Kathy Neuzil and others at Vanderbilt University, pregnant women are hospitalized at a fairly high rate (*7*); therefore, the vaccine is now recommended for pregnant women or women who would be in their second or third trimester of pregnancy during the flu season.

The other interesting issue that has come up, is whether or not influenza transmission can actually be prevented in communities by vaccinating children. This idea has been debated for a while, because of the unique role that children play in a community's transmission of influenza. The issue has been whether or not, by vaccinating children routinely, there can be an impact on transmission to high-risk individuals by preventing the propagation of outbreaks.

In support of this concept are data published in 2001 that looked at the rates of influenza in Japan during a period when influenza vaccine was routinely given to schoolchildren (*8*). After the 1957 pandemic in Japan, schoolchildren began to be routinely vaccinated each year against influenza, and during that time, there was a significant reduction in influenza-related mortality in Japan. The interesting thing is that this reduction in influenza-related mortality occurred in an elderly population that was not being targeted for vaccination (Figure 1). When the policy of school vaccination was discontinued towards the end of the 1980s, the rate of influenza-related mortality in Japan began to rise, suggesting that vaccination in schoolchildren might be an effective way of preventing influenza in the general population.

Although current vaccines are clearly very effective and their use should be expanding,

FIGURE 1. Pneumonia and influenza mortality in Japan and in the United States, 1950–1998.



Source: Reichert et al. The Japanese experience with vaccinating schoolchildren against influenza. *New Engl J Med* 2001;344:889–896

there are still several areas in which vaccine performance could be improved, and there is active development in all of them. These include improvements in production, with an effort to reduce the dependence on embryonated eggs as the substrate for vaccine production, as well as efforts to improve vaccine efficacy, particularly in high-risk groups. In this regard, it has been noted that despite the increased vaccine utilization in such groups, pneumonia and influenza mortality in United States hospitals has not been decreasing substantially (*9*). This suggests that other strategies may be needed to effectively control this problem. We see that the indication for this vaccination might expand to new population targets, particularly children, and, of course, we still feel that we need vaccine strategies to respond to pandemics.

The remainder of the chapter will review some of the new strategies under consideration in influenza vaccine development. Some of these entail considering the use of different doses than the one currently used in the inactivated vaccine, adding adjuvants to the inactivated vaccines, generating the vaccine in substrates other than embryonated eggs, and intranasal approaches that might use both live vaccines and inactivated vaccines.

An issue that has come up in regards to the relative shortage of vaccine in 2000 and 2001, is whether or not it is always necessary to use the currently accepted dose of approximately 15 μg of hemagglutinin. We have looked at what is the immune response to the vaccine at lower doses, and it has been shown that even a reduction to 7.5 μg results in a measurable decrease in immune response (*10*). The ratio of geometric mean titers (GMTs) between a full and a half dose is about 20% better after vaccinating with the full dose, and the difference in those responding again is about a 5% to 10% lower rate in those receiving the half dose (Figure 2). That level of decrease is something that is certainly measurable, although it appears to be relatively small. It might be considered when vaccines supplies are limited.

Influenza vaccines are currently formulated without adjuvants, and there is considerable interest in the possibility of improving vaccine immunogenicity by adding such agents. In this regard, it is important to keep in mind that there is very little room for increased reactogenicity for any adjuvant for influenza vaccine, since this is traditionally one of the main reasons why people don't get vaccinated in the first place. In addition, an ideal adjuvant

FIGURE 2. Immune response differences at different vaccination doses.

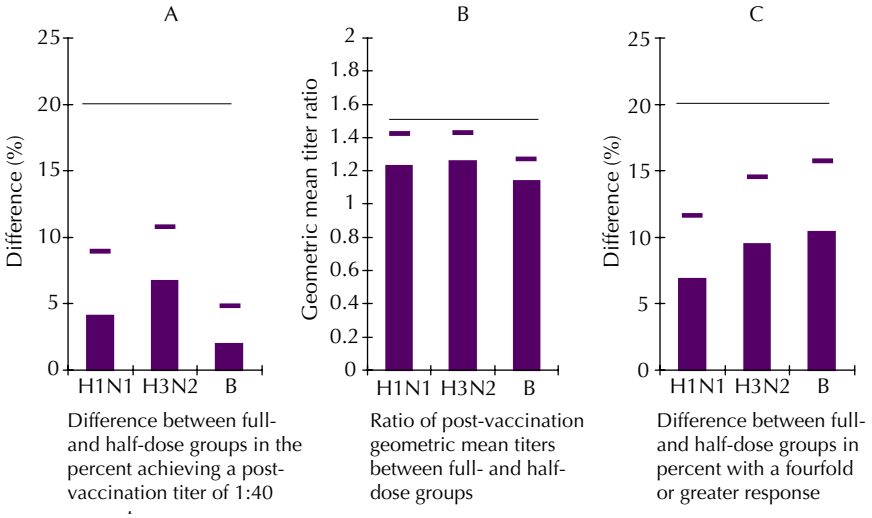
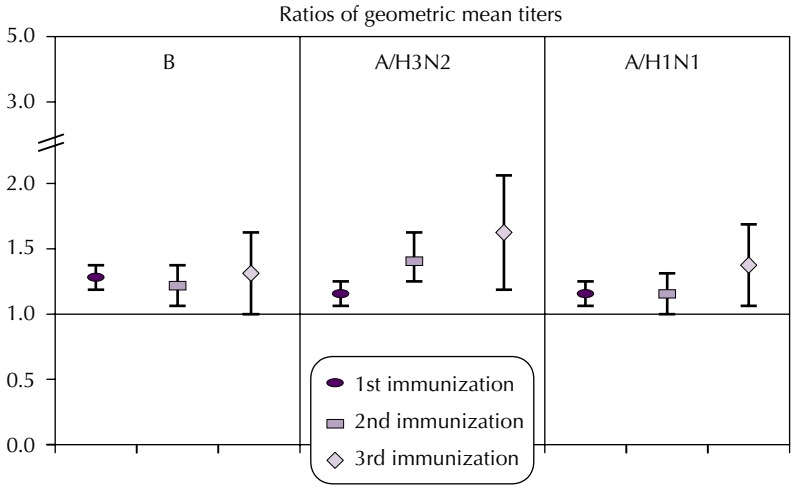


FIGURE 3. FLUAD® immunogenicity meta-analysis: increased immune response in elderly subjects.



Source: Podda A. The adjuvanted influenza vaccines with novel adjuvants: Experience with the MF59-adjuvanted vaccine. *Vaccine* 2001;19:2673–2680.

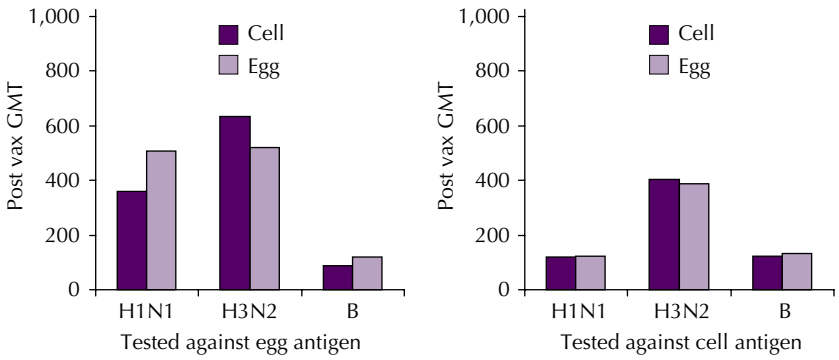
would not result in substantially increased cost.

There have been several studies of potential adjuvants, and more potent reformulations of the vaccine have been performed. The largest clinical experience has been with the recently licensed oil-in-water emulsion MF59. Figure 3 shows data describing the experience with MF59 that was recently presented by Dr. A. Podda (*11*) at the international meeting on infectious diseases in Singapore, which he graciously made available to me. The figure shows the ratio of post-vaccination HI GMTs between adjuvanted and non-adjuvanted vaccines in a group of elderly subjects. It can be seen that for each of the adjuvants that they looked at, the formulation with MF59 resulted in modest but significant response increases.

Current vaccines are generated in embryonated hen's eggs, which have several disadvantages, including somewhat tenuous supply and the potential for selection of avian-like variants in the HA which could potentially have less protective efficacy. Therefore, there has been considerable interest in developing vaccines generated in substrates other than eggs. Figure 4 shows the immune responses to an inactivated vaccine generated in Madin-Darby Canine Kidney (MDCK) cells, a mammalian cell line (*12*). As can be seen, there are very few differences between it and the egg-derived vaccine that could be measured in immune response to vaccination. Both vaccines were equally effective in eliciting antibody as measured by the HI test. One of the interesting things about this study was that these viruses showed no differences whether or not they were tested against egg-derived or cell-derived antigen. In part, this may be due to the fact that the seed viruses had already been selected in eggs, so the possibility remains that an MDCK-cell selected virus could have a different antigenic specificity.

An alternative to using mammalian cells is to use insect cells, with expression by high-yield baculovirus vectors. This is especially attractive in situations where handling the live influenza virus could be dangerous, such as

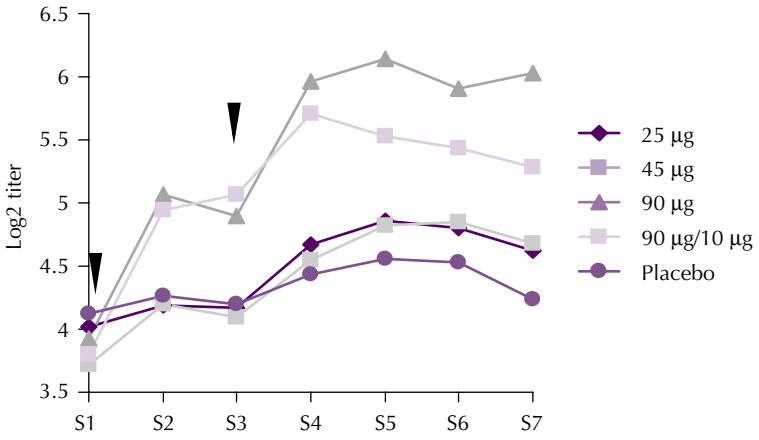
FIGURE 4. Evaluation of immune responses to Madin-Darby Canine Kidney (MDCK) cell-derived vaccine in adults.



Source: Halperin SA, Smith B, Mabrouk T, et al. Safety and immunogenicity of a trivalent, inactivated, mammalian cell culture-derived influenza vaccine in healthy adults, seniors, and children. *Vaccine* 2002;20:1240–1247.

with the recent H5 viruses. When these cases of lethal H5N1 influenza were first reported in Hong Kong, there was immediate interest in whether a clone of the HA gene, expressed in insect cells by recombinant baculovirus, would be an effective approach to an H5 vaccine. When observed in healthy adults (Figure 5), the baculovirus-derived HA was found to be immunogenic, and it induced neutralizing antibodies against the A/Hong Kong/156/97 (H5N1) virus (*13*). However, the vaccine was relatively less immunogenic than it was hoped, and, in fact, it took relatively large doses of antigen to elicit neutralizing antibodies. This is

FIGURE 5. Neutralization titers against A/Hong Kong/156/97 (H5N1) influenza.



Source: Treanor JJ, Wilkinson BE, Masseoud F, et al. Safety and immunogenicity of a recombinant hemagglutinin vaccine for H5 influenza in humans. *Vaccine* 2001;19:1732–1737.

consistent with our previous experience using baculovirus-expressed HAs of more conventional influenza viruses. However, similarly poor immunogenicity for H5 influenza also was reported for more traditional egg-derived vaccines (*14*).

An interesting development is the concept of using an intranasal delivery system for influenza vaccine, which has the advantages of inducing a mucosal immune response. Two approaches have been considered: using live attenuated vaccines and using inactivated vaccine. The use of live attenuated influenza viruses has a long history. The concept was first suggested by A. A. Smorodintsev very shortly after the influenza virus was isolated and after experiments showed that individuals who were experimentally infected with influenza virus developed resistance to reinfection (*15*). After many years of empiric tinkering, the approach that has been used most successfully has been the development of so-called cold-adapted viruses, by John Maassab (*16*). These viruses have been used as master donor viruses. This strategy takes advantage of the natural ability of influenza viruses to undergo reassortment of gene segments, in order to rapidly attenuate new antigenic variants by generating viruses that contain genes that encode attenuation from an attenuated master virus, and the genes encoding the new HA and NA from the wild type antigenic variant (Figure 6).

These cold-adapted reassortant vaccines have been shown to have many desirable properties for a live attenuated vaccine. They exhibit reproducible levels of infectivity and attenuation, which are very important properties when you consider that you need to generate new reassortants every year. In addition, they are not efficiently transmitted to susceptible contacts. They are phenotypically stable, even on prolonged replication in young children, because the genes encoding attenuation are multiple and there are multiple attenuation mutations. However, it's important to bear in mind that the level of infectivity of these viruses depends somewhat on the age of the recipient and the level of prior immunity to influenza virus.

The best data showing the protective efficacy of these vaccines was generated several years ago by Robert Belshe and others, in a study in which children were vaccinated with

FIGURE 6. Rapid attenuation of new antigenic variants by genetic reassortment.

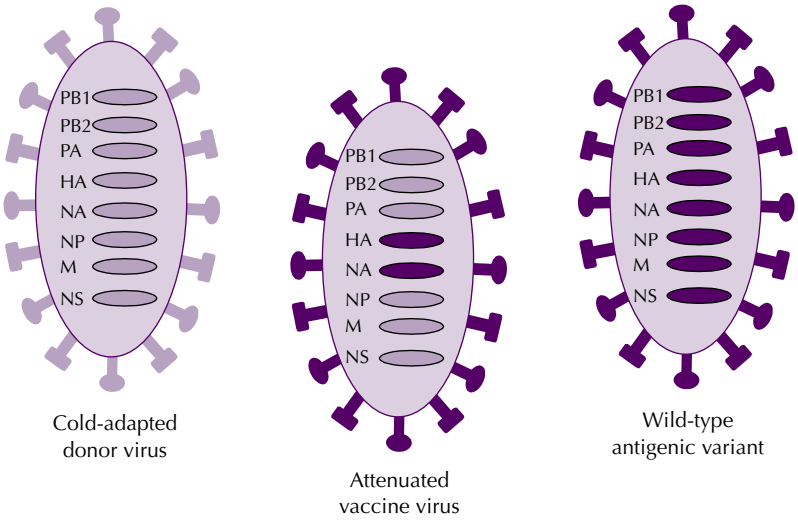


TABLE 1. Protective efficacy of trivalent, cold-adapted influenza vaccine in children.

Group	No. of subjects	No. (and percent) of subjects laboratory documented		
		Influenza A[a]	Influenza B[b]	Either
Placebo[c]	532	64 (12.0)	37 (7.0)	95 (17.8)
Vaccine	1,070	7 (0.7)	7 (0.7)	14 (1.9)

[a] Protective efficacy against influenza A is 95% (CI_{95} 88%, 97%).
[b] Protective efficacy against influenza B is 91% (CI_{95} 79%, 96%).
[c] Six children in the placebo group had both influenza A and influenza B.

TABLE 2. Protective efficacy against the drift variant, A/Sydney/95.

Group	No. of subjects	No. (and percent) of subjects with illness due to influenza A/H3N2 viruses that were		
		Wuhan-like[a]	Sydney-like[b]	Either
Vaccine	917	0 (0)	15 (2)	15 (2)
Placebo	441	4 (1)	51 (12)	55 (12)

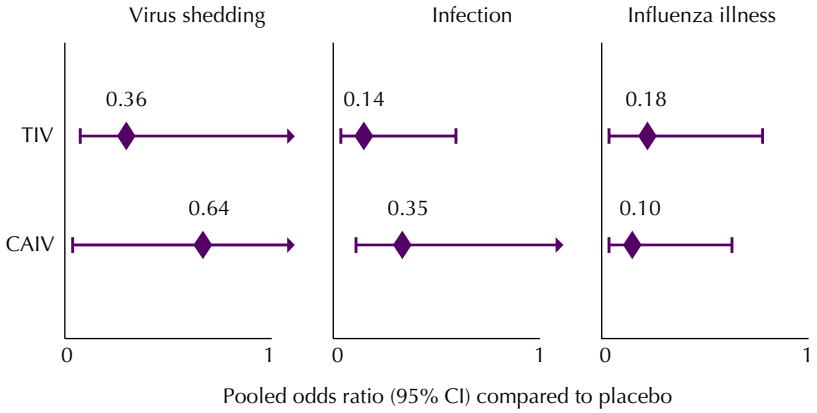
[a] Protective efficacy against Wuhan-like virus was 100% (54%, 100%).
[b] Protective efficacy against Sydney-like virus was 86% (75%, 92%).

wild vaccine or were given intranasal placebo, and then followed for the development of influenza (*17–19*). The vaccine was highly protective against both influenza A and influenza B in the study, with an overall protective efficacy of more than 90% (Table 1). In the study's second year, the children were challenged to an influenza virus that was a significant antigenic drift from the virus that was in the vaccine, and even so the vaccine provided very solid protection against the drifted virus (Table 2). This may be a unique feature of the live vaccine to provide a broader type of immunity that also would protect against the antigenic drifted viruses.

In adults, the data on protective efficacy is not quite as extensive. We have done some studies with a trivalent formulation of the cold-adapted vaccine, comparing it to inactivated vaccine in a model system where protective efficacy is assessed by artificial infection of the volunteers with the wild type viruses. In this study, we looked at either the cold-adapted vaccine or at the inactivated vaccine for the ability to protect against all three of the components contained in the vaccines by doing separate challenges with H1, H3, and B viruses (*20*). Figure 7 shows the pooled results, examining the odds ratio of developing either virus shedding, infection, or influenza illness, which was defined as presence of an infection plus a clinical illness after receipt of either trivalent or cold-adapted vaccine, compared to a placebo. What we saw is that both vaccines were protective. In this model, the inactivated vaccine looks somewhat better than the cold-adapted vaccine, but if you look at the primary end point—the development of influenza illness—these findings are statistically significant.

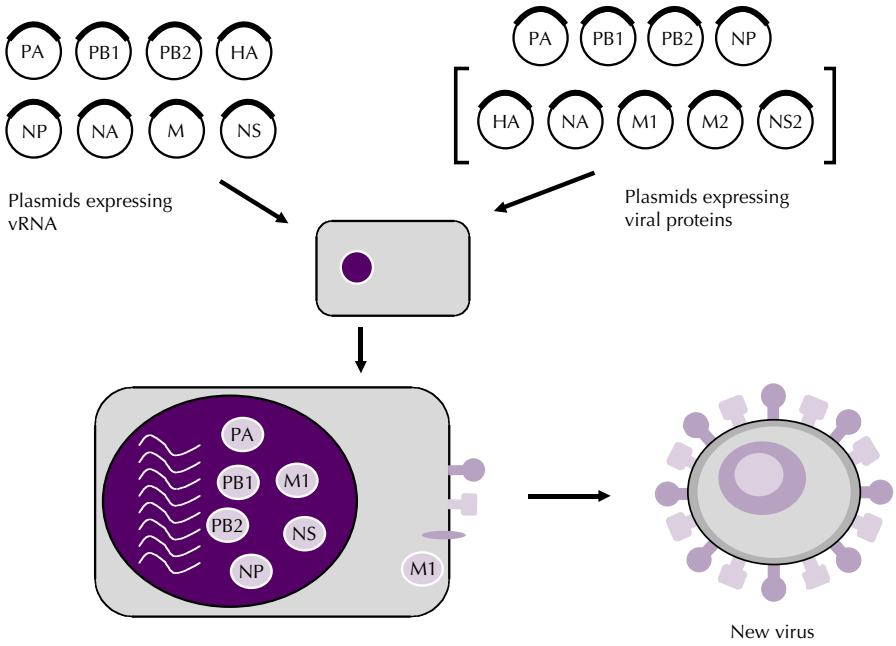
In the elderly, however, these vaccines appeared to be relatively poorly immunogenic, because they don't replicate well in the presence of the prior immunity that older people tend to have. So, we've been looking for alternative approaches. One of these would be the use of reverse genetics techniques to develop vaccines with specific mutations. In the past, this was a very cumbersome procedure that required the construction of artificial gene segments in vitro, but recently a new way of

FIGURE 7. Pooled results of experimental infection studies in adults.



doing this has been developed, involving a plasmid system in which viruses with any mutation one wishes to derive can be made completely from plasmids (*21*) (Figure 8). By eliminating the need for a helper virus, it is now possible to generate vaccines that have a number of mutations. One which has been proposed so far includes vaccine with mutations in the NS1 gene, which has been proposed to be an interferon antagonist (*22*). Other ap-

FIGURE 8. Virus derivation from plasmids.



Source: Neumann G, Watanabe T, Ito H, et al. Generation of influenza A viruses entirely from cloned cDNAs. *Proc Nat Acad Sci U S A* 1999;96:9345–9350.

BOX 1. Intranasal approaches to influenza vaccine.

Live Viruses	Inactivated Vaccine
• Replicating system	• Nonreplicating system
• Induction of all arms of the immune system	• Not as dependent on prior immunity?
• Need to titrate attenuation and immunogenicity.	• Requires adjuvant or formulation.
• Safety: genetic stablity and reassortment	• Safety: nasal irritation, neural?

proaches include viruses in which the NS2 protein has been deleted and which are therefore noninfectious, and viruses with changes in the M2 ion channel or with deleted neuraminidase, all of which look very promising.

As for the inactivated intranasal approaches, it has been known for a long time that if you put inactivated virus in the nose you generate an immune response, although not a very efficient one. Therefore, several adjuvants have been assessed for their ability to stimulate nasal vaccine responses. The one that has received most attention has been the use of cholera toxin, which has been shown to be a powerful mucosal adjuvant. In addition, some data suggest that administration of inactivated vaccine intranasally with cholera toxin generates a form of cross protective immunity that does not depend on HA-specific antibody, and this is not understood very well (*23*). The problem with this approach has been that the beta subunit of these toxins binds very tightly to gangliosides which are present in neural tissue, and are subsequently transported into the olfactory bulb, at least in rodents (*24*). The significance of this finding in terms of toxicity in humans is not clear, but it is a concern for using these toxins for human intranasal approaches.

Various other intranasal inactivated approaches are being considered, including the use of proteosomes or HA molecules formulated with the outer membrane proteins (OMP) of *N. meningitides* (*25*). Preliminary studies in humans have suggested that these vaccines elicit reasonable systemic antibody responses and excellent mucosal responses in healthy adults, and this approach is now in testing in the experimental challenge model by John Oxford, and others (*26*).

Box 1 summarizes the differences between live and inactivated intranasal approaches. The two approaches are similar in many respects, but live vaccines are replicating systems in which the immune response can be amplified by in situ replication. Inactivated vaccines, on the other hand, don't replicate, generally requiring the addition of adjuvants to be immunogenic. Consequently, each approach has a different set of safety concerns. Both ultimately aim at generating a mucosal immune response in the upper respiratory tract, however.

In conclusion, we are still working on multiple paths towards improved performance of influenza vaccines. All these developments are critically important because they prepare us for the next pandemic that may threaten us in the future.

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VACCINE PROSPECTS FOR RESPIRATORY SYNCYTIAL VIRUS

Peter F. Wright[1]

INTRODUCTION

Respiratory syncytial virus (RSV) is a leading cause of respiratory illness in infants, young children, and the elderly. It is estimated that more than two million deaths of children each year are due to acute respiratory infections. RSV's contribution to this toll is approximately 64 million cases and nearly 200,000 deaths. The road leading to the development of a vaccine for the prevention of RSV has been so difficult and the prospects for a vaccine remain so daunting that only the impact of the disease provides the imperative for researchers to continue in their quest.

This chapter will provide background on the significance of RSV as a cause of illness, as well as a brief history of the attempts to develop an effective vaccine and the progress to date.

BIOLOGY

RSV is a single-stranded RNA virus with two envelope glycoproteins that have been the major antigenic targets for vaccine development: the F, fusion protein, and the G, attachment protein. Two subgroups are recognized, RSV-A and RSV-B, that differ most strikingly in their G protein. As a point in favor of vaccine development, there is not the progressive antigenic change over time seen with influenza.

EPIDEMIOLOGY

The public health significance of RSV in the United States has been well-defined (*1*). Globally, lower respiratory tract infections are a leading cause of death in children (Figure 1), and RSV is clearly the single most important cause of severe respiratory illness in infants. RSV is a ubiquitous infection, with three-quarters of infants infected in the first year of life and virtually all by the end of the second year. In the United States, RSV is responsible for an estimated 100,000 hospitalizations and 500 deaths, with medical costs in excess of US$ 300,000,000 per year (*1*).

There is a strong seasonal pattern of isolation of RSV virus, which is prevalent only during the winter months. At Vanderbilt University, in Nashville, Tennessee (U.S.A.), we have conducted surveillance for many years, which has enabled us to produce a reliable composite graph of seasonal illness (Figure 2). There is only small temporal variation in the epidemics from year to year, with the peak occurring in either December, January, or February each year. There are small differences in severity from year to year, as reflected in hospitalization numbers, but a predictable epidemic occurs every year. There are no clues as to the

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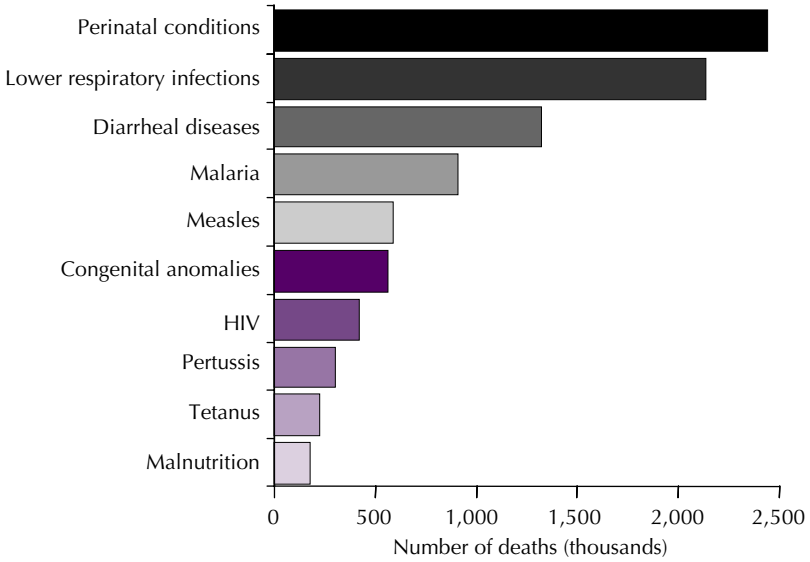
FIGURE 1. Estimated burden of disease in children under 5 years old, worldwide, 2000.



Source: World Health Organization, Global Burden of Disease 2000 Project.

FIGURE 2. Seasonality of respiratory syncytial virus infection, Nashville, Tennessee.

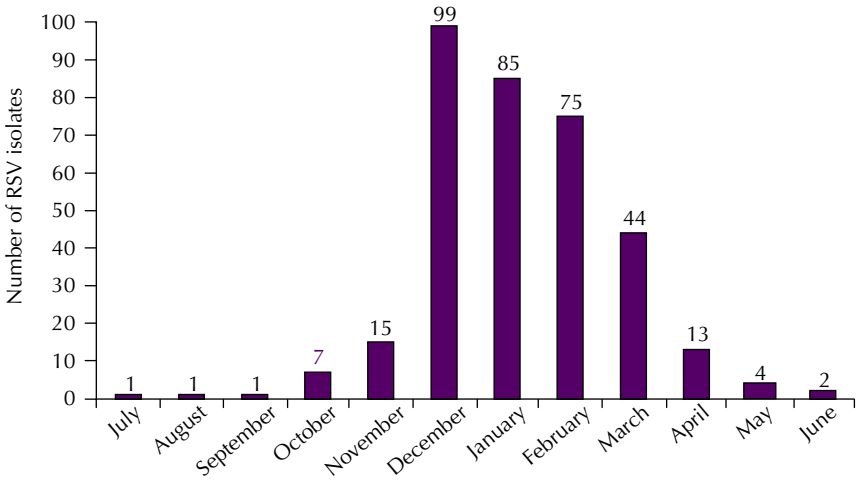


FIGURE 3. Percentage of total upper respiratory infections assignable to culturable respiratory viruses.

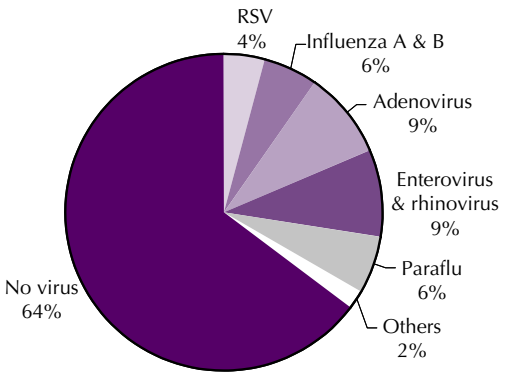
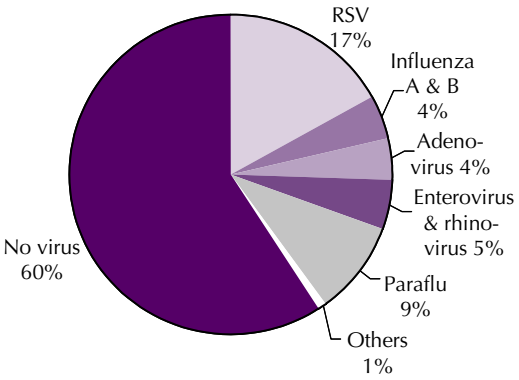


FIGURE 4. Percentage of lower respiratory tract illnesses assignable to culturable respiratory viruses.



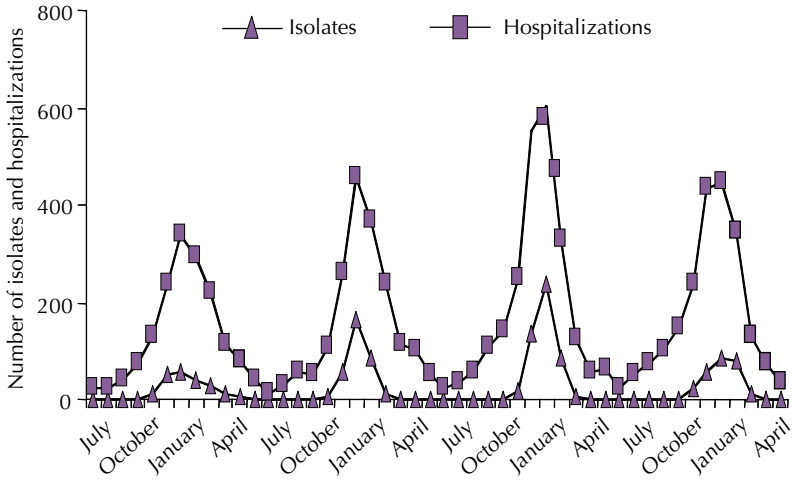
whereabouts of the virus during the summer months. The epidemicity in tropical climates is less well defined. Securing a better understanding of variations in RSV seasonality is one of the objectives in a study conducted by the World Health Organization (WHO) that will be described later in this chapter.

At Vanderbilt, we have looked over a 25-year period at the isolation of respiratory viruses from children with respiratory infections. Figure 3 indicates the isolation of viruses from children with upper respiratory infections. RSV, at 4% of the total, does not appear to be particularly prominent and is comparable to or slightly less than the influenza A and B, adnenovirus, enterovirus, and parainfluenza groups. However, when we examine lower respiratory tract illnesses (Figure 4) we see that RSV has advanced to cause 17% of the illnesses. Notably, we are still left with 60% of patients from whom we have not been able to isolate a virus using traditional tissue culture methodology. It is not clear when polymerase chain reaction (PCR) or newer tissue culture techniques will be able to provide better clues as to the unexplained respiratory illness. However, it is a reasonable postulate that there are new viruses still to be discovered as causes of both upper and lower respiratory tract illness in children and that the current estimate of the disease burden of viruses such as RSV is merely a minimal estimate.

Some of the most impressive evidence for the impact of RSV comes from the remarkable temporal association of isolates of RSV with hospitalizations for bronchiolitis and pneumonia in children (Figure 5). Influenza accounts for some of these wintertime respiratory illnesses, and it is becoming clear that human metapneumovirus can temporally and clinically mimic RSV (2). However, the consistent overlay of RSV isolation and bronchiolitis and pneumonia that fill hospitals in the United States each winter makes the causative association of RSV and severe respiratory illness undeniable.

A WHO-supported study of the incidence of lower respiratory infection due to respiratory syncytial virus in children younger than 5 years old is examining the impact of RSV at four international sites: Indonesia, Mozambique, Nigeria, and South Africa. The seasonality of disease seems quite idiosyncratic with geographically close sites in eastern South Africa and Mozambique having differing seasonality to their epidemics. The overall incidence of severe lower respiratory tract infection, particularly in the first year of life, is higher in the developing country sites than in the United States, but the rates of RSV-associated severe respiratory illness are quite

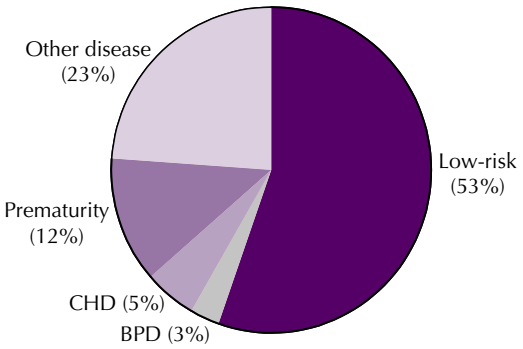
FIGURE 5. Correlation of pediatric hospitalizations for bronchiolitis and pneumonia with isolates of respiratory syncytial virus, Vanderbilt Hospital, representative four consecutive years of data.



comparable. Completed analysis of this data and the detailed studies of Weber and colleagues (*3*) will establish the impact of RSV in the developing country settings. What we believe will emerge from these studies is that RSV is as important a cause of illness in developing countries as in the United States, but that there is an overlay of additional severe respiratory illness in countries such as Mozambique and South Africa which may be bacterial, potentially pneumococcal, disease. The epidemiology and impact of RSV are now being shown to be influenced by HIV infection in countries such as South Africa (*4*).

When RSV-related hospitalizations in the United States are examined, it is clear that RSV causes disproportionately more frequent severe disease in certain high-risk populations (Figure 6). However, when RSV hospitalizations are examined—based on data from the state of Tennessee—53% of the children hospitalized for RSV had no identifiable risk (*5*). As with influenza, hepatitis B, and other illnesses, targeting high-risk people with vaccine approaches or other preventative approaches probably will not have a major impact on the disease as a whole.

FIGURE 6. Underlying illnesses in children hospitalized with respiratory syncytial virus.

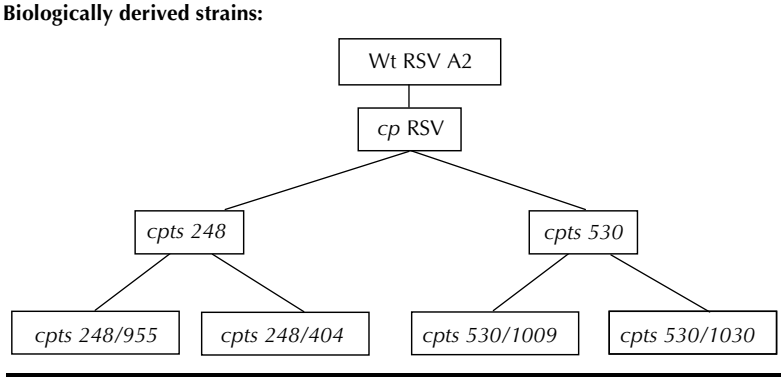


Source: Boyce TG, Mellen BG, Mitchel EF Jr, Wright PF, Griffin MR. Rates of hospitalization for respiratory syncytial virus infection among children in Medicaid. *J Pediatr* 2000;137(6): 865–870.

RSV Vaccine Development

The road to the successful development of a vaccine preventing RSV has been a long one, and while we are still not at its end, promising discoveries and progress have been made. There was an early unfortunate experience

FIGURE 7. Derivation of live attenuated respiratory syncytial virus vaccines.



with inactivated vaccine in which enhanced disease was seen after administration of an inactivated parenteral vaccine (*6*). This led to a great deal of work to try to understand the correlates of immunity and the pathogenesis of enhanced disease in animal models. Operationally it has led us to focus almost entirely on live attenuated vaccine approaches, and, in particular, on intranasal delivery of vaccines.

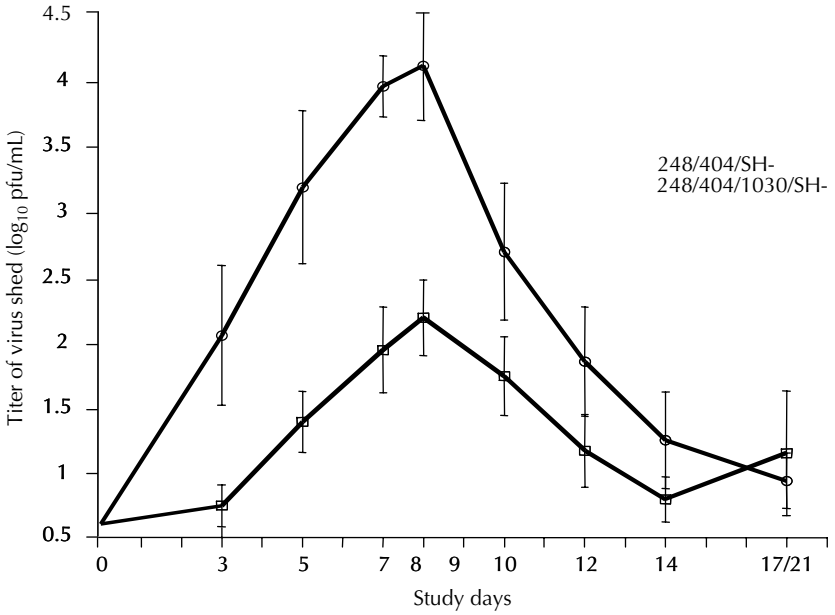
The original vaccine developed was a cold-passaged (cp) RSV virus vaccine which was evaluated in adults and younger children (*7*). At that time we also looked at some temperature-sensitive (ts) RSV vaccines derived by mutagenesis (*8*). This early work established much of the proof of principle of delivery of a vaccine by the nasal route, but the vaccines were either insufficiently attenuated or genetically unstable. Then ensued about 15 years when the vaccine effort was basically in the freezer. Then, an effort was made to go back and further mutagenize the cold-adapted virus, giving rise to the cluster of cpts viruses that appear on the top half of Figure 7. These viruses were comprehensively evaluated in animal models to develop a gradient of increasing temperature sensitivity which correlated with increasing attenuation (*9*).

The horizontal line in Figure 7 represents another very significant advance in our capacity to develop live attenuated RSV vaccines. This was the contribution by Peter Collins of reverse genetics and molecular techniques that allowed the introduction of stabilized mutations and deletion of individual viral proteins (*10*). In the viral genome, there are at least four proteins that have been deleted: NS1, NS2, SH, and M2. Mutations from the original cold passage material labeled "cp" have been kept and others introduced. We now have an array of attenuated vaccines and can examine the process of their evaluation as suitable candidates for human use.

RSV Vaccine Assessment

The evaluation of any vaccine for infants is an extremely time-consuming process. Adults can be assessed relatively quickly, as can seropositive children. We have developed as a criterion for a childhood RSV vaccine that it must not replicate in adults or seropositive children, if

FIGURE 8. Patterns of virus recovery after intranasal inoculation of two respiratory syncytial virus vaccines, 248/404 and 248/404/1030/ΔSH, in seronegative children 4–24 months old.

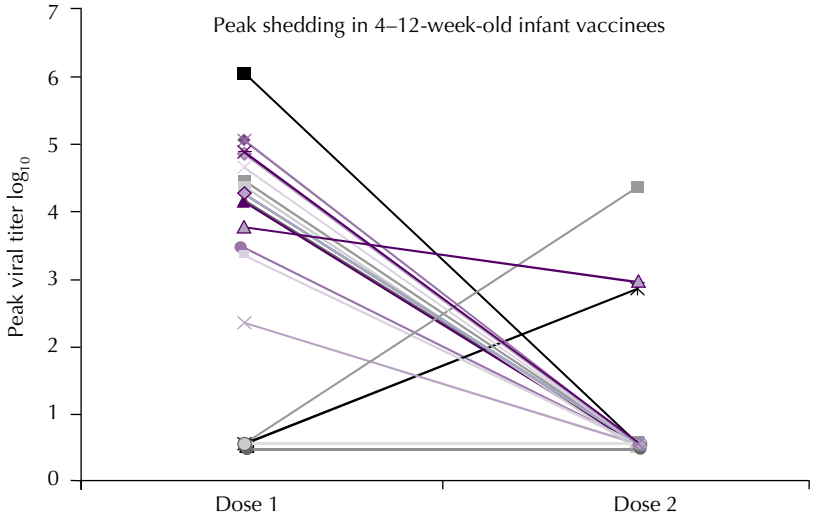


we expect it to be appropriately attenuated for seronegative children. We have seen little that differentiates a child who has been infected with RSV from an adult who has been infected many times with RSV in their resistance to infection with an attenuated RSV vaccine.

We then look at seronegative children 4 to 24 months of age. In this group we see substantial virus shedding that is influenced by the relative attenuation of the vaccine (Figure 8). This is a strong indication of immunity to RSV, since prior natural experience with the virus almost abrogates vaccine virus shedding that is otherwise seen in a seronegative child of 4–5 logs of virus per ml of nasal wash for multiple days. Figure 8 shows the effect of additional mutations in decreasing virus shedding: 248/404, shown as the top line, is further attenuated by the additional deletion of SH and 1030 mutation to form the virus designated 248/404/1030/ΔSH. Both vaccines, 248/404 and 248/404/1030/ΔSH, appeared entirely safe in this age group in moderate-sized phase 1 studies. There was a broad and quite consistent humoral and mucosal antibody response to attenuated RSV vaccines in this age group. Consideration has been given to attempting to license a vaccine, such as 248/404, to limit the impact of secondary RSV infections with resultant otitis media and occasional lower respiratory tract illness.

However, since RSV is a virus that causes its most severe illness within the first three months of life, the target has been for a vaccine that could be given in the first months of life. Two vaccines, cpts248/404 and cpts248/404/1030/ΔSH, have been given in this age group (*11*). With 248/404, mild upper respiratory tract illness of less than one day's duration was seen 8–10 days after vaccine administration. No illness was seen with the more attenuated 248/404/1030/ΔSH. In children 1 month of age the shedding pattern looks almost the same as it does in children 6 to 24 months of

FIGURE 9. Virus recovery with the first and second dose of respiratory syncytial virus vaccine 248/404 in 1–2-month-old infants.



Source: Wright PF, Karron RA, Belshe RB, Thompson J, Crowe JE Jr, Boyce TG, *et al.* Evaluation of a live, cold-passaged, temperature-sensitive, respiratory syncytial virus vaccine in infancy. *J Infect Dis* 2000;182:1331–1342.

age (data not shown). Thus, vaccine virus shedding in the upper respiratory tract is not influenced by the level of maternal antibody. The same observation has been made in natural infection (*12*).

In children 1–2 months old, the immune response was sharply modulated downwards by maternal antibody. Specifically, this age group showed no neutralizing antibody response after the first dose of vaccine. We could detect a serum IgA response to the G protein. Of particular interest was that despite the absence of a neutralizing antibody response to the first dose of vaccine, almost no virus shedding was seen after a second dose a month to six weeks later (Figure 9). The protection when a second dose of vaccine was given appeared to correlate with the IgA response to the G protein (*11*).

Even with the second dose of vaccine, the most consistent response that could be measured was still in the IgA class in the serum, and the second dose did not generate a measurable neutralizing titer. This is not dissimilar to natural infection with RSV in a 1–2-month-old child. Only about 30–50% of children sick and hospitalized with RSV, in spite of exhibiting substantial virus shedding, will demonstrate a neutralizing antibody response to primary infection (*12*).

There were a few children who did not shed virus with the first dose of vaccine who shed virus with the second dose of vaccine, indicating the value of a second dose as a fill-in.

A very intriguing alteration in virulence is conveyed by the NS2 deletion. This protein suppresses the interferon response (*13*). Deletion of NS2 has a very impressive effect in suppressing replication of virus even in seronegative children. Therefore, this deletion is a very attractive direction to continue to pursue.

Through all this developmental pathway we look very carefully, in view of the enhanced illness seen with inactivated vaccines, at all illness seen in post-vaccination surveillance. In all of the experience with live attenuated vaccines there has been nothing that resembled

the enhanced illness seen after inactivated vaccine. In fact, although not specifically sought, there is evidence of protection afforded by these vaccines (*11*).

Summary

RSV has been shown to have a substantial impact in developing countries as well as in the industrial world. Much of RSV's impact is in otherwise healthy children. We believe that live intranasal vaccines remain the most promising approach to prevention. We have powerful tools with which to achieve an appropriate level of attenuation. We have indications from giving second doses of vaccine and from protection shown in surveillance that, although we will not prevent RSV reinfection, we will have a vaccine in the near future that can very substantially modulate the severity of illness and thus prevent the tremendous burden of hospitalization caused by RSV.

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Part IV
The Quest

A NEW GENERATION OF TUBERCULOSIS VACCINES

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INTRODUCTION

Recent advances in genomics and proteomics, and our understanding of the immunology of *Mycobacterium tuberculosis* (Mtb), have led to the development of a new generation of vaccine candidates for the prevention and treatment of tuberculosis (TB). Human clinical studies are beginning for a handful of these new TB vaccines, while characterization of more protective antigens, preclinical testing, and development of new technologies for delivering TB vaccines continues. While it is clear that the widely used BCG vaccine is not effective in preventing adult pulmonary TB in many regions of the world, new clinical investigations of BCG are addressing important questions about immune correlates and comparative immunization of target populations. Together with the creation of clinical site infrastructure, they are laying a foundation for the testing of new TB vaccines in endemic countries. Clinical evaluation of new TB vaccines will need to address many critical issues, such as the use of these vaccines in populations that are infected with Mtb and/or HIV, have active TB disease, or have been vaccinated with BCG. The quest for developing and introducing new effective TB vaccines into countries with the greatest need will depend upon a cooperative effort from many partners in the TB community and, most importantly, the engagement of the health care staff in nations endemic for tuberculosis.

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THE NEED FOR NEW EFFECTIVE VACCINES TO PREVENT TUBERCULOSIS

Along with the need to develop vaccines for malaria and AIDS, finding new, effective vaccines for tuberculosis for the developing world remains one of our biggest challenges. The risk of infection with Mtb and rates of mortality due to tuberculosis are at staggering levels worldwide. Throughout the world about 200 people die each hour from tuberculosis, and there are almost eight million new cases of TB per year (*1*). The AIDS epidemic, poor economic conditions, drug-resistant Mtb, and lack of available treatments are among the factors contributing to these confounding levels of tuberculosis. Although implementation of antibiotic treatment through the "Stop TB DOTS" program has helped control TB in many regions, it is becoming clear that without an effective immunization program it will be difficult to stop the transmission of TB. In support of this claim, modeling studies have predicted that a TB vaccine that is only 50% effective would save thousands of lives in the next 10 years (*2*, *3*).

After a resurgence of TB in developed countries in the early 1990s, there has been a rapid increase in funding for tuberculosis research. One investigative focus has been on the immunopathogenesis of *Mycobacterium tuberculosis*, which has led to the identification of several new antigens that show promise as components for new TB vaccines. The quest for new effective TB vaccines also has galvanized the public health community, and resulted in the formation of new programs to accelerate and coordinate the development of new TB vaccines. New TB vaccine initiatives at the United States National Institutes of Health, at the World Health Organization, and within the structure of the European Union have been launched. Nongovernmental organizations such as the Sequella Global TB Foundation and the Bill and Melinda Gates Foundation also have mobilized resources to facilitate preclinical and clinical testing of TB vaccines. Researchers, clinicians, industrial partners, and health care staff from developing nations have recently convened to map out a strategy for accelerating TB vaccine development (*4*). Among the recommendations from the TB community is the proposal to begin clinical testing of the most promising TB vaccine candidates, while continuing to identify additional novel vaccine candidates via basic research and preclinical testing programs.

Lessons to Be Learned from BCG Vaccine Studies

We can still learn much from our investigation of the BCG vaccine in clinical studies. Many countries immunize with BCG vaccine at birth, and there is convincing evidence that it is effective in reducing TB complications in infants (*5*). A number of efficacy trials have been performed to determine the vaccine's efficacy against preventing adult pulmonary TB (*6*, *7*). Clinical studies such as those performed in the United Kingdom and in the central and western United States have shown that BCG vaccine is greater than 80% effective in preventing TB. Other trials, for example the large efficacy trial in India, have indicated that BCG vaccine is completely ineffective. One interpretation of the differences observed among various BCG vaccine trials is that the vaccine shows less efficacy in populations exposed to environmental bacteria (*8*). In a recent BCG vaccine study, the same BCG vaccine was used to immunize young adults in Malawi and in the United Kingdom (*9*). Although the vaccine was very effective (~80% efficacy) in preventing TB in the United Kingdom, it was ineffective (0% efficacy) in Malawi. Measurement of IFNγ release in PBMCs stimulated with PPD in these study subjects showed a significant difference between pre- and post-immunized subjects in the UK as might be expected for an effective vaccine. However, the pre-immunization cytokine levels in the Malawi study subjects were so great that any increase due to vaccination was masked. These data support the idea that pre-exposure to environmental mycobacteria in the African population interferes with BCG-induced immunity and blocks any benefits of BCG vaccination (*10*).

Experimental evidence for this pre-exposure hypothesis has been provided by Brandt and colleagues (*11*). These researchers demonstrated that guinea pigs that are exposed to mycobacteria other than Mtb (MOTT), including *M. avium*, prior to immunization with BCG vaccine, fail to control growth of Mtb in lung tissues as well as guinea pigs immunized with BCG vaccine only. They also have shown that the persistence of the live BCG in guinea pigs that were pre-exposed to MOTT is significantly decreased and, therefore, effective immunity elicited by the BCG vaccine is reduced. This MOTT-interference hypothesis has important implications for the testing and introduction of new vaccines. It provides experimental evidence to support the long-held idea that revaccination with BCG has little effect in preventing adult TB; in fact, findings of the recent human BCG vaccine revaccination study performed in Brazil (*12*) showed that a second immunization with BCG given to school age children affords no better protection against pulmonary TB than one primary

immunization given at birth. It also has provided evidence for the lack of BCG vaccine efficacy in populations more likely to be exposed to MOTT. The hypothesis also provides justification for the use of non-viable subunit or DNA vaccines for boosting BCG vaccine.

A prime-boost strategy using novel subunit vaccines to boost the BCG vaccine presents a practical tactic for introducing new TB vaccines into the many global regions that immunize with BCG at birth. Brooks and colleagues (*13*) have shown that this strategy can produce effective results in an animal model for TB. Using guinea pigs immunized with BCG vaccine, they found that after boosting with a vaccine composed of purified mycobacteria Antigen 85A together with MPL-A and IL-2 adjuvants at 9 and 15 months, there was significantly reduced growth of Mtb in the lung, compared with animals immunized with BCG or the subunit vaccine only. This prime-boost strategy is currently being tested in phase 1 human clinical studies in the United Kingdom, where individuals previously vaccinated with BCG are being boosted with Antigen 85 expressed from a vaccinia vector (*14*).

TABLE 1. Vaccine formulations likely to be in phase 1 clinical testing by the end of 2003.

Vaccine types	Candidates
Live attenuated Mtb	• Mtb Δ pan[1]
Recombinant BCG	• rBCG + Ag 85[2]
Killed vaccine	• Inactiv. *M. vaccae*[3]
Viral vector	• MVA + A[4]
Protein vaccine	• Ag85-ESAT6[5]
	• 72 fusion protein[6]
DNA vaccine	• Hsp65 DNA[7]

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New TB Vaccine Candidates

In the summer of 2001, the TB community was challenged by WHO's TB Vaccine Advisory Committee to test a minimum of five novel TB vaccines in phase 1/2 studies by 2005 (*4*). Remarkably, by the end of 2003 it is likely that six new TB vaccine formulations will be in phase 1 clinical testing (Table 1). In addition to the vaccinia–vectored Ag85 vaccine mentioned above, clinical testing of two subunit vaccines should soon begin. A fusion protein vaccine consisting of the Mtb39a antigen (*15*) and a 43 kDa antigen, both shown to elicit human T cell responses, is being produced and tested by Corixa, Inc. (S. Reed, personal communication). Another polyvalent subunit vaccine that is likely to be tested in humans soon, is an ESAT6 and 85A antigen fusion construct that has been well characterized by Peter Andersen's laboratory (*16*). Both of these vaccines have been shown to elicit effective TH1-type immune responses, and provide protection against Mtb challenge in more than one animal model for TB. Since these vaccines have been combined with adjuvants for which there is limited human experience, additional safety and toxicity testing will likely be required during human trials. A multi-peptide vaccine containing peptides from mycobacterial antigens that have been selected to contain promiscuous human MHC Class II domains is being conjugated with a novel adjuvant for human testing (*17*). One limitation of this approach is the difficulty in obtaining relevant immunological and efficacy data in animal models for TB, since the peptides have been designed to interact specifically with human epitopes. In this circumstance, a decision to proceed with

human clinical testing will likely have to be made without evidence of vaccine efficacy in preclinical studies. A recombinant live BCG overexpressing Antigen 85 has been shown to be more efficacious than BCG vaccine in a TB challenge model (*18*). Since this is a live BCG vaccine, it may not be useful as a booster vaccine, but could substitute in primary immunization programs if shown to be more effective than the current BCG vaccines. Because BCG vaccine is commonly contraindicated for use in immunocompromised individuals, however, it is unlikely that this vaccine will be used in populations with a high incidence of AIDS. A vaccine consisting of heat-killed *M. vaccae* organisms has been tested, with limited success, as an immunotherapeutic adjunct to antituberculous antibiotic therapy. This killed preparation is currently being studied in a phase 2 clinical trial as a vaccine to prevent TB in HIV-positive patients in Tanzania (*19*).

More than 200 candidate TB vaccines have been triaged through preclinical testing programs including the NIAID, NIH animal testing contract (*20*). As a result, many more intriguing TB vaccines may soon be available for human testing. Among these are live attenuated Mtb strains that have stable gene deletions that lead to substantially reduced virulence while providing effective immunity—for example, Mtb auxotrophs (*21*) or strains lacking the RD1 region, a major deletion found in BCG strains (*22*). The target population and use of these live Mtb vaccines in humans is a matter of controversy but they have been shown to be safe and effective in animal models for TB. It should be noted that there is no accepted process for selecting which TB vaccine candidates should go forward into human clinical testing programs. There also is no set of safety and immunological parameters for determining the success of a new TB vaccine in phase 1/2 human clinical investigations, nor is there criteria for subsequently selecting vaccines for testing in large phase 3 trials. The development of standardized criteria for selecting the best candidates for phase 3 testing remains a great need within the TB vaccine community.

TB Vaccines for Targeted Populations

To be effective at reducing TB disease and transmission, new TB vaccines must do no harm while at the same time elicit protective immunity in a number of different target populations. If used in countries that are endemic for tuberculosis, TB vaccines will eventually be used among the following population groups:

- those already infected with Mtb,
- those who may have active tuberculosis,
- those infected with MOTT,
- those infected with HIV,
- those who have been immunized with BCG at birth, and
- neonates and infants

Possible use of vaccines in individuals that are infected with Mtb or have undetected active disease makes it important to test these vaccines in assays that assess the possibility of vaccine-induced Koch reactions (*23*) or other immunoreactions that may exacerbate disease. Clinical testing of TB vaccines in populations previously immunized with BCG makes it difficult (or probably impossible) to use PPD skin reactivity as a measure of infection with Mtb. New diagnostic tools are needed to distinguish between BCG vaccination and Mtb infection, as well as immunization with the test vaccine. At least three major questions will need to be addressed during the development of TB vaccines for use in endemic countries. First, since BCG vaccine is effective in preventing complications of TB in infants, particularly TB meningitis, how can a new TB vaccine be substituted for the existing BCG vaccine? This problem has implications both for clinical testing of new TB vaccines in pediatric populations and for introduction of a new TB vaccine into regions where low-cost BCG vaccines are supplied by WHO. Second, can a safe, effective post-infection vaccine be developed that prevents adult pulmonary TB? Lastly, can TB vaccines be developed that can be used safely and effectively in HIV-positive populations? It is clear that coordination among many groups

interested in vaccines and public health will be needed to address these challenges. A clinical trials network for TB vaccine testing should be established that incorporates new TB diagnostics and ideas from other vaccine development programs such as malaria and AIDS. Most importantly, vaccine trials should engage health care workers from the endemic countries to provide a consistent and sustained vaccination effort. A new immunization program modeled on the Expanded Program on Immunization also may be needed for the immunization of adults. Developed nations that have more, need to do more. Within the global community, it should be recognized that the failure to discover new vaccines and treatments for tuberculosis occurring largely in poor nations will soon put everyone at risk.

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A NEW POLIO VACCINE?

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INTRODUCTION

Poliovirus is on the verge of being globally eradicated (*1*). The almost total elimination of wild type (*wt*) polioviruses has been achieved through the widespread use of two excellent vaccines: oral polio vaccine (OPV) and inactivated polio vaccine (IPV) (*2*, *3*). Due to this success, the World Health Organization (WHO) has proposed the use of OPV and IPV in the final stages of poliovirus eradication as well as in the containment of a polio outbreak in the posteradication era (*4*). However, based on the following observations, there are compelling reasons for the development of a new polio vaccine. First, outbreaks of poliomyelitis associated with vaccine-derived poliovirus were discovered in Egypt (*5*), Haiti and the Dominican Republic (*6*), the Philippines (*7*), and Madagascar (*8*). Second, it has been documented that individuals who are deficient in humoral immunity can excrete vaccine strains of poliovirus for prolonged periods (from a few months to up to 10 years) (*9*, *10*). These findings clearly indicate that poliomyelitis could re-emerge from OPV-derived viruses in an ever-increasing nonimmune population in the posteradication era.

In view of these possible complications, IPV, which is currently being used in most developed countries (*2*), offers great advantages over OPV. It does not cause vaccine-associated paralytic poliomyelitis, it cannot circulate and thereby lead to vaccine-derived neurovirulent poliovirus variants, and it will not establish persistent infections in persons with immune deficiency disorders (*2*). IPV, however, poses a different risk. The seed viruses currently used for the production of IPV are *wt* strains, precisely the virulent viruses that are being eradicated at great cost. There are documented cases of the re-introduction of *wt* strains from a production facility into the community (*11*). Therefore, *wt* poliovirus could cause a possible catastrophe in the posteradication and postvaccination eras if released accidentally or intentionally from an IPV vaccine production facility into a population with minimal or no immunity to the virus.

Against this background, it is quite apparent that there are risks associated with the use of OPV and IPV either in the final stages of poliovirus eradication or should containment of a poliovirus outbreak become necessary in the posteradication era. It has been argued that

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it is too late to plan for the development of a novel poliovirus vaccine (*10*). Whereas this may hold true for OPV, we argue that the development of highly attenuated substrates for IPV is warranted.

Sabin vaccine strains of poliovirus have been used as substrates for IPV (*12*, *13*). The resulting vaccines, however, showed a lower potency than the conventional IPV (*14*, Dr. K. Chumakov [personal communication]). The most likely explanation for this finding is that the mutations in the coat proteins of Sabin strains that alter the properties of the viral capsid (as compared to the *wt* capsid) also influence production and immunogenicity of Sabin-derived IPV (*15*). One way to circumvent this problem is to generate highly attenuated poliovirus strains without modifying the amino acid sequence of the *wt* viral capsid. In line with this approach, our investigations of the last few years show that the neuropathogenicity of poliovirus can be attenuated by genetic modifications in the 5′ nontranslated region (5′ NTR) without modifying the open reading frame (ORF) of the poliovirus genome (*16–18*). This chapter describes a number of such attenuated poliovirus strain derivatives that can be used as substrates for a new IPV.

Candidate Strains for Inactivated Polio Vaccine

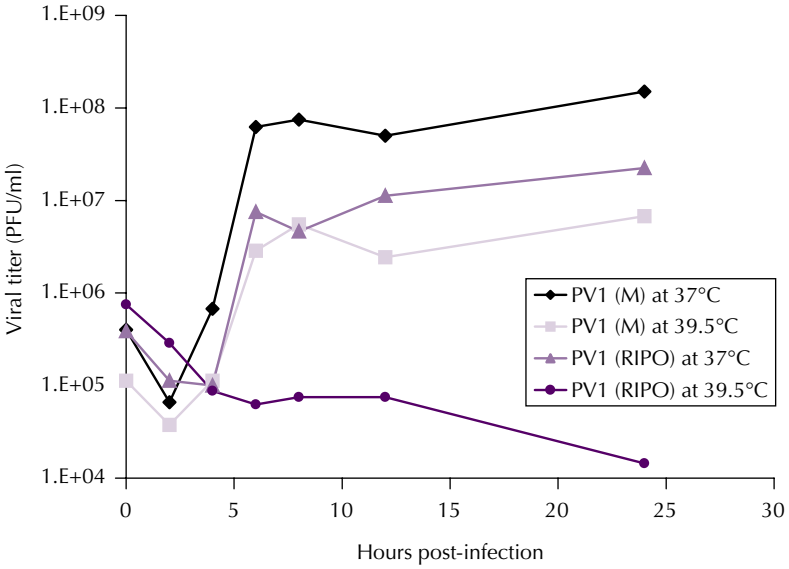
Strains Generated by Exchanging the Poliovirus Internal Ribosomal Entry Site with Its Counterparts from Human Rhinovirus Type 2

Poliovirus employs one of the simplest genetic systems known for proliferation (*3*, *19*). The virus enters the cell after attaching to the cellular receptor CD155 (*20*, *21*). Immediately after entering and uncoating inside the cell, the viral genomic RNA is translated under the control of the internal ribosomal entry site (IRES) (*22*, *23*). The IRES, which binds ribosomes to the viral mRNA independently of the structure of the 5′ end, is part of the 5′ NTR not only of poliovirus, but also of other picornaviruses and hepatitis C virus (*3*, *24*). These genetic entities have been recognized by their function, not by their structure. Indeed, IRES elements of different viruses may have only minor, if any, apparent homology (*3*, *25*, *26*), yet they are interchangeable from virus to virus, leading to novel chimeric infectious viruses (*16*, *27*, *28*).

We have constructed intergeneric poliovirus chimeras, of which PV1 (RIPO) is the prototype (*16*, *17*). In this chimera, the cognate IRES of poliovirus type 1 (Mahoney) [PV1 (M)] was replaced with that of human rhinovirus type 2 (HRV2). PV1 (RIPO) showed reduced growth (*16*) and temperature-sensitive (ts) phenotype (Cello J, De Jesus N, Welker R, Gromeier M, and Wimmer E, unpublished data) in SK-N-MC neuroblastoma cells (a human cell line of neuronal origin) (Figure 1). These results led us to speculate that poliovirus chimeric genomes, translated under the control of the HRV2 IRES, may express considerably decreased virulence in motor neurons. This was confirmed when the neuropathogenicity of PV1 (RIPO), PV1 (M), and Sabin 1 strain were compared in CD155 transgenic (tg) PVR21 mice expressing the human poliovirus receptor (CD155) (*15*). The results showed that PV1 (RIPO) was 100 and 10,000 times less neurovirulent than the attenuated poliovirus strain (Sabin 1) and the *wt* PV1 (M), respectively. In addition, a substantial fraction of mice inoculated with PV1 (RIPO), in contrast to those inoculated with *wt* and vaccine strains, survived and recovered from transient paralysis. Moreover, similar results were obtained when an IRES recombinant between HRV2 and neurovirulent *wt* Leon/37 poliovirus type 3 was tested in the mouse model (*15*).

Finally, a comprehensive neurovirulence testing of PV1 (RIPO) in nonhuman primates was carried out according to WHO's guidelines for neurovirulence assessment of the attenuated vaccine strains of poliovirus (*15*). The mean histological lesion score for the PV1 (RIPO) inoculated-monkeys (0.92 ± 0.42) was similar to the value obtained with monkeys inoculated with the U.S. Sabin neurovirulence reference (0.87 ± 0.38). These results validated

FIGURE 1. One-step growth curves of wild type poliovirus (PV) 1 (M) and PV1 (RIPO) at 37°C and 39.5°C in SK-N-MC cells.

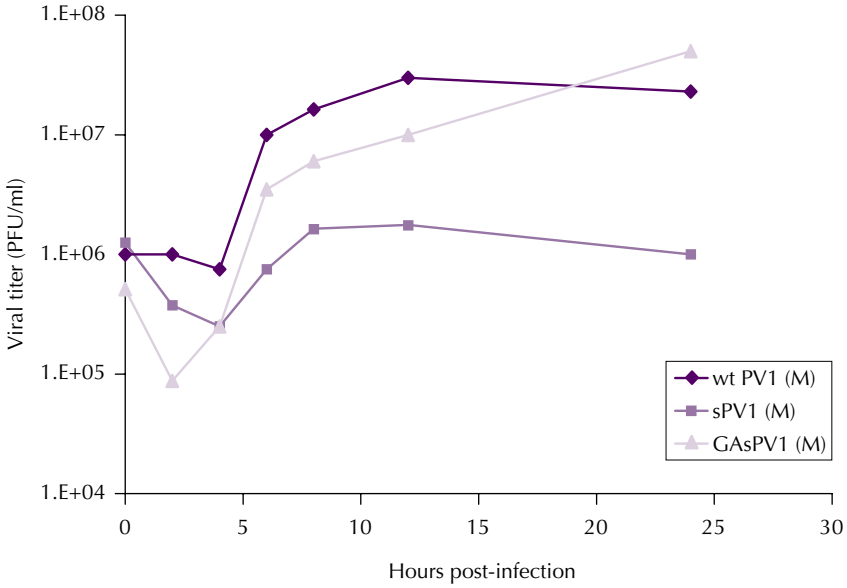


the nonneuropathogenic phenotype of PV1 (RIPO).

Strains Generated by Genetic Alterations of the Region between the Cloverleaf and Internal Ribosomal Entry Site of Poliovirus

Recently, we demonstrated that infectious poliovirus can be generated by *in vitro* chemical-biochemical synthesis (*18*). The assembly of oligonucleotides of plus and minus strand polarity led first to poliovirus-specific double-stranded DNA (complementary DNA, or "cDNA"), roughly 7,500 base pairs in length. To ascertain the identity of the synthesized poliovirus sequence, we engineered 27 nucleotide substitutions into the sPV1 (M) cDNA as genetic markers. The synthetic cDNA was then transcribed with T7 RNA polymerase into viral RNA (*29*). Incubation of the synthetic RNA in a cell-free extract of uninfected HeLa cells resulted in the generation of a synthetic virus with biochemical and pathogenic characteristics of poliovirus. Unexpectedly, however, the synthetic poliovirus [referred to as sPV1 (M)] was 10,000 times less neurovirulent than the *wt* PV1 (M) in the CD155 tg mouse model (*18*). With the exception of one mutation, none of the 27 nucleotide substitutions engineered into the synthetic viral genome resulted in a change of the amino acid sequence of viral proteins. The exception is a mutation mapping to the coding region of polypeptide 2B, where it leads to an amino acid substitution. This change, however, had been previously shown to have no effect on the replication phenotype of poliovirus in tissue culture (*30*). Two of the mutations ($U_{102}A_{103}\rightarrow G_{102}G_{103}$) mapped to a sequence in the 5′ NTR between the cloverleaf and the IRES. Previous studies had determined that these mutations did not influence replication in tissue culture either (*31*). Therefore, it was surprising that the 27 substitutions introduced into the sPV1 (M) genome exerted such a strong influence on the neuropathogenic phenotype of the virus. To identify the mutation (s) that influences the mouse neurovirulence phe-

FIGURE 2. One-step growth curves of wild type poliovirus (wt PV) 1 (M), sPV1 (M), and GAsPV1 (M) at 39.5°C in SK-N-MC cells.



notype of sPV1 (M), we determined the entire nucleotide sequence of virus isolated from spinal cords of paralyzed mice. A comparison of this nucleotide sequence with that of sPV1 (M) disclosed only one change. As mentioned, sPV1 (M) carries the $U_{102}A_{103}\rightarrow G_{102}G_{103}$ substitutions in the 5′ NTR. In virus isolated from the central nervous system of paralytic mice, the 102/103 locus had partially reverted to $G_{102}A_{103}$ (Cello J, Paul AV, and Wimmer E, unpublished data).

When the revertant virus [referred to as GAsPV1 (M)] was inoculated intramuscularly into CD155 tg mice, the animals developed paralysis within three days. A similar incubation period (2.8 days) was observed with animals inoculated intramuscularly with *wt* PV1 (M). In contrast, mice inoculated intramuscularly with sPV1(M) developed neuropathogenic symptoms only after an incubation period of five days. We then isolated virus from *CD155* tg mice that were inoculated intramuscularly with sPV1 (M) and sequenced its genome. Interestingly, a genetic variation at the 102/103 locus was again observed. This time, however, G_{102} had changed to A_{102}, that is, the variation was $G_{102}G_{103}\rightarrow A_{102}G_{103}$ (Cello J, Paul AV, and Wimmer E, unpublished data). Apparently, the $G_{102}G_{103}$ pair at the 102/103 locus attenuates the virus in *CD155* tg mice but exerts little, if any, influence on replication in tissue culture (*18*, *31*). The molecular basis of this startling attenuation phenotype is currently under investigation.

Another significant difference between these virus strains was observed when one-step growth curve experiments were carried out in SK-N-MC cells. Growth of sPV1 (M) was highly impaired at 39.5°C, whereas the revertant strain [GAsPV1 (M)] replicated well and exhibited growth characteristics similar to that of *wt* PV1 (M) (Figure 2) (Cello J, De Jesus N, and Wimmer E, unpublished data).

Altogether, these results indicate that the two nucleotide changes at the 102/103 locus in the 5′ NTR strongly attenuate the neurovirulence of *wt* PV1 (M). Our findings also suggest that these attenuating mutations are unstable upon replication, since all genetic variants isolated from the spinal cord of paralyzed mice

had a (single) nucleotide change in the 102/103 locus. These variants exhibited neurovirulence phenotype(s) similar to that of *wt* PV1 (M). Based on these observations, we think it may be possible to generate a more stable attenuation phenotype by insertions of large nucleotide sequences into the 102/103 locus. To test this hypothesis, we are currently examining a mutant derived from PV1 (M), in which a rescuer cis replication element sequence has been inserted between the cloverleaf and IRES (*32*). Preliminary results showed that this virus is viable and the insert is retained in the viral genome after six passages in HeLa cells.

Conclusions

There are two highly effective and safe vaccines available against poliomyelitis, which have been used successfully for over 40 years. Thus, the development of new vaccines is a questionable pursuit, mainly because of the need to demonstrate safety and efficacy comparable to that of the existing vaccines. However, the possibility that polio could re-emerge in the posteradication era from OPV-derived strains or from the intentional or unintentional introduction of *wt* poliovirus, whether originating from vaccine production facilities or from laboratories, has raised new concerns. Because of these circumstances, it has now become clear that the development of a new polio vaccine should be considered. Based on our experience, we believe that the highly attenuated poliovirus strains combining changes at the 102/103 locus with exchanges of the IRES elements could be developed to lead to the production of a novel IPV. A very important feature of these attenuated viruses is that their capsid proteins retain *wt* sequences. Thus, the immunogenicity of the new attenuated polioviruses should be similar to that of *wt* poliovirus strains. Moreover, isolation procedures and the mode of inactivation of the novel poliovirus strains should also be very similar, if not identical, to those applied to the *wt* poliovirus strains that are currently used for the production of IPV. As it is anticipated that vaccination against poliovirus may cease by 2010–2012 (*33*), enough time may be available for the development of a new IPV, even for the posteradication era. We believe that such a development is highly desirable.

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THE QUEST FOR A PREVENTIVE VACCINE AGAINST HIV/AIDS

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THE URGENT NEED FOR AN HIV VACCINE

Just twenty years after its recognition, HIV/AIDS has become the most important infectious disease; it is the leading cause of death in sub-Saharan Africa and the fourth most common worldwide. From approximately 60 million people who have been infected with HIV since the beginning of the epidemic, 20 million have already died of AIDS, about 3.1 million in 2002 alone. Today, an estimated 42 million people are living with HIV/AIDS, 95% of them in developing countries, especially in sub-Saharan Africa, which is home to more than 29 million of those infected. The average HIV prevalence in the adult population in sub-Saharan Africa is 8.8%. There are seven countries, all of them in the southern cone of Africa, where more than 20% of adults already are infected with HIV.

The epidemics in Latin America and the Caribbean are well established, with an estimated 1.9 million adults and children living with HIV/AIDS (*1*, *2*). Twelve countries in the Region have estimated HIV prevalences of 1% or higher among pregnant women. Adult HIV prevalence rates in several Caribbean countries are surpassed only by those in sub-Saharan Africa, making the Caribbean the second most affected region in the world. Haiti is the most affected country in the Americas, with an estimated national adult HIV prevalence of more than 6%.

Despite intense national and international efforts to control the AIDS epidemic, HIV continues to spread at a rate of nearly 15,000 new HIV infections every day, 95% of them in developing countries. This represented more than 200,000 new infections in Latin America and the Caribbean in 2002 alone. These sustained rates of transmission emphasize the need to develop additional biomedical and preventive tools that are simple, effective, and affordable, such as microbicides and preventive vaccines (*3*, *4*).

CHALLENGES IN THE DEVELOPMENT OF AN HIV VACCINE

The development of HIV vaccines has encountered a number of financial and logistic challenges. These challenges are related to the relatively low level of public and private investment on HIV vaccine research (*4*), as well as to the complexities of conducting multiple human trials, especially in developing countries (*5*). The major obstacles for the development of an HIV vaccine, however, are mostly of a scientific nature (*6*).

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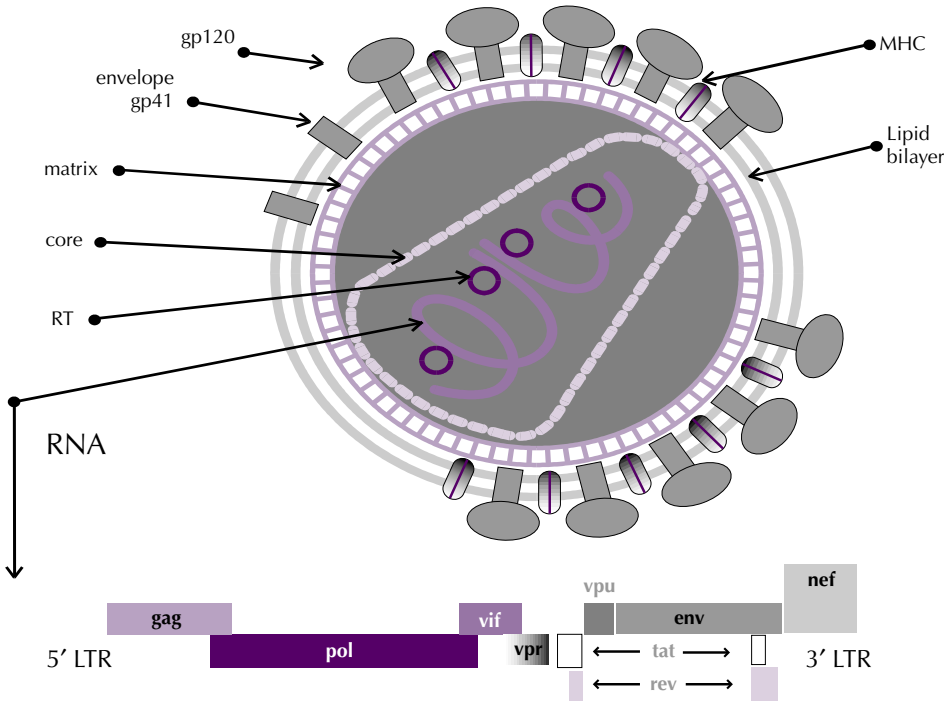
Immune Correlates of Protection

A major stumbling block for the rational development of HIV vaccines has been the lack of information on the immunological correlates of protection against HIV/AIDS. With most vaccine-preventable diseases, naturally occurring (or vaccine-induced) immune responses correlate with protection against infection or disease. In contrast, even though most people infected with HIV develop a broad range of immune responses against the virus, in most cases these immune responses neither control the infection nor prevent progression to AIDS. Natural history and animal protection experiments have failed to produce conclusive results, although most scientists believe that a combination of both humoral and cell-mediated immune responses may be needed for effective protection (which, in turn, could be improved if a mucosal immune component was added) (*7–9*). Ongoing HIV vaccine development strategies are targeted at these two major types of immune responses.

HIV Genetic Variability

Genetic analysis of HIV-1 strains isolated from different parts of the world has revealed that several HIV genes exhibit extensive sequence variability, particularly the *env* gene, which codes for the viral envelope glycoproteins (the gp160 precursor, which is then cleaved into gp120 and gp41) (Figure 1).

FIGURE 1. Structure of the HIV-1 virion and genome.



Note: Each HIV-1 virion has two molecules of genomic RNA enclosed into a protein "core," which is surrounded by a lipid bilayer. Two glycoproteins are associated with the virus "envelope," gp120 and gp41, both derived from a gp160 precursor. The envelope and core proteins are coded by the *env* and *gag* genes respectively, and they constitute the two major targets for vaccine development. The virus genome also codes for a reverse transcriptase (RT) and for several non-structural (regulatory) proteins, two of which (*tat* and *nef*) are also being used as vaccine antigens.

This genetic variability has been used to classify HIV-1 strains into groups and subtypes. Most HIV infections are caused by viruses belonging to HIV-1 group M (or "major"), which, in turn, is divided into at least nine pure genetic subtypes or clades (A–D, F–H, J, and K). Viruses from different subtypes also can recombine among themselves, generating inter- and intra-subtype recombinants with mosaic genomes. The most successful mosaic viruses become established as circulating recombinant forms (CRFs), which are associated with several established or emerging epidemics in different parts of the world (*10*).

Pure HIV-1 subtypes and CRFs have unequal geographical distributions (*11*). Most infections in the world are being caused by subtype C, which is prevalent in southern Africa and India. In addition to subtype C viruses, subtypes A and D and a CRF (CRF02_AG) are causing the epidemic in Africa. In the Americas, most infections are being caused by subtype B, although a number of BF recombinant forms also have been identified in several South American countries (*12–18*), including a CRF (CRF12_BF), associated to heterosexual transmission in Argentina.

Although much is known about the genetic variability of HIV, it is unclear how it could relate to potential vaccine-induced protection. For example, it is not known whether the genetic subtypes define immunological types or whether specific vaccines will need to be designed for each subtype. The results of future human trials with candidate vaccines that are based on different subtypes may provide the answer to that question.

Animal Protection Experiments

Several experimental vaccines have induced different degrees of protection in primate models, including chimpanzees challenged with HIV-1, or macaque monkeys challenged with the analogous simian immunodeficiency virus (SIV) or with SIV/HIV chimeric viruses (SHIV). An important observation is that most experimental vaccines tested in macaques have failed to fully protect against infection ("sterilizing immunity"). Instead, vaccines mediate an attenuation of the infection, with reduction of virus load and slower progression to disease in immunized animals who become infected after challenge. Animal experiments also have failed to provide clear information on potential immune correlates of protection. Moreover, it is unclear whether the animal results will be predictive of vaccine-induced protection in humans. Such information will only be obtained from human trials.

Evolution of Vaccine Paradigms and Clinical Trials

Despite the scientific uncertainties discussed above, a number of candidate vaccines have been developed in the laboratory and are being tested in animal models. The most promising products have also moved to clinical trials in humans (*19*). The first phase 1 trial of an HIV vaccine was conducted in the United States in 1987. Since then, more than 10,000 healthy human volunteers have participated in more than 80 phase 1/2 trials of more than 30 different candidate vaccines. Various vaccine approaches (or vaccine concepts) have been tested in three successive overlapping "waves," which have been dominated by different vaccine development paradigms (*4*) (Box 1).

First "Wave": Induction of Antibodies

The first "wave" of HIV candidate vaccines and clinical trials was based on the concept that antibodies would be sufficient to confer protection. It resulted in the design of candidate vaccines based on the envelope glycoproteins of HIV (especially gp120) or on synthetic peptides representing the V3 loop of gp120. The first generation of envelope vaccines involved mainly monomeric molecules based on laboratory-adapted strains of HIV (X4 strains) produced by genetic engineering in mammalian cells (*20*). With the elucidation of the co-receptor use by different strains of HIV-1,

BOX 1. The three "waves" of HIV vaccine paradigms and clinical trials.

First "wave:" Induction of neutralizing antibodies
Recombinant gp160 produced in a baculovirus system
Recombinant gp160 produced in mammalian cells
Recombinant gp120 produced in mammalian cells
Synthetic V3 branched peptides
Recombinant V3 protein produced in bacterial system

Second "wave:" Induction of cell-mediated immunity
Vaccinia vectors
Canarypox vectors (ALVAC-HVI)
Attenuated modified vaccine Ankara (MVA) vectors
DNA constructs
Lipopeptide constructs
First generation BCG vectors
Prime-boost combinations (live vectors and envelope antigens)

Third "wave:" Better and broader immune responses
Adenovirus vectors
Venezuelan equine encephalitis replicons
Fowlpox vectors
Vesicular stomatitis virus vectors
Adeno associated virus (AAV) vectors
Yeast vectors
Second generation BCG vectors
Salmonella vectors
Different novel DNA constructs
Novel multi-epitope peptides
Novel envelope protein constructs
Regulatory proteins (*tat, nef*)
Multiple prime-boost combinations of some of the above

novel envelope candidate vaccines also included in their design envelopes from primary isolates (R5 strains) of HIV (*21*).

Envelope-based candidate vaccines were found to be safe and immunogenic in diverse populations, inducing neutralizing antibodies in essentially 100% of the volunteers, but not cytotoxic T lymphocytes (CD8+ CTL). A limitation of the existing envelope vaccines is that the antibodies they induce are mostly directed to laboratory-adapted strains of HIV, with weak or no ability to neutralize primary isolates. In addition, reflecting the variability of the gp120 molecules, those neutralizing antibodies are subtype-specific, with little cross-reactivity with other subtypes.

Second "Wave": Induction of Cell-mediated Immunity

The second "wave" of HIV vaccine research started in the mid-1990s, with the recognition of the importance of cell-mediated immune responses in the control of HIV infection (*22*). This paradigm led to the development (or refinement) of live recombinant viral vectors, especially poxvirus vectors, capable of delivering HIV-1 antigens in the context of the MHC

class I pathway. The antigens expressed by these candidate vaccines include products of the *env* gene, but more specifically from *gag* and from two of the regulatory genes of HIV-1 (*tat* and *nef*). Prime examples of this approach have been the different constructs of replication-deficient canarypox-HIV recombinant vectors, collectively known as ALVAC-HIV from Aventis-Pasteur (*23*). Other more recent candidate vaccines being developed under the cell-mediated immunity paradigm include different types of DNA constructs (*24*), vectors based on the attenuated modified vaccinia Ankara (MVA) (*25*, *26*), and lipopeptides (*27*).

Different ALVAC-HIV constructs have been extensively tested in clinical trials, usually in prime-boost regimens together with gp120 vaccines (*23*). These trials have shown that the prime-boost combinations are safe and well tolerated, inducing proliferative responses (mostly to gp120) in 50%–100% of the volunteers. Binding antibodies to gp120, and neutralizing antibodies to the HIV-1 MN strain, are induced in essentially 100% of the volunteers, although little or no neutralization of primary HIV isolates has been detected. The prime-boost regimens also are capable of inducing CTL responses to different HIV-1 proteins in 15%–20% of the volunteers at any one time, with different estimates of cumulative responses over time. Those trials have shown that some vaccinated volunteers develop cross-reactive CTL responses against different HIV-1 subtypes, and this provides some encouragement regarding the possibility of developing broadly protective vaccines (*28*, *29*).

Third "Wave": Better and Broader Immune Responses

The third "wave" of HIV vaccines began with the new century, and it should see much work aimed at optimizing immune responses by existing, or yet to be developed, candidate vaccines. The goals of this new "wave" of HIV vaccine research are to develop candidate vaccines that can induce antibodies capable of neutralizing primary (R5) strains from all HIV-1 subtypes and/or high levels of long-lasting, cross-reactive CTL responses against different HIV-1 structural and regulatory proteins. In fact, some believe that a successful vaccine against HIV will need to "stimulate the innate immune system, generate high levels of neutralizing antibodies, strong cellular immune responses, and mucosal immunity" (*30*). Not an easy challenge to meet!

A range of novel candidate vaccines is being developed to meet that challenge, and some already are moving to human trials. One of these novel candidate vaccines is represented by a replication incompetent Adenovirus type 5 vector expressing *gag* (being developed by Merck), which in a DNA-prime/Adenovirus-boost regimen in the SHIV/macaque model induced high levels of CTL, resulting in marked attenuation of infection after challenge (*31*). Phase 1 clinical trials of both the DNA and Adenovirus type 5 vector, alone or in prime-boost combinations, are ongoing. In addition, results from primate experiments indicate that a heterologous prime-boost regime, using the Adenovirus type 5 vector followed by an ALVAC-HIV vector, is capable of eliciting high levels of antiviral T-cell responses. This approach will soon enter phase 1 clinical evaluation (*32*). Other novel candidate vaccines already in clinical trials include different DNA constructs containing *gag* and *pol* from clade B and *env* from clades A, B, and C (being developed by the Vaccine Research Center of the U.S. National Institutes of Health) (*33*, *34*), DNA-MVA combination regimes (*25*, *26*, *35*), and a combination of gp120 and NefTat fusion protein formulated in the clinically tested adjuvant AS02A (from GlaxoSmithKline) (*36*).

Other candidate vaccines under preclinical development include different configurations of HIV envelope glycoproteins (*37–40*), multiepitope immunogens based on multiple Th lymphocyte epitopes (*41*), Tat-based vaccines (*42*, *43*), and a number of novel bacterial and viral vectors, including salmonella and shigella (*44*), bacillus Calmette-Guerin (*45*), fowlpox virus (*46*), vesicular stomatitis virus (*47*), and Venezuelan equine encephalitis replicons (*48*).

Of course, research continues into development of more effective prime-boost combinations, the use of cytokine adjuvants (*49*), and different delivery systems. Results from clinical trials with these novel candidate vaccines will be eagerly awaited.

Clinical Trials in Developing Countries

Clinical trials in developing countries are necessary because:

- the vast majority of HIV infections are occurring in these countries, where an effective vaccine is most needed;
- phase 3 trials need to be conducted in populations with high HIV incidence, many of which are in developing countries;
- the variability of HIV may necessitate testing of candidate vaccines in different areas of the world where different subtypes and strains are prevalent; and
- it may be necessary to evaluate how different routes or cofactors of transmission and host genetic background could influence vaccine induced protection.

The first HIV vaccine trial in a developing country was conducted in 1993 and, since then, 20 phase 1/2 trials and one phase 3 trial have been conducted in developing countries (Table 1).

The first trial was conducted in China with a synthetic peptide vaccine representing part of gp120 (the V3 loop), and it was rapidly followed in 1994 by additional trials with the same candidate vaccine in Thailand and Brazil, two of the countries with WHO-sponsored National AIDS Vaccine Plans (*5*, *50*). Most of the subsequent trials conducted in developing countries between 1995 and 2000 were done in Thailand, testing different envelope vaccines based on gp120 from B and E subtypes of HIV-1 (*21*, *51*, *52*), one of which entered phase 3 trial evaluation in 1999 (*53*). In the meantime, other developing countries also initiated phase 1/2 trials and in 1996, a multi-epitope polypeptide V3 candidate vaccine was tested in Cuba (*54*).

The second "wave" of HIV vaccines reached the developing world in 1999, when Uganda, another country with a WHO-sponsored National AIDS Vaccine Plan, conducted its first (U.S.-NIH sponsored) clinical trial with the already well-studied subtype B ALVAC vCP205, at a time when cross-subtype CTL reactivity was being recognized (*55*). Since then, two series of ALVAC-HIV prime-boost phase 1/2 trials have been conducted (or are being conducted) in Thailand and in the Americas (Brazil, Haiti, Peru, and Trinidad and Tobago) with candidate vaccines based on E or B subtypes of HIV-1. Another vaccine concept that is being evaluated since 2001 in two African countries (Kenya and Uganda) is driven by the International AIDS Vaccine Initiative (IAVI) and is based on a prime-boost combination using DNA and MVA candidate vaccines expressing a number of genes from a clade A HIV-1 strain (*25*, *26*, *56*).

TABLE 1. HIV vaccine trials in developing countries.

Year(s) of initiation	Candidate vaccines	HIV subtype	Countries
1993–1996	Envelope-based candidate vaccines (gp120, V3 peptides, and V3 protein)	B	Brazil, China, Cuba, and Thailand
1997–1998	Envelope-based candidate vaccines (gp120)	B, E	Thailand
1999–2002	Canarypox and modified vaccinia Ankara vectors, DNA constructs, and prime-boost combinations	B, E, A	Brazil, Haiti, Kenya, Peru, Thailand, Trinidad and Tobago, and Uganda
2003 (proposed)	Multiepitope DNA vaccine, Adenovirus *gag* vector	B	Several countries in Africa, Asia, and the Americas

Additional phase 1/2 trials are expected to start in 2003 in Botswana, using a multiepitope DNA candidate vaccine from Epimmune (*41*), and in several developing countries in Africa, the Americas, and Asia, with the clade B Adenovirus type 5 vector from Merck.

Efficacy Trials of HIV Vaccines

As discussed above, large-scale phase 3 trials represent the only way to assess the efficacy of candidate vaccines for preventing infection or disease. The first phase 3 trials of an HIV candidate vaccine were initiated in North America in June 1998 and in Thailand in March 1999, using two different versions of bivalent gp120 candidate vaccines based on locally prevalent subtypes of HIV-1 (BB for the trial in North America, and BE for the trial in Thailand), produced by VaxGen (*21*, *53*). The North American trial, which involved sites in Canada, the Netherlands, and the United States, enrolled 5,095 men who have sex with men and 308 women at higher risk of HIV infection. The trial in Thailand enrolled 2,545 volunteers, all of them recovering injecting drug users, and is being conducted in collaboration with the Bangkok Metropolitan Administration and the U.S. Centers for Disease Control and Prevention (*57*). The North American/European trial was completed at the end of 2002 and the trial in Thailand will be completed at the end of 2003.

Preliminary Results from the First Efficacy Trial

Preliminary results from the phase 3 North American/European trial of the gp120 BB manufactured by VaxGen were announced in February 2003. The study showed that the candidate vaccine was ineffective overall, with the rate of infections in the vaccine group not significantly different from that in the placebo group. However, a preliminary subset analysis of less than 10% of the enrolled volunteers suggested vaccine efficacy among black volunteers, and these results do not appear to have been due to faulty randomization of the volunteers. Preliminary data also suggest that women produced higher levels of antibodies than men, and that vaccinated volunteers preferentially excluded viruses resembling vaccine antigens (virus sieving) (*58*). What is not clear at this time (April 2003) is whether those results are statistically correct and significant. A careful evaluation of all the data will be needed to make decisions regarding any future work with this candidate vaccine.

Results from the ongoing phase 3 trial in Thailand with the VaxGen gp120 BE candidate vaccine, expected by the end of 2003, may provide some additional information relevant to the gp120 vaccine concept. It is important to recognize, however, that because the Thai volunteers have different routes of HIV-1 transmission than the North American/European volunteers, it might not be appropriate to extrapolate results from one population to another.

The Next Efficacy Trial

The next phase 3 trial also is planned to be conducted in Thailand, as a collaboration between the Thai Ministry of Public Health, the United States Military HIV Research Program, and the United States National Institutes of Health. The trial is planned as a community-based trial, involving 16,000 volunteers in the Rayong and Chon Buri provinces in central Thailand, to assess the efficacy of a prime-boost combination using an ALVAC-HIV clade E recombinant vector (vCP1521, from Aventis-Pasteur) and gp120 BE (from VaxGen). Results from this trial would be available in 2008.

Future Efficacy Trials

In order to accelerate the development and future access to HIV vaccines, it is essential to increase efforts to move additional candidate vaccines to clinical trials, including phase 3 trials. There is a special urgency to develop and test vaccines relevant for use in Africa and other heavily affected areas of the world. With the presently available resources, a potential

best-case scenario is that three candidate vaccines could move to phase 3 in 2004–2005, including a clade B Adenovirus-HIV-*gag* vector (being developed by Merck); a multiclade (A,B,C) prime-boost combination of DNA and an Adenovirus-HIV vector (being developed by the Vaccine Research Center of the U.S. National Institutes of Health); and a clade A prime-boost combination of DNA and an MVA-HIV vector (being developed with support from IAVI).

Future Access to HIV Vaccines

Early planning is essential to ensure that future effective HIV vaccines are made available to all populations in need without unnecessary delay. For this purpose a number of actions must take place including the identification of policies and strategies for vaccine introduction and use in different communities, countries, and regions, as well as the development of estimates of needs and probable vaccine uptake according to different estimates of vaccine efficacy (*59*, *60*). Of special importance would be to ensure that the introduction of a future vaccine is coordinated with, and be complementary to, the overall HIV/AIDS prevention effort.

Current Challenges and Lessons Learned

Two major questions remain to be answered in the area of HIV vaccine research:

- Are any of the current candidate vaccines protective? and
- Can we do better in vaccine design?

Answering the above questions will require a systematic approach to identify the specific challenges, lessons learned, and potential ways to move forward. It is necessary to:

1. Do more basic and clinical research to rationally develop novel vaccine concepts and candidates;
2. Conduct multiple trials with different vaccine candidates, to obtain information on the type of immune response(s) required for protection (antibodies, CTL, helper T cells, or combinations of immune responses);
3. Develop, standardize, and validate better laboratory assays to evaluate vaccine-induced immune responses;
4. Design clinical trials with the appropriate sample size and end-points to obtain information on the efficacy of candidate vaccines in preventing infection, progression to AIDS, and/or HIV transmission;
5. Conduct trials in different populations around the world, to obtain information on the ability of the candidate vaccines to protect against different HIV-1 subtypes, different routes of HIV transmission, and in populations that may differ on their genetic background or health status; and
6. Pay due attention to ethical aspects, community involvement, and the needs and expectations from developing countries (*61*, *62*).

In conclusion, a well coordinated effort will be required to accelerate the development of effective HIV vaccines, and this should involve the full participation of developing countries. Once a vaccine (or vaccines) is developed, international solidarity will be essential to make those vaccines available to all populations and countries in need.

Summary

The best long-term hope for controlling the HIV/AIDS pandemic is a safe, effective, and affordable preventive vaccine. The development of such a vaccine, however, has encountered unprecedented scientific challenges, including the lack of information on immune correlates of protection, the genetic variability of HIV-1, and the limitations of available animal models. Despite these uncertainties, more than 80 phase 1/2 clinical trials of 30 different candidate vaccines have been conducted since 1987. Most of these trials have been conducted in the United States and Europe, but several

also have been conducted in developing countries (including several countries in the Americas, such as Brazil, Cuba, Haiti, Peru, and Trinidad and Tobago). Preliminary results from the first phase 3 trial with a gp120 candidate vaccine indicated that the vaccine had no overall efficacy in preventing HIV infections. Nevertheless, fifteen years of HIV vaccine research have provided important lessons that can be used to decide on future strategies. It is clear, however, that to accelerate the development of an effective HIV vaccine, multiple candidate vaccines would have to be evaluated in both industrialized and developing countries, and this will require intense international collaboration and coordination.

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DENGUE VACCINES

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Dengue disease rates in the tropics have increased dramatically since World War II. More than 2.5 million people are at risk for dengue by virtue of living in areas infested with the principal vector mosquitoes, *Aedes aegypti* and *A. albopictus*. Each year, there are an estimated 50–100 million cases of dengue, including a half million cases of dengue hemorrhagic fever (DHF) (*1*).

The focus of the United States Department of Defense dengue vaccine development program is to develop a tetravalent dengue vaccine for travelers. However, the people at highest risk for dengue are the one billion children living in dengue endemic areas. It is hoped that a vaccine suitable for both groups will soon be identified.

A Historical Perspective

The story of dengue in many ways starts in the Americas. Dr. Benjamin Rush made the first good clinical description of dengue (*2*). He was in charge of hospitals under General George Washington in the Continental Army and described the dengue outbreak in Philadelphia in 1780: "This fever generally came on with rigor, but seldom with a regular chilly fit. The pains which accompanied this fever were exquisitely severe in the head, back, and limbs. The pains in the head were sometimes in the back parts of it, and at other times they occupied only the eyeballs. A few complained of their flesh being sore to the touch, in every part of the body. Its general name among all classes of people was, the break-bone fever." Dengue today presents with the same fever, headache, eye pain, myalgia, and arthralgia.

The U.S. military's dengue research efforts started just after the Spanish-American War, sparked by the very many dengue casualties in the Philippines. A dengue commission was established in 1900, and Ashburn and Craig were sent to the Philippines to determine the etiology of dengue and to devise countermeasures. Through a series of experiments they deduced that dengue was caused by "an ultra microscopic and nonfilterable agent," or a virus (*3*). This was only the second human viral pathogen identified after the yellow fever virus that was identified by Walter Reed (*4*). Ashburn and Craig confirmed that the virus could be transmitted from person to person by both mosquito and by syringe; they made careful descriptions of the disease to include leukopenia. Important for vaccine development, they demonstrated that immunity following infection was absolute; they could only make healthy volunteers sick with dengue one time.

The Army's first dengue vaccine candidate was developed in 1929 by Simmons et al. (*5*). They fed 2,000 *A. aegypti* mosquitoes on febrile

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and, therefore, viremic volunteers (*6*). They allowed virus replication within the mosquitoes for two weeks before triturating the mosquitoes and inactivating the virus with phenol and formalin. The supernatant was given as a vaccine after careful studies to assure sterility. The five volunteers who received the vaccine were not protected from dengue upon challenge with wild-type virus, although their symptoms were reported to be milder than usual. This vaccine might have worked. The investigators gave two doses of vaccine, which was appropriate for an inactivated vaccine, but did so only four days apart. This did not provide adequate time for good primary and booster immune responses. Secondly, they challenged the volunteers only one week after immunization; again, probably not giving enough time for a mature immune response to develop. Today, the Walter Reed Army Institute of Research is pursuing a similar approach.

During World War II, Japan and the United States of America had large dengue research programs. Dr. Hotta and Dr. Kimura in Japan isolated the dengue serotype 1 virus (DENV-1) shortly before Dr. Sabin and Dr. Schlesinger did so in Hawaii. Sabin and Schlesinger were the first to isolate DENV-2. Dr. Sabin made the first effective live attenuated DENV-1 vaccine by passing the virus serially in mice (intracerebral inoculation). After seven passages, the virus lost its ability to induce illness. Volunteers developed a mild rash and leukopenia, but otherwise remained well (*7*). Due to concerns about adventitious agents in a live-mouse-brain-derived vaccine, this approach was eventually dropped, however.

In the 1950s, the face of dengue changed dramatically with the widespread recognition of DHF. The Army and the Air Force sent Dr. Bill Hammond to investigate the 1956 outbreak of hemorrhagic fever in the Phillipines. He worked with Philippine and Thai scientists to isolate DENV-3 and DENV-4 (*8*). The most important pathological process that distinguishes DHF from dengue fever is plasma leakage that can lead to shock and death. Examples of plasma leakage include pleural effusion and ascites. A combination of ascites and plural effusion can lead to respiratory embarrassment. Untreated, DHF has a mortality rate of around 10%. With careful fluid management, however, mortality rates drop to below 1% (*9*). DHF can occur in any age group, but it is most common among children living in dengue hyperendemic areas. Hyperendemic areas are considered to be those where multiple dengue virus serotypes co-circulate.

Virology, Serology, and Pathogenesis

Dengue virus is a member of the family *Flaviviridae* and genus *Flavivirus*, as are yellow fever virus, Japanese encephalitis virus, tick-borne encephalitis virus, and West Nile virus. It is a single stranded RNA virus with just less than 11,000 bases that code for three structural proteins (envelope protein, membrane protein, and capsid protein) and seven nonstructural proteins. There are four dengue virus serotypes named types 1, 2, 3, and 4 (*10*). The four dengue virus serotypes can all elicit the full spectrum of clinical disease severity, from subclinical infection (most common) to severe plasma leakage, shock, hemorrhage, and, in some cases, death (*6*).

Serologically, a distinction can be made between first and subsequent dengue virus infections with heterologous serotypes. During a primary dengue virus infection, the IgM antibody response is predominant over the IgG antibody response. Following a secondary or sequential dengue virus infection, there is an anamnestic rapid rise in IgG antibody (*11*).

In 1973, Scott Halstead published his immune-enhancement theory of dengue pathogenesis (*12*). He wrote that cross-reactive dengue antibody from a previous dengue virus infection could bind but not neutralize the new infecting serotype. This antibody virus complex more easily enters Fc-receptor bearing cells such as monocytes and macrophages. Enhanced virus replication may lead

to an exaggerated immune response that can lead to plasma leakage in some cases (*13*).

Vaccine Development Efforts

No good animal disease models have been established for dengue. The typical sequence in dengue vaccine development is to evaluate candidate vaccine immunogenicity in mice; protection from viremia in monkeys; and finally safety, reactogenicity, and protective efficacy in people. If you challenge rhesus macaques with wild-type dengue virus, they will not become ill, but they will become viremic. If a candidate vaccine prevents viremia in monkeys, it will likely prevent disease in people. Recently, the Walter Reed Army Institute of Research redeveloped a human challenge model for dengue viruses. DENV-1 and DENV-3 candidates have been identified that consistently make people ill with approximately three days of fever, headache, and malaise. Similar efforts have been unsuccessful to date for DENV-2 and DENV-4 (*14*). A human model of vaccine protective efficacy may prevent a suboptimal vaccine from going forward to field trials that will likely include tens of thousands of children living in endemic areas.

Table 1 summarizes many of the active approaches to dengue vaccine development. The approaches are ordered in terms of the number of genes that are presented to the vaccine recipient. The rationale is that more genes should result in a broader immune response leading to better protection. It also is hypothesized that live approaches should be superior to genome or subunit approaches. On the other hand, "non-live" approaches potentially offer advantages in terms of safety, tolerability, and vaccine storage. Due to theoretical concerns about immune enhancement, dengue vaccines should be tetravalent to immunize against all four dengue virus serotypes simultaneously. Listed first in Table 1 are live-attenuated vaccine (LAV) approaches followed by live chimeric approaches, followed by DNA, inactivated virus, and recombinant subunit approaches.

Live Attenuated Vaccines

Live attenuated vaccines (LAV) developed at Mahidol University in Bangkok, Thailand, are being commercially developed by Aventis Pasteur (*15*). These vaccine viruses were attenuated by serial passage in primary dog kidney

TABLE 1. Partial list of active dengue vaccine development efforts.

Approach	Number of dengue virus genes provided to recipient per serotype	Status (proponents)[d]
Live attenuated, PDK[a]	10	Tetravalent phase 2 trials (Mahidol University and AvP)
Live attenuated, FRhL[b]	10	Tetravalent phase 2 trials (WRAIR and GSK)
3'Mutation	10	Preclinical (FDA and WRAIR)
DENV[c]-4 chimera	Chimera 2 + 8	Phase 2 for DENV-4 (NIH)
DENV[c]-2 chimera	Chimera 2 + 8	Preclinical (CDC)
Yellow fever chimera	Chimera 2	Phase 1 for DENV-2 (Acambis/AvP)
DNA	2 or more	Preclinical (NMRC, WRAIR, JHU, CytoPulse, Powderject, Maxygen)
Purified inactivated	3	Preclinical (WRAIR)
Recombinant DENV[c]-2 envelope	<1	Preclinical (HGI, WRAIR, NMRC, IP)

[a] PDK, primary dog kidney cells.
[b] FRhL, fetal rhesus lung cells.
[c] DENV, dengue virus.
[d] AvP, Aventis Pasteur; WRAIR, Walter Reed Army Institute of Research; GSK, GlaxoSmithKline Biologicals; FDA, Food and Drug Administration; NIH, National Institutes of Health; CDC, Centers for Disease Control and Prevention; NMRC, Naval Medical Research Center; JHU, Johns Hopkins University; HBG, Hawaii Biotechnology Incorporated; IP, Institute Pasteur.

cells for DENV-1, 2, and 4 and in primary green monkey kidney cells and fetal rhesus lung cells for DENV-3. A series of phase 1 and 2 clinical trials have been completed, most recently a trial in Thailand among 103 children 5-to-12 years of age. They received two doses of vaccine at time zero and 3 to 5 months later, with a booster dose approximately 12 months after the second dose. Two tetravalent formulations were evaluated along with a positive control arm of rabies vaccine. So far, this vaccine has proven to be safe, although there is some reactogenicity that includes fever, headache, and rash. One 13-year-old girl had fever for about three days, essentially a mild dengue fever. The Mahidol/Aventis group uses a symptom index to score reactogenicity comprised of select symptoms scored 1 to 3 for severity and multiplied by the number of days that each symptom is experienced. The team considers a score under 20 to be acceptable. The mean symptom index following the first dose was 10. They found that the two tetravalent dengue vaccine formulations were more reactogenic than the rabies vaccine. Dengue vaccine reactogenicity was dramatically reduced with the second and third doses. This might be due to the DENV-3 component to which there is increased reactogenicity and 100% seroconverion with the first dose. There may be reduced reactogenicity with the second and subsequent doses, as the DENV-3 component is quickly neutralized. That is one theory. In terms of immunogenicity the vaccine is poorly immunogenic following the first dose. Tetravalent seroconversion approached 100% by the third dose, however.

The Walter Reed Army Institute of Research has partnered with GlaxoSmithKline Biologicals to develop a similar tetravalent dengue vaccine (*16*). The vaccine viruses were attenuated by serial passage in primary dog kidney cells and finished in fetal rhesus lung cells. Two doses are given six months apart, and to date this vaccine has been safe in 160 volunteers. The Walter Reed Army Institute of Research/GlaxoSmithKline Biologicals group also uses a symptom score with an average score around 10, although the scoring system is slightly different to that used by Aventis Pasteur. Likewise, reactogenicity is decreased with the second dose. Seroconversion rates alos are similar to the Aventis product, with an 83% tetravalent seroconversion rate after two doses. When given to monkeys this vaccine protects them from viremia, and a small number of adult volunteers have been protected from disease in a study just completed. The short-term plans for this vaccine are to expand testing in infants and in partially immune adults.

There are two concerns with LAVs regarding possible immune enhancement. First, if you give live dengue vaccine viruses to someone who has pre-existing dengue antibody, you risk increased reactogenicity from increased replication of the vaccine viruses. At Walter Reed Army Institute of Research, 15 partially immune volunteers who had yellow fever, Japanese encephalitis, or dengue virus exposure in the past were given tetravalent LAV without an increase in reactogenicity (*16*). This is a small number of volunteers, however, and severe dengue occurs only among a small proportion of those infected. This risk will need to be evaluated empirically in large numbers of volunteers who are partially immune to dengue virus. The other concern with dengue vaccines (any approach) is that following administration, if tetravalent protection is not long-lasting, antibody from the vaccine could then enhance replication of a dengue virus naturally encountered years later.

The United States Food and Drug Administration (FDA), in collaboration with Walter Reed Army Institute of Research, is taking a molecular approach to develop LAVs. Working with DENV-2, the FDA made a series of chimiric viruses between dengue virus and West Nile virus. The chimera only involves the last 90 nucleotides of the dengue virus (*17*). A particular chimera, referred to as Mutant F, or mutF, was restricted for growth in insect cells, yet grew normally in mammalian cells. Today, infectious clones of DENV-1, 2, 3, and 4 have been made, and the mutF mutation has been

introduced into each clone. A series of experiments in monkeys had been completed with the DENV-1 mutF vaccine candidate. Seroconversion rates have been 100% with uniform protection from viremia following wild-type challenge even 17 months after a single dose of vaccine.

Live Chimera Vaccines

At the National Institutes of Health they are taking two molecular approaches to a tetravalent dengue vaccine. Their efforts started with the development of the first full-length infectious clone of a dengue virus for DENV-4 in the early 1990s (*18*). From this clone they removed 30 nucleotides to attenuate the virus (DENV-4 delta 30) to produce a DENV-4 vaccine that has been given to more than 100 persons (*19*). Seroconversion rates have been near 100%. To date, the vaccine has been safe and well tolerated in volunteers. One approach to a tetravalent vaccine is to introduce the delta 30 mutation and other mutations into the other three serotypes to produce four LAV as discussed in the previous section (*20*). The other approach is to use the DENV-4 as a molecular backbone to produce three chimeric viruses where the genes coding for the membrane and envelope proteins of the DENV-4 with the genes coding for the same proteins from the other serotypes. The final tetravalent vaccine then consists of an attenuated DEN-4 virus and 3 chimeric viruses.

The Centers for Disease Control and Prevention is taking a similar chimeric approach (*21*). Rather than using a DENV-4 as the backbone, however, they are using the DENV-2 LAV virus from the Mahidol/Aventis vaccine as the backbone, and replacing the structural genes to produce chimeric vaccine viruses for DENV-1, 3, and 4 (*22*). This is currently being evaluated in monkeys, and there is yet no human data.

At Acambis, they are using the highly successful yellow fever 17D vaccine virus as the molecular backbone and replacing structural genes to produce yellow fever-dengue chimeras. They have demonstrated proof of principle with a similar vaccine to protect against Japanese encephalitis (ChimeriVax^{TM}-JE) (*23*). The Japanese encephalitis vaccine has been safe and immunogenic in human volunteers. They have produced a tetravalent dengue vaccine that protects monkeys from each of the four dengue virus sereotypes (*24*). They just completed their first phase 1 trial with a Dengue 2 monovalent vaccine. Initial results indicate that the vaccine was safe, well tolerated, and immunogenic. A tetravalent vaccine phase 1 trial is planned.

DNA Vaccines

Other candidate vaccines have yet to undergo clinical evaluation. These include DNA-based vaccines being developed at the Navy Medical Research Center (*25*) and at Walter Reed Army Institute of Research (*26*) in collaboration with several corporate partners. DNA vaccines offer significant advantages over LAVs, as they are unlikely to cause dengue-like illness upon administration; they also should be more stable, thus reducing cold chain requirements. There are some obstacles to be overcome, however. To date, neutralizing antibody production in nonhuman primates has been relatively low-titered and short-lived, as has been protection from viremia. Efforts are being made to add molecular adjuvants, to identify more efficient delivery systems, and to evaluate prime-boost strategies with inactivated vaccines (*27*, *28*). Maxygen, working with the National Medical Research Center, is developing "gene shuffling" as an approach to a single molecule DNA vaccine that provides tetravalent protection.

Inactive Vaccines

Similar to the approach of Simmons in 1929, Walter Reed Army Institute of Research is growing dengue viruses to high titer and then inactivating in formalin to serve as vaccines (purified inactivated vaccines, or PIVs) (*29*). Simmons used live mosquitoes to grow virus, but the group at Walter Reed is using Vero

cells. This vaccine should go to phase 1 trials in the coming year.

Last are recombinant subunit protein vaccines. Hawaii Biotechnology Incorporated has developed a tetravalent candidate consisting of a portion of the dengue envelope protein. One microgram of the DENV-2 vaccine protected monkeys from viremia. There are theoretical concerns with both the PIV and recombinant subunit approaches that protection may be relatively short-lived, as neutralizing antibody wanes from protective levels to potentially enhancing levels. These approaches may be acceptable as travelers' vaccines or as part of a prime-boost strategy with DNA vaccines.

Conclusion

Dengue disease incidence has increased dramatically since World War II. Dengue vaccine development efforts also have increased, as commercial vaccine developers have joined the effort to bring a tetravalent dengue vaccine to the market to protect both persons living in endemic areas and those traveling to those areas. Some candidate vaccines are in phase 2 testing, with other vaccines now moving to clinical trials. A human challenge model may allow selection of the most promising vaccines before moving to large field efficacy trials. The challenges for developing a successful dengue vaccine are many, including a lack of an animal model, the need for four vaccines rather than just one, and the important theoretical concern of immune enhancement. The success of other flavivirus vaccines (yellow fever, Japanese encephalitis, and tickborne encephalitis), however, makes it possible to hope that a dengue vaccine is close at hand.

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PROGRESS TOWARD A MALARIA VACCINE

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INTRODUCTION

Forty percent of the world's population is at risk for malaria, which causes 300 to 500 million cases of disease every year, and between 1.4 and 2.7 million deaths a year (*1*). It is a recognized research and development (R&D) priority, both for a vaccine and for drugs that will circumvent drug resistance.

Human disease is principally caused by *Plasmodium* parasites. *P. falciparum* causes the greatest number of deaths, while *P. vivax* has the greatest geographic distribution. It is not difficult to identify the child with clinical disease during the rainy season; he will be visibly ill and, if the case is severe, may be comatose with cerebral malaria (*2*). It is more difficult to identify those who have the parasite and exhibit no symptoms, which is a frequent occurrence in semi-immune populations in highly endemic areas. Young children and infants suffer disproportionately, and are more likely to die and suffer from severe anemia, cerebral malaria, and acute respiratory syndrome. Together, these symptoms represent the most severe manifestations of malaria (*3*).

Thus, the question is, if malaria affects so many people and has been recognized for many years, why there is no malaria vaccine today? In general, two misperceptions have hampered the development of a malaria vaccine:

1. Malaria vaccines are not technically feasible and, even if they are, they present a high risk as a development project;
2. Market forces cannot support the development of a malaria vaccine.

However, several observations support the feasibility of a malaria vaccine. First, it has long been known that people in endemic regions become clinically immune, and rarely exhibit clinical symptoms (*4*). The second observation is that passive transfer of antibodies protects human research volunteers (*5*). In the field, antibodies are generated after multiple infections, and when nonimmune persons are passively transferred this hyperimmunoglobulin, the disease is ameliorated, indicating again that the human immune system is able to generate an antibody response that can impact the disease process. Third, irradiated *Plasmodium* sporozoites protect human volunteers from malaria challenge (*6, 7*). This is not a simple exposure, however. The sporozoites are live-attenuated and presented in a series of six to 10 exposures involving 1,000 immunizing mosquito bites over many months. When the volunteers are challenged with malaria, up to 90% are protected for a short period of time. This human challenge model has been validated for evaluating the efficacy of preerythrocytic vaccines, has been reviewed for

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safety and efficacy, and is established in the United States, Europe, and Australia. These observations support the fundamental concept that humans do generate a protective immune response to *Plasmodium* through vaccination.

More recently, evidence from three vaccine studies indicates that they afford some level of protection, although not all of them have thus far generated protection from clinical disease in human subjects. The most advanced candidate is GlaxoSmithKline's (GSK) RTS,S preerythrocytic vaccine, a virus-like particle made up of the circumsporozoite antigen and hepatitis B surface antigen. RTS,S demonstrated efficacy in small numbers of volunteers challenged with *Plasmodium* (*8*). It demonstrated 70% protection against infection in the field, but only for about two months (*9*). The "Combination B" vaccine made in Australia contains the blood-stage antigens MSP1, RESA, and MSP2 and demonstrated genotype-specific protection of the included *Plasmodium* allelic variants (*10*). Oxford University has recently presented data for a prime-boost strategy using two pox vectors that demonstrated some protection in its first human challenge trial. All three vaccines are proceeding with studies to replicate or further test the observations.

Research is starting to generate candidate vaccines that are beginning to reveal the critical elements of protection. A number of other candidates, representing all stages of the parasite's life cycle, will enter human trials in the coming years, creating a wealth of new information for scrutiny. Some of these are already formulated as combination vaccines.

The Economics of Malaria Vaccines

Another reason that malaria vaccines have not received as much attention as vaccines for diseases that cause illness and death in both the North and South is the contrast of the potentially low return on investment in a malaria vaccine over the product's lifetime, compared to a profitable vaccine, such as pneumococcal conjugate vaccine or hepatitis B vaccine. The vaccine industry fails to identify how costs will be covered, let alone how it will turn a profit. Malaria vaccines will not be less complex or less expensive to develop than other vaccines at either the preclinical or clinical trial phases (*11*, *12*). The relatively small market of travelers or military personnel and the very large—but donor-dependent—indigent market for a malaria vaccine has not been sufficient to drive industrial vaccine development efforts (*13*).

At the time of the creation of the Malaria Vaccine Initiative (MVI) in 1999, funding of some key projects in Australia and in the vaccine industry had been halted; these projects were searching for external funding to continue. That year, the United States National Institutes of Health (NIH) conference, Meeting the President's Challenge on Malaria, HIV, and Tuberculosis Vaccines, laid out a rather ambitious, yet realistic plan of what it would take to support R&D efforts for a malaria vaccine. At the same time, the European Union recognized malaria vaccines as a priority. Models for industry-public sector partnerships had already been crafted by the Medicines for Malaria Venture (MMV) and the International AIDS Vaccine Initiative (IAVI). However, IAVI deals with a disease of global rather than geographically limited impact, and MMV focuses solely on drugs, which already have proof of concept and a better understood market, and thus function under some different pressures.

In 2002–2003, funding priorities again shifted. The good news is that the creation of the Vaccine Fund for financing of childhood vaccines for the poorest countries gives credibility to the hypothesis that if someone makes an HIV or malaria vaccine, there will be resources and mechanisms to get those vaccines to people. However, biodefense R&D is consuming many of the players in the vaccine arena. People working on adjuvants and platform technologies are now looking to biodefense preparedness funding to further develop them. Hopefully, this may create opportunities to generate data and technologies that will be useful across vaccines.

The 2001 report of the World Health Organization (WHO) Commission on Macroeconom-

ics and Health, *Macroeconomics and Health: Investing in Health for Economic Development*, demonstrated the enormous impact of malaria on financial stability and economic development in Africa. At the same time, advocacy efforts to translate this information into enhanced visibility and support on the global stage are much stronger for HIV and tuberculosis than for malaria and the other diseases that predominately affect the developing world. This is reflected in the world's relatively weak commitment to malaria research, drug and vaccine development efforts, or control.

NIH is the biggest funder annually of malaria vaccine R&D, having both a strong extramural program as well as an intramural Malaria Vaccine Development Unit (MVDU). Six other funding organizations—the European Commission (EC), European Malaria Vaccine Initiative (EMVI), MVI, the United Nations Development Program (UNDP)/World Bank/World Health Organization Special Programme for Research and Training in Tropical Diseases, the United States Agency for International Development (USAID), and the United States Department of Defense/Naval Medical Research Center—spent a total of approximately US$ 33 million on malaria vaccine R&D annually over the last two years, far less than the commitment of NIH alone of about US$ 350 million for HIV vaccine R&D. It is not that the HIV/AIDS amount is too large, but rather that the amount committed to malaria is too small to drive forward the number of candidate vaccines that should be evaluated. In addition, the cost to bring a prescription drug to market has increased—driven by increased clinical trial and regulatory costs—which only exacerbates the problem for malaria vaccines.

Life Cycle of the Parasite

To try to explain what is happening in the field of malaria vaccine research, it is useful to remember the complex life cycle of the malaria parasite. The mosquito vector injects the parasite into the human host during a blood meal, and within minutes invades the liver, where it matures for one to two weeks (*12*). The merozoite emerges from the liver and invades red blood cells within seconds. The parasite grows, and the blood cells lyse and then enter a replicative cycle in the human host. Eventually, some of the parasites differentiate into sexual forms and, when picked up by the next mosquito, mature in the mosquito gut to continue the cycle upon the next human bite. The bad news is that the parasite has evolved over thousands of years to get around the natural human immune response and, more recently, drugs (*14*). The good news is that this can create multiple targets for intervention by the immune system. We have to be smart enough to select and then downselect to identify the appropriate combination of antigenic targets.

The discussion of what the target immune response should be continues. Genomics has verified that it is stage-specific (actually the proteomic data are showing that more antigens are expressed in multiple stages) and complex. It is probably enough to say that the natural immune response is complex, and for some stages, the thinking right now is that both antibodies and cell-mediated immunity are important. This supports the current malaria vaccine hypothesis: that a broad immune response to a number of antigens will be required for effective, long-term protection.

Vaccine Targets

To simplify the analysis, it is useful to consider somewhat artificial separation into three different and unique vaccine targets: the first is the pre-erythrocytic vaccine, which, if 100% efficacious, would prevent disease; the second is the blood-stage vaccine, which would ameliorate the disease; and the third is the transmission-blocking vaccine, in which antibodies generated in humans would prevent replication of the parasite in the mosquito.

The vaccine that usually receives the least attention is the transmission-blocking vaccine (*15*). Conceptually, large populations, such as entire villages or geographic areas, would

be immunized to reduce transmission among the population. The transmission-blocking vaccine has a number of strengths, among which is that there is a fairly well developed transmission-blocking in vitro assay that allows for comparison and rapid screening of candidates. Theoretically, the presence of high levels of active antibodies in phase 1 trials defines a pathway to a proof of concept. In addition, transmission-blocking vaccines have the potential for enormous impact whether used to control epidemics, control malaria in areas of low endemicity (thereby shrinking the malaria map), or as part of a combination vaccine to prevent escape mutants. The candidates preparing for clinical evaluation are being produced by the NIH/MVDU. Compatible efforts are under way in Europe and investigators in Japan and at the Johns Hopkins University, in the U.S., are working on transmission-blocking DNA vaccines. The MVDU's first phase 1 vaccine trial is ongoing. The strength of the development pathway for a stand-alone transmission-blocking vaccine is complicated by the ultimate goal: the need to immunize—not just vaccinate—virtually every person in a community, regardless of age or other conditions, to benefit not the individual but the community.

Next are the blood-stage antigens, of which 35 to 40 have some supporting data to indicate that they could be potential vaccine candidates (*16*). These are generally expressed on the surface of blood-stage merozoites, and data documenting protection in parallel rodent and primate versions of their own species-specific malaria exist for many of them. A number of phase 1 trials presented at the third Multilateral Initiative on Malaria Pan-African Conference on Malaria indicate important progress in the field (*17*). Absent a way to validate which candidate or groups of candidates will be efficacious, field trials are necessary to validate the value of those vaccine candidates that can be produced under Good Manufacturing Practices (indicating that production is reproducible) and found to be safe and immunogenic in early clinical trials.

The last type of vaccine is the disease-prevention vaccine targeting the pre-erythrocytic stage of the parasite. Several antigens, including liver-stage antigens, have been studied over the years (*18*, *19*). The one that has received the most scrutiny is the circumsporozoite antigen.

Malaria Vaccine Trials

RTS,S, a vaccine developed by GSK and the Walter Reed Army Institute of Research, has protected in the human challenge model at an efficacy of 30% to 80%. RTS,S is the most advanced candidate vaccine, as it was developed over a number of clinical trials in a total of more than 1,000 volunteers, probably because it offered the potential to serve as a travelers' vaccine. The RTS,S vaccine has shown a good safety profile in children and will be tested in a phase 2b trial in Mozambique in 2003. Initial results from that trial are expected in 2004. Other approaches are being developed, including a virus-like particle based on the hepatitis B core antigen.

The prime-boost delivery system is being evaluated, as are several viral vectors. Data and reports about progress in these are expected in the coming years.

There are at least eight malaria vaccines in clinical trials, with many more being prepared for their first phase 1 trial. This represents substantial progress compared to the situation three years ago. A number of researchers are pushing forward their candidates and getting support from a variety of sources; this, along with new funding sources, such as the Bill and Melinda Gates Foundation, is responsible for this progress. We need to plan for success—with forward thinking about the impact of a vaccine, defining the market, and developing options to ensure access and availability—as well as consider what alternatives should be pursued if those in the pipeline fail. There is certainly a growing sense of momentum.

At the same time, it is important to remember the not-so-good news. If phrased in terms of the challenges still faced, many of the vac-

cine projects critically need the rigor of clinical development—not just clinical research, but rather the kind of thinking fostered by the vaccine industry in the development process. Many difficult decisions will have to be made regarding downselection and clinical and regulatory pathways. The number of vaccines in the pipeline exceed the available resources—particularly human resources—committed to malaria research right now. Many of the strategies that are being tested are complex, and the technical and opportunity risks for most industrial sponsors remain high.

The Role of Partnerships

This is where the concept of partnerships with the public sector comes into play. The mission of MVI "is to accelerate the development of malaria vaccines and to ensure their availability and accessibility to the developing world" (*20*). Early on, MVI received wonderful research proposals and had to decide not to fund research. MVI has focused on development, not discovery. The advantage of malaria is that with anything from 200 to 1,000–2,000 volunteers, it is possible to obtain a preliminary estimate of efficacy against clinical disease in one malaria transmission season. That information can rapidly feed back into the development process.

Science, like infectious diseases, knows no borders. MVI works with partners to define the scope of work, lay out the funding plan, negotiate responsibilities and obligations, and track progress. Candidates come from all over the world, and MVI supports projects on five continents. Several other organizations are supporting other candidates, including NIH, EMVI, USAID, the EC, and the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases. These efforts will be joined by new ones, including the European and Developing Countries Clinical Trial Partnership. Collaboration can avoid the worst-case scenario, in which each funding agency would develop and control access to testing sites, creating an unnecessary barrier to testing multiple vaccines at multiple sites in the future.

Why is the field taking a parallel approach and driving a variety of candidates to preliminary efficacy (phase 2b) trials? Until candidates, particularly blood-stage vaccines, progress into field trials, whether alone or in combination, we will not know the role each can play. Clinical data will be fed back into development. When the goal is not to develop a single proprietary vaccine, but rather to make a vaccine that works, it is rational to ensure that the pipeline is full enough to yield candidates that can go forward to advanced development and licensure. However, as valid candidates are identified, questions arise about how to ensure that a manufacturing facility is built in a timely manner to avoid delays in access to vaccines.

The MVI approach has been to develop and manage partnerships using milestone-based funding and joint vaccine development committees to manage the partnership; these are not grants. Experience shows that it works and is key to the development process. To actively manage intellectual property for the public sector, MVI is working with other groups that are dealing with access to intellectual property rights for product development for developing countries. These can be very complex issues. MVI attempts to be a very neutral broker for technology and science and to develop commercialization strategies that will help catalyze the broader field.

The publication of the human, malaria, and mosquito genomes will lead to a lot of new research which, in the long term, may be extremely helpful. The problem is that high-throughput approaches are just being developed and already they potentially define about 1,400 proteins. In a single stage, three-quarters of the 684 proteins that have been defined are either hypothetical, have never been identified, or are irrelevant. We are not yet sure what we have. Genomics has documented stage-specific proteins. The question for the vaccine target, at least against something as complicated as malaria, is how to screen these proteins when

complex evaluation in model systems (human or animal) is required.

Conclusion

Our collective challenges, from an industrial point of view, are to continue to refine product profiles, encourage partnerships, and establish and live by "go" and "no go" decisions. It will be important to be able to make hard choices and stop a project when it does not meet the criteria for success. Unfortunately, the history of malaria vaccines has been marked with delays, uncertain funding, inability to make these difficult decisions, lack of collaboration, and poorly powered development efforts. At this time, with persistence, leadership, adequate resources, and just a little bit of luck, the field is poised to overcome this history.

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HOOKWORM IN THE AMERICAS: PROGRESS IN THE DEVELOPMENT OF AN ANTI-HOOKWORM VACCINE

Peter J. Hotez[1]

INTRODUCTION

This chapter will focus on human hookworm infection. Some consider hookworm infection as the most important helminthiasis in humans and possibly even the second most important parasitic disease of humans next to malaria. Hookworm, together with ascariasis and trichuriasis are the three major soil-transmitted helminth (STH) infections. New WHO data indicate that as many as two billion people are infected with STHs. Both STH infections and schistosomiasis may account for as much as 40% of the morbidity of all infectious diseases, exclusive of malaria (*1*). In the Americas, the overall prevalence of STH infections ranges between 10% and 19% (*2*), with 84, 100, and 50 million people infected with Ascaris, Trichuris, and hookworms, respectively (Table 1). The highest rates of STH infections occur in tropical and subtropical regions, especially in areas of poverty. It is, therefore, no surprise that Central America, the Caribbean, and tropical regions of South America exhibit the highest prevalence and intensity of STH infections.

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As shown in Table 2, the highest nationwide rates of American hookworm infection in the Region occur in Guatemala and Paraguay (*3*), but Brazil and other Latin American countries also have focal areas of extremely high endemicity. For example, in some villages of Minas Gerais State, Brazil, the prevalence exceeds 80%, with high hookworm burdens leading to clinical disease. Overall, 50 million of the estimated 740 million cases of hookworm worldwide occur in the Americas (*2*).

Hookworm is a particularly pathogenic nematode because it can cause intestinal blood loss. Each adult hookworm can cause up to 0.2 ml of blood loss per day. Therefore, individuals who are chronically infected with large numbers of hookworms may experience deficiencies in blood components such as iron and protein. In areas of rural poverty, where dietary intake of protein and iron is low, there is a linear relationship between the number of hookworms present in an individual's intestine and the degree of his iron deficiency and anemia (*4*). Hookworm can account for the major component of iron-deficiency anemia in endemic regions, particularly among vulnerable populations with borderline iron reserves, such as children and women of childbearing age (*5*). In areas of epidemiological overlap with malaria and HIV-AIDS, human hookworm infection will further exacerbate the

TABLE 1. Prevalence estimates of soil-transmitted helminth infections in the Americas.

Soil-transmitted infection	Population at risk (in millions)	Prevalence of infection (%)	Total cases (in millions)
Ascaris	514	16	84
Trichuriasis	523	19	100
Hookworm	346	10	50

Source: Based on data from Brooker S, de Siliva N, Hotez P, Montresor A, Engels D, Savioli L. Soil-transmitted helminth infections: updating the global picture. Manuscript submitted to *Trends Parasitol.*

TABLE 2. Hookworm prevalence, selected countries of the Americas.

Prevalence (%)	Country
45–60	Paraguay
25–45	Guatemala
5–25	Belize Bolivia Brazil Colombia Cuba Ecuador El Salvador Guyana Haiti Honduras Peru Suriname Venezuela
< 5	Argentina Canada Costa Rica Dominican Republic Chile Mexico Nicaragua United States Uruguay

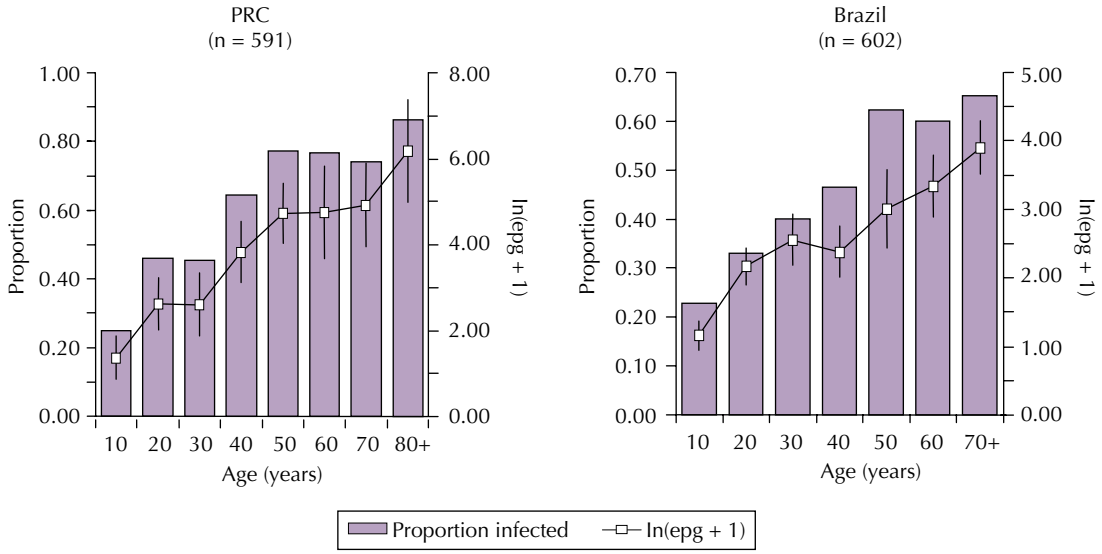
Source: Based on data from Brooker S, de Siliva N, Hotez P, Montresor A, Engels D, Savioli L. Soil-transmitted helminth infections: updating the global picture. Manuscript submitted to *Trends Parasitol.*

anemia caused by these conditions. Based on recent estimates of disability-adjusted life years (DALYs) that incorporate the association between hookworm and iron-deficiency anemia, hookworm outranks hepatitis B infection, dengue, leprosy, schistosomiasis, Chagas' disease, and many other conditions as a cause of disease burden (*6*).

Both STH infections and schistosomiasis are generally thought of as having their highest prevalence and intensity in children, especially children of school age. Indeed, hookworm is a major problem among children of this age group in many regions of the Americas (*3*). However, new data from cross-sectional surveys conducted over the past decade indicate that hookworm infection often exhibits age versus prevalence or age versus intensity curves, which are quite different from other STH infections (*7*). Whereas worm burdens and prevalence for ascariasis and trichuriasis peak in the school-aged group, hookworm rates often increase linearly with age. Figure 1 shows data from China and Brazil that suggest that hookworm has emerged as an important infection among adults and even the elderly (*8*, *9*).

The basis by which hookworms establish chronic infections in humans and increase in intensity with age is not known. However, unpublished studies conducted in collaboration with Drs. Rodrigo Correa-Oliveira and Jeff Bethony in Minas Gerais State, Brazil, under the auspices of an Institutional Review Board at FIOCRUZ-Belo Horizonte, point to the possibility that hookworms induce a state of host anergy. Peripheral blood mononuclear cells obtained from patients with chronic hookworm infection exhibit minimal lymphoproliferative capacity when the cells are stimulated with either crude hookworm antigens or recombinant hookworm proteins. The cells produce large quantities of the cytokine interleukin 10 (IL-10) and IL-5, but almost no IL-4. Hookworm-infected patients who are coinfected with other parasites such as schisto-

FIGURE 1. Prevalence and intensity of infection with *Necator americanus* in two endemic areas: Hainan Province, People's Republic of China, 1999, and Minas Gerais, Brazil, 2000.



Note: The prevalence and intensity of infection with *Necator americanus* increases with age in two endemic areas: Hainan Province, PRC (1999) and Minas Gerais, Brazil (2000). Data are from cross-sectional studies. Analysis of variance showed that egg counts were significantly different ($P < 0.001$) among the age intervals, and that the eldest 4 age intervals were significantly different ($P < 0.05$) from the younger age intervals, but not different from each other.

Source: Reproduced from Hotez PJ, Zhan B, Bethony JM, Loukas A, Williamson A, Goud GN, Hawdon JM, Dobardzic A, Dobardzic R, Ghosh K, Bottazzi ME, Mendezz S, Zook B, Wang Y, Liu S, Esiet-Gibson I, Chung-Debose S, Xiao SH, Knox D, Megher M, Inan M, Correa-Oliveira R, Vilk P, Shepherd HR, Brandt W, Russell PK. Progress in the development of a recombinant vaccine for human hookworm disease: the human hookworm vaccine initiative. *Int J Parasitol* 2003; in press.

somes also exhibit blunted lymphoproliferative responses and diminished IL-4 production to schistosome antigens. Of interest, successful patient treatment with anthelminthic chemotherapy (with removal of adult hookworms from their intestines) restores the ability of some hosts to respond to hookworm antigens. These patients exhibit restoration of their lymphoproliferative responses, as well as renewed IL-4 production. It is as though anti-hookworm chemotherapy reconstitutes a hookworm-infected patient's immune system! Currently we are examining whether hookworm-induced immunosuppression may render the human host susceptible to intercurrent infections, including malaria and HIV-AIDS.

Several anthelminthic drugs are available to remove hookworms from the human intestine. The most widely used drugs, albendazole and mebendazole, are of the benzimidazole class and work by binding to unique sites on parasite microtubules. For the most part, the benzimidazoles are cheap, safe, and effective. However, these agents have so far not been effective in controlling hookworm worldwide, because of the high rates of reinfection that occur following anthelminthic chemotherapy. Presumably because of the capacity of hookworms to induce a state of immunosuppression, humans living in endemic areas are susceptible to reinfection almost immediately following anthelminthic deworming. WHO-sponsored studies indicate that reinfection to pretreatment levels occurs within 4 to 12 months (*10*). Despite this observation, periodic deworming is currently the only major method of STH infection control in practice in developing countries. Generally this occurs

through school-based programs that target children with high Ascaris and Trichuris worm burdens. Such programs ignore adults infected with hookworm, including the estimated 44 million pregnant women with hookworm (*11*).

The Search for a Vaccine

As an alternative or complementary approach to hookworm control, we have been examining the feasibility of developing a hookworm vaccine. Because exposure to the parasite does not confer natural immunity, the development of a successful anti-hookworm vaccine would require stimulating the host immune response in a manner that does not ordinarily occur in nature. In this sense, the development of anti-hookworm vaccine requires us to overcome hurdles similar to those faced by scientists working to develop vaccines against HIV/AIDS, tuberculosis, and malaria.

Evidence for the feasibility of hookworm vaccine development is derived from three observations (*9*). First, work conducted during the 1930s and 1940s at the Johns Hopkins School of Hygiene and Public Health (now known as the Bloomberg School of Public Health) demonstrated that small doses of the third-stage infective larvae (L3) of the canine hookworm *Ancylostoma caninum*, either injected subcutaneously or administered orally, made for an effective immunogen that rendered laboratory animals resistant to large challenge doses of L3. This principle was used to develop a commercial canine anti-hookworm vaccine comprised of two doses of L3 that were attenuated by ionizing radiation. Both vaccines relied on the observation that antigens secreted by L3 during host entry were linked to protective immunity. Second, our cross-sectional human investigations in Hainan Province, China, revealed that subjects with low hookworm burdens, despite regular and frequent exposure to hookworm L3, exhibit a unique immunological profile. Specifically, these putatively immune individuals exhibit high levels of circulating IgE antibody directed against one of the major L3 secreted antigens, a protein known as ASP-2. The final piece of evidence for the feasibility of anti-hookworm vaccine development rests on studies showing that it is possible to vaccinate sheep with either L3 secreted antigens (including ASP orthologues) or antigens derived from the alimentary canal of the adult parasite of the sheep blood worm *Haemonchus contortus*. *H. contortus* is a veterinary trichostrongyle nematode of tremendous economic importance, and a parasite that is phylogenetically related to hookworms.

Based on these three avenues of feasibility, we embarked on an antigen discovery program to identify the major L3 secreted antigens of hookworms, as well as antigens from the alimentary canal of the adult hookworm. Box 1 lists the major candidate antigens; they were identified, cloned, sequenced, and ultimately expressed in our laboratory.

The most abundant antigens released by L3 hookworms are two cysteine-rich secretory

BOX 1. Lead candidate vaccine antigens for the Human Hookworm Vaccine Initiative.

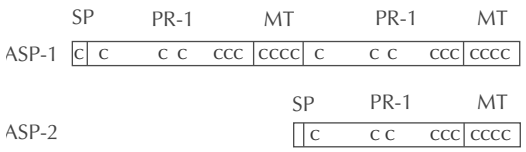
Antigens from third-stage infective larvae (L3)

Antigen	Description	MW	Expression vector
ASP-1	Ancylostoma secreted protein 1	45 kDa	Yeast (Pichia)
ASP-2	Ancylostoma secreted protein 2	24 kDa	Insect cells
MTP-1	Metalloprotease (astacin type) 1	62 kDa	Baculovirus

Antigens from adult hookworm alimentary canal

Antigen	Description	Expression vector
APR-2	Aspartic protease (pepsin type) 2	Insect cell/baculovirus
MEP-1	Metalloprotease (neprilysin type) 1	Baculovirus
CP-2	Cysteinyl protease 2	Yeast (Pichia)

FIGURE 2. Schematic diagram of the protein domain structures of ASP-1 and ASP-2.



Note: ASP-1 corresponds to a heterodimorphic repeat of the single domain ASP-2.

Source: Reproduced from Hotez PJ, Zhan B, Bethony JM, Loukas A, Williamson A, Goud GN, Hawdon JM, Dobardzic A, Dobardzic R, Ghosh K, Bottazzi ME, Mendezz S, Zook B, Wang Y, Liu S, Esiet-Gibson I, Chung-Debose S, Xiao SH, Knox D, Megher M, Inan M, Correa-Oliveira R, Vilk P, Shepherd HR, Brandt W, Russell PK. Progress in the development of a recombinant vaccine for human hookworm disease: the human hookworm vaccine initiative. *Int J Parasitol* 2003; in press.

proteins (CRISPs) that belong to the pathogenesis related protein (PRP) superfamily and are known as Ancylostoma secreted protein 1 (ASP-1) and ASP-2 (Figure 2). Their function is unknown, although the identification of similar proteins in both free-living and plant-parasitic nematodes suggests that they may not have a direct role in the mammalian host-parasite relationship. The observation that both ASP-1 and ASP-2 are secreted in vitro by L3 hookworms only in response to host serum factors might suggest otherwise, however (*12–14*). The ASPs from *H. contortus* are also protective antigens in sheep and guinea pigs challenged with homologous L3 (*9*). Another antigen secreted by L3 hookworms in response to host serum is a zinc metalloprotease belonging to the invertebrate astacin class of proteases (*15*). Known as MTP-1, the hookworm astacin may have a role in parasite invasion through the tissues. In addition to its functional catalytic domain, MTP-1 also contains C-terminal epidermal growth factor and CUB domains, which may have a regulatory role in the host-parasite relationship. Both ASP-2 and MTP-1 are immunodominant molecules that are recognized by sera obtained from hookworm-infected patients who exhibit low hookworm burdens.

From adult hookworms, the lead candidate antigens are proteases that line the brush border membrane of the parasite alimentary canal. The proteases function to help the parasite digest host hemoglobin as a key nutrient. These antigens were selected because their orthologues from *H. contortus* are protective vaccine candidates (*9*). It is presumed that these antigens are good vaccines because antibodies produced against them in a vaccinated host would interfere with parasite hemoglobin digestion. At least three major proteases of different classes—an aspartic protease (APR-2), a cysteinyl protease (CP-2), and a metalloprotease (MEP-1)—from MTP-1 work together to degrade host hemoglobin sequentially (*16–19*). Also under study are several parasite macromolecules that are secreted at the site of attachment, such as a fatty acid binding protein (FAR-1), an anticoagulant (AP), and a tissue inhibitor of metalloprotease (TMP), which also presumably function in the host-parasite relationship (*20–22*).

Because it is not possible to isolate sufficient quantities of natural product parasite antigens from hookworms, their development as vaccine antigens for either animal or human testing requires their expression in either a prokaryotic or eukaryotic vector. However, in the case of *H. contortus* antigens, it is possible to isolate sufficient quantities for vaccine testing. Therefore, the Haemonchus system is a useful paradigm for selecting corresponding hookworm antigens. Studies on *H. contortus* conducted during the 1980s and 1990s revealed that both the ASPs and the gut-derived proteases (hemoglobinases) are effective vaccines at reducing both parasite burdens and parasite egg production (*23–28*). In the case of the ASPs, immunity was noted to depend on host production of antigen-specific IgE (*29*), much like the situation of humans with reduced hookworm burdens in China. However, it was further noted that cloning and expression of these *H. contortus* antigens in *Escherichia coli* failed to produce proteins that either folded correctly or reproduced vaccine protection of the natural product (*30*). This indicated the necessity of abandoning *E. coli* expression in favor of more expensive eukaryotic expression vectors.

Our experience with the lead candidate hookworm antigens has been similar to the experiences of our colleagues in the Haemonchus field. Most of the *E. coli* antigens fail to protect laboratory animals challenged with L3 hookworms, except in a few animals that acquire exceedingly high antibody titers. In some cases, protection is observed only in animals that develop antigen-specific IgE antibody titers. Even in these animals, however, antibody acquired to the *E. coli* expressed antigens often fails to immunoprecipitate the corresponding native antigen from parasite extracts. This indicates to us that the *E. coli* expression protein fails to fold correctly in order to produce conformational epitopes. Indeed, many of the hookworm antigens are highly rich in cysteines and have large numbers of disulfide bonds. This feature may account for some of the incorrect folding that occurs during expression by *E. coli*.

To solve this problem, we have invested heavily both in terms of funds and human energy to re-engineer all of the lead candidate antigens in eukaryotic vectors. Most of the antigens are expressed in parallel yeast and insect cell-baculovirus systems, since it is usually not possible to predict which of the two will be most successful for expressing any given antigen. Our choice for a yeast expression vector has been the methanol utilizing organism *Pichia pastoris*, which produces proteins in high yield and at lower costs than some other yeast systems. (See Box 1 for a list of the lead candidate antigens and their expression vector.) To date, all of the proteases expressed in either eukaryotic system have exhibited enzymatic activity. Similarly, antibody to the ASPs expressed in eukaryotic systems immunoprecipitates native antigen from parasite extracts. Therefore, eukaryotic expression has helped us to overcome a major obstacle in human hookworm vaccine development.

A second hurdle has been the modest immunogenicity of the antigens expressed in eukaryotic systems versus *E. coli* proteins. To date, most of the immunogenicity testing has been done using aluminum based adjuvants such as alum or alhydrogel. In an effort to augment immunogenicity, the lead candidate antigens are being formulated with new generation adjuvants. Our goal is to elicit high titers of antigen-specific antibody, preferably of the Th2 type. A secondary goal is to elicit antibodies of the IgE subclass, because of the importance of ASP-specific IgE in protecting both sheep against *H. contortus* (*29*) and humans against hookworm.

Based on both human serological cross-reactivity and animal testing, ASP-2 has emerged as a lead L3 candidate. Studies *in vitro* indicate that anti-ASP-2 antibodies block L3 invasion through skin, suggesting a possible mode of action, although it is not clear why this mechanism would rely on IgE as opposed to other immunoglobulin classes. The lead candidate adult hookworm proteases are undergoing testing in laboratory animals as well as human serologic studies. Our immediate goal is to take at least one L3 candidate antigen and one adult hookworm antigen into process development and pilot manufacture, prior to Phase 1 human testing. A clinical development plan is also pending.

Conclusion

The obstacles to developing a hookworm vaccine product are formidable. No human clinical trials have ever been conducted with a recombinant nematode vaccine, and we anticipate that chronically infected patients will not respond to hookworm vaccine antigens unless their immune system is first reconstituted through anthelminthic chemotherapy. Because hookworm is a disease of the most impoverished in developing countries, there is little or no traditional commercial market for an anti-hookworm vaccine. Therefore, GMP product manufacture and distribution will rely heavily on the public sector, or a public-private sector partnership. Innovative steps to building these partnerships are now under way.

It is likely that the first tests of vaccine efficacy for an anti-hookworm vaccine will be conducted in the Americas. As of this writing,

several hookworm-endemic regions in Brazil and in Central America are being seriously considered.

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Part V

New Concepts for Vaccine Development, Adjuvants, and Delivery Systems

MUCOSAL VACCINES TO INDUCE CELLULAR IMMUNITY AGAINST HIV AND OTHER VIRAL INFECTIONS

Jay A. Berzofsky[1] *and Igor M. Belyakov*[1]

INTRODUCTION

This chapter discusses new vaccine strategies that are being studied in animal models, in this case in mice and monkeys (rhesus macaques), for the design of new mucosal vaccines. We focus primarily on HIV as an example, though we will also mention smallpox, where mucosal immunity is relevant as well, given its risk in bioterrorism. Approximately 42 million people are infected with HIV, the vast majority—over 29 million—in sub-Saharan Africa. In the Americas, more than 2.5 million people are infected (*1*). HIV is tragic, particularly for those who cannot afford treatment and who will most likely die of AIDS, but also for their families. For example, over 13 million children are estimated to have been orphaned by AIDS. The vast majority of AIDS orphans live in sub-Saharan Africa, but more than a half million live in the Americas (*2*). The AIDS epidemic also massively affects the economies of the most highly affected countries by incapacitating and claiming the lives of a large number of young and working-age persons.

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Clearly, a vaccine is needed, but what is the rationale for a mucosal vaccine? HIV is naturally transmitted through mucosal routes, either genital or gastrointestinal, and many other viruses are transmitted via the respiratory mucosa, including influenza and smallpox (*3–5*). A major site of replication of the AIDS viruses is the gastrointestinal mucosa, which contains more T cells than all the other lymphoid organs combined (*6*, *7*). Therefore, focusing on the mucosal immune system and induction of immunity at these mucosal sites may be critical for protection against or control of viral infections (*8–13*).

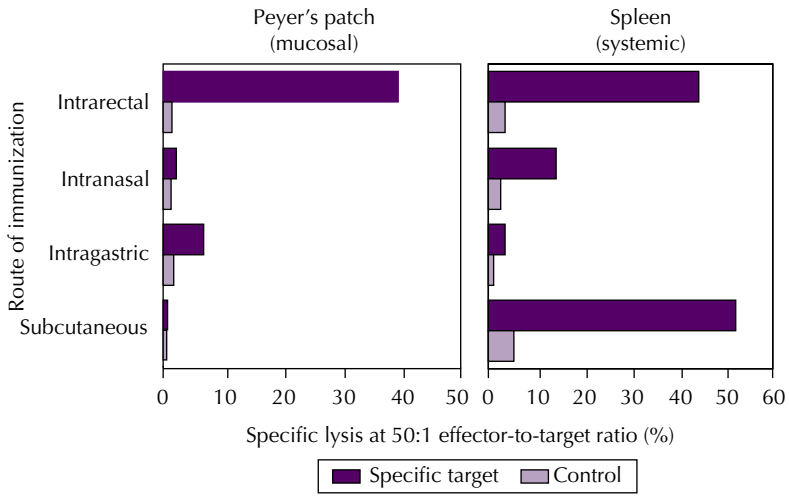
In the mucosal immune system in the small intestine, there are at least two areas of immune cells. The first is the Peyer's patches, which are lymphoid organs in the wall of the intestine that are believed to be the inductive site of the immune response. The second is the lamina propria, where the effector arm of the immune system acts to protect against infections. We wanted to find out whether we could induce immunity in both of these sites. We used peptide vaccines as a prototype; however, most of what we will discuss applies to almost any type of vaccine (*14–16*). These peptide vaccines were constructed of helper epitopes that we had defined in parts of the HIV envelope protein, and a major cytotoxic T lym-

phocyte (CTL) epitope that we had defined as well (*17–19*). We then improved on these by modifying the amino-acid sequences of some of these epitopes (*20, 21*).

Asymmetry in Mucosal versus Systemic Immunization

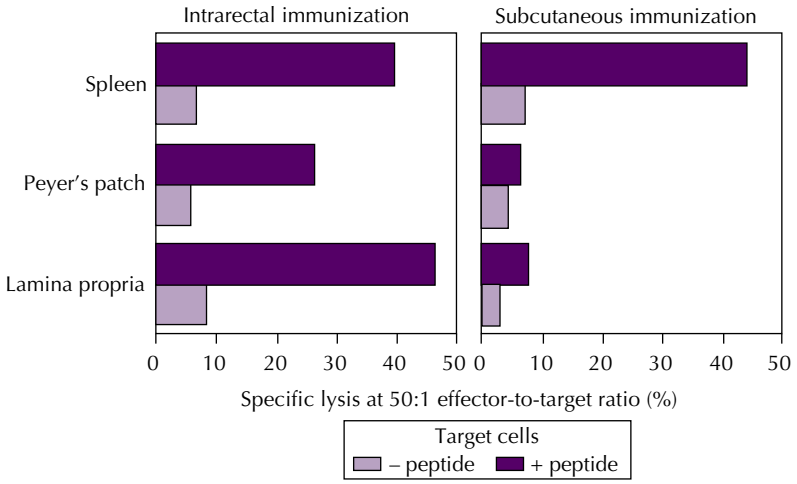
First, we will discuss the importance of mucosal CTLs for virus clearance and prevention of mucosal transmission of HIV recombinant vaccinia viruses as a surrogate virus in mice. We will then examine their application to nonhuman primates (rhesus macaques) in a comparison of mucosal and systemic immunization, followed by mucosal challenge with a real AIDS virus, a pathogenic SHIV, which is a chimera between HIV and a simian AIDS virus called simian immunodeficiency virus (SIV). We first looked at different routes of mucosal immunization—intrarectal, intranasal, intragastric—as well as the subcutaneous route, comparing CTL responses in the Peyer's patches (inducing mucosal immunity) and in the spleen (inducing systemic immunity) (Figure 1) (*22*). Subcutaneous immunization prompted a good CTL response in the spleen, but barely any in the mucosal site. The mucosal routes prompted some response in both sites. The intrarectal route was best for producing both mucosal and systemic CTLs, so we focused on that. Responses to the different routes of immunization were asymmetrical: subcutaneous immunization induced systemic, but not mucosal immunity, whereas mucosal immunization via the intrarectal route induced systemic immunity and mucosal immunity both in the Peyer's patches and in the lamina propria (Figure 2) (*22*). This asymmetrical response to a peptide vaccine applies not only to peptides, but to viruses. For example, experiments with a recombinant vaccinia virus vaccine, MVA 89.6, show that intraperitoneal immunization induces a very good CTL response in the spleen, but virtually none in the lamina propria and Peyer's patches in the mucosa (Figure 3) (*23*). In contrast, intrarectal mucosal im-

FIGURE 1. Comparison of mucosal and subcutaneous routes of immunization for the induction of mucosal or systemic cytotoxic T lymphocytes.



Source: Modified (with permission) from Belyakov IM, Derby MA, Ahlers JD, Kelsall BL, Earl P, Moss B, *et al*. Mucosal immunization with HIV-1 peptide vaccine induces mucosal and systemic cytotoxic T lymphocytes and protective immunity in mice against intrarectal recombinant HIV-vaccinia challenge. *Proc Natl Acad Sci U S A* 1998;95:1709–1714.

FIGURE 2. Comparison of mucosal (intrarectal) and subcutaneous routes of immunization with synthetic peptide HIV vaccine for the induction of cytotoxic T lymphocytes (CTLs) in mucosal (Peyer's patch and lamina propria) and systemic (spleen) compartments.



Note: BALB/c mice were immunized four times with HIV-1 peptide vaccine and two weeks after the last immunization, spleen, Peyer's patch, and intestinal lamina propria cells were stimulated with specific peptide and assayed for CTL activity against target cells with specific peptide or no peptide (control).

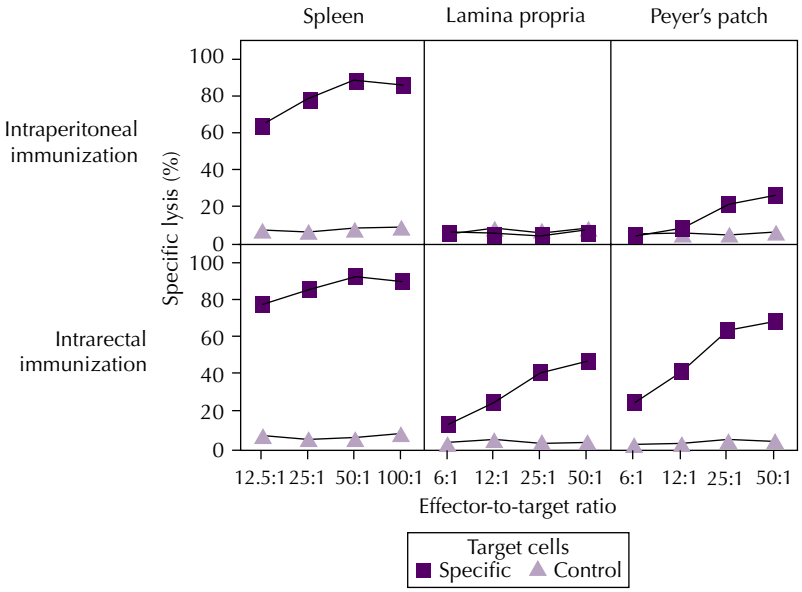
Source: Modified (with permission) from Belyakov IM, Derby MA, Ahlers JD, Kelsall BL, Earl P, Moss B, *et al.* Mucosal immunization with HIV-1 peptide vaccine induces mucosal and systemic cytotoxic T lymphocytes and protective immunity in mice against intrarectal recombinant HIV-vaccinia challenge. *Proc Natl Acad Sci U S A* 1998;95:1709–1714.

munization prompts a very good CTL response in all three sites (Figure 3). This asymmetry applies not only to this replication-incompetent vaccinia MVA, but to replication-competent vaccinia, with which systemic immunization—in this case intraperitoneal—prompts a very good systemic response in the spleen, but none in the mucosal sites (data not shown) (*23*). Thus, in order to obtain mucosal immunity, one must immunize mucosally.

Protection against Mucosal Transmission Requires CTLs in the Mucosa

Though other routes can induce mucosal immunity, the most effective is a mucosal route. Given that mice cannot be challenged with HIV, we challenged them intrarectally with a recombinant vaccinia virus (*24*) expressing HIV antigen (the gp160 envelope protein of HIV-1 primary isolate strain 89.6) as a surrogate virus (*23*) to determine if mucosal immunity would protect against virus challenge. Since this virus likes to replicate in the ovary, virus titer in the ovary is measured on a log scale here. Compared to the unimmunized mice, with 10^8 pfu, intrarectally immunized mice had a four log reduction in virus titer (Figure 4). That protection is completely dependent on cytotoxic T lymphocytes, which were depleted with an antibody to CD8, which completely abrogated protection (*25*). The fact that that protection is mediated by CTLs does not by itself mean that those CTLs have to be in the mucosa, since mucosal immunization produces CTLs in the spleen (the systemic immune compartment), as well as in the mucosa

FIGURE 3. Comparison of intrarectal and intraperitoneal MVA 89.6 immunization for the production of mucosal (in Peyer's patches and lamina propria) and systemic (in the spleen) cytotoxic T lymphocytes (CTLs).

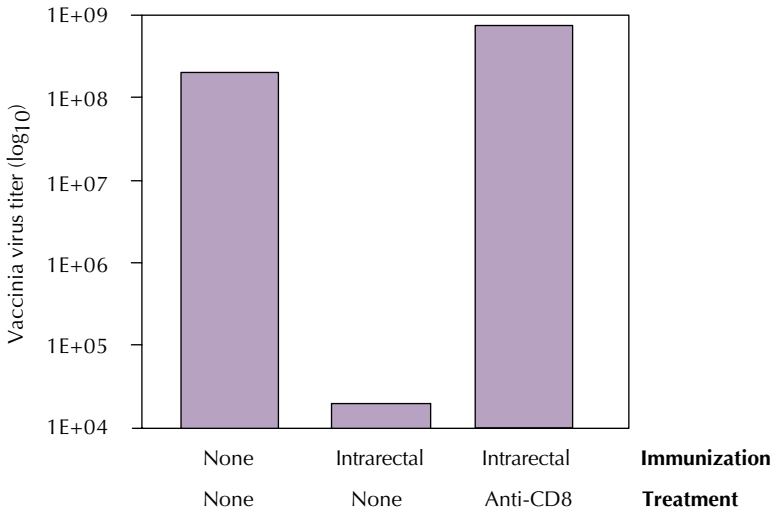


Note: Asymmetry between mucosal and systemic immunization applies to live viral vector vaccines as well as peptide vaccines. BALB/c mice were immunized intraperitoneally (upper panels) or intrarectally (lower panels) with 100 million pfu of replication-incompetent vaccinia vector MVA 89.6 expressing HIV-1 envelope protein. Four weeks later, spleen cells, Peyer's patch cells, and lamina propria lymphocytes were restimulated with specific peptide for one week and assayed for CTL activity on specific (squares) or control (triangles) target cells.

Source: Based (with permission) on data from Belyakov IM, Wyatt LS, Ahlers JD, Earl P, Pendleton CD, Kelsall BL, *et al*. Induction of mucosal CTL response by intrarectal immunization with a replication-deficient recombinant vaccinia virus expressing HIV 89.6 envelope protein. *J Virol* 1998;72: 8264–8272.

(Figure 2). To determine if systemic CTLs were sufficient to confer protection, we looked at animals that we immunized subcutaneously that had an equally good systemic response, but did not have the mucosal CTLs. If the systemic CTLs were sufficient to confer protection, these animals should be equally protected, but if CTLs in the mucosa were needed to protect against mucosal transmission, then only the mucosally immunized mice would be protected. The results were very clear, as only the intrarectally immunized mice were protected (Figure 5) (25). The subcutaneously immunized mice were not protected at all. Thus, we concluded that to be protected against mucosal transmission, one has to have local mucosal immunity. The CTLs in this case must be in the mucosa. This is a strong argument for a mucosal route of vaccine delivery. We concluded so far that natural transmission of HIV and smallpox, for example, are through mucosal surfaces. For CTLs to prevent transmission of a virus across the mucosal barrier, we showed that these must be present in the local mucosa at the site of transmission. However, since mucosal immunization induces both mucosal and systemic immunity, whereas systemic immunization may induce only systemic immunity, mucosal delivery of an HIV or smallpox vaccine may therefore be the most effective route.

FIGURE 4. Protection induced by mucosal peptide HIV immunization against viral challenge.



Note: Mucosal peptide immunization protects against mucosal viral challenge, with protection dependent on CD8+ T cells. Mice were immunized intrarectally and then challenged intrarectally with a recombinant vaccinia virus expressing HIV-1 gp160 as a surrogate virus, since mice cannot be infected with HIV itself. Six days later, virus titers were measured in the ovaries, a major site of replication of this virus (log scale on ordinate). Some mice were treated with anti-CD8 to deplete CD8+ cells prior to virus challenge, and this treatment abrogated protection.

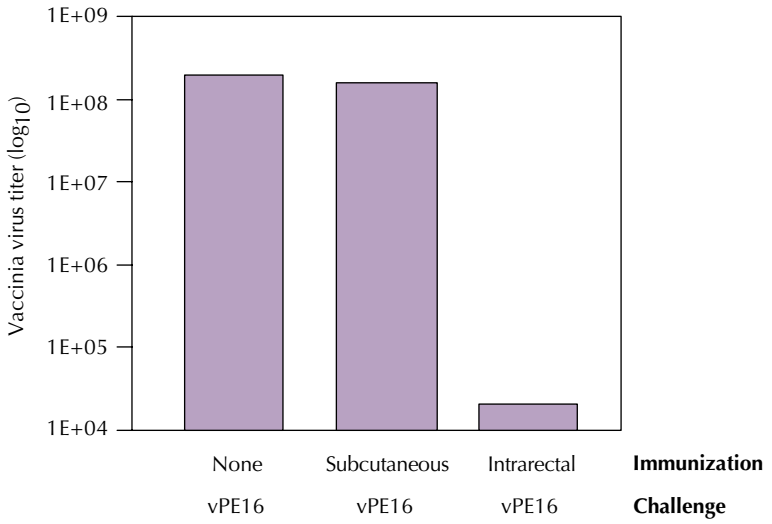
Source: Modified (with permission) from Belyakov IM, Ahlers JD, Brandwein BY, Earl P, Kelsall BL, Moss B, *et al*. The importance of local mucosal HIV-specific CD8+ cytotoxic T lymphocytes for resistance to mucosal-viral transmission in mice and enhancement of resistance by local administration of IL-12. *J Clin Invest* 1998;102(12):2072–2081.

Importance of Clearance of Gut AIDS Virus Reservoir by Mucosal CTLs Induced in Macaques by Mucosal Vaccine

To reproduce those results in a primate infected with a real AIDS virus, we conducted studies in rhesus macaques with similar peptide vaccines with helper epitopes. We used the same helper epitopes from the envelope protein, but with different CTL epitopes from SIV gag or pool genes that were chosen because they have been found to be presented by the first class I major histocompatibility (MHC) molecule—the equivalent of human leukocyte antigen (HLA) in humans defined in rhesus macaques as Mamu-A*01 (*26*). All the animals were selected to have that MHC type. We immunized three groups of animals with a mixture of those four peptides: the control group received adjuvant alone, one group received the 4 peptides subcutaneously, and the other group received the same 4 peptides intrarectally. Each group received a set of four immunizations, followed by biopsies to study immunity, then another set of immunizations, another biopsy, and a final boost two weeks before being challenged with the pathogenic strain of SHIV (*26*).

Table 1 summarizes the responses of each group. In the intrarectally immunized group, all but one of the animals had a CTL response to at least one of the peptide epitopes, both in the mesenteric lymph nodes and in the colon (i.e., the mucosal site) as well as distally in the peripheral blood and in the axillary lymph

FIGURE 5. Mucosal immunization with HIV-1 peptide and induction of protective immunity against intrarectal recombinant HIV-vaccinia challenge.



Note: Protection against mucosal viral transmission requires cytotoxic T lymphocytes (CTLs) to be present in the local mucosa. To determine if CTLs in the spleen were sufficient to confer protection, mice immunized by the subcutaneous (systemic) or intrarectal (mucosal) route were challenged intrarectally. Only the mice immunized intrarectally were protected, whereas those immunized subcutaneously were not, showing that mucosal CTLs were required for protection.

Source: Modified (with permission) from Belyakov IM, Ahlers JD, Brandwein BY, Earl P, Kelsall BL, Moss B, *et al.* The importance of local mucosal HIV-specific CD8+ cytotoxic T lymphocytes for resistance to mucosal-viral transmission in mice and enhancement of resistance by local administration of IL-12. *J Clin Invest* 1998;102(12):2072–2081.

TABLE 1. Cytotoxic T lymphocyte (CTL) responses to intrarectal and subcutaneous immunization in rhesus macaques (with CTLs to at least one epitope).

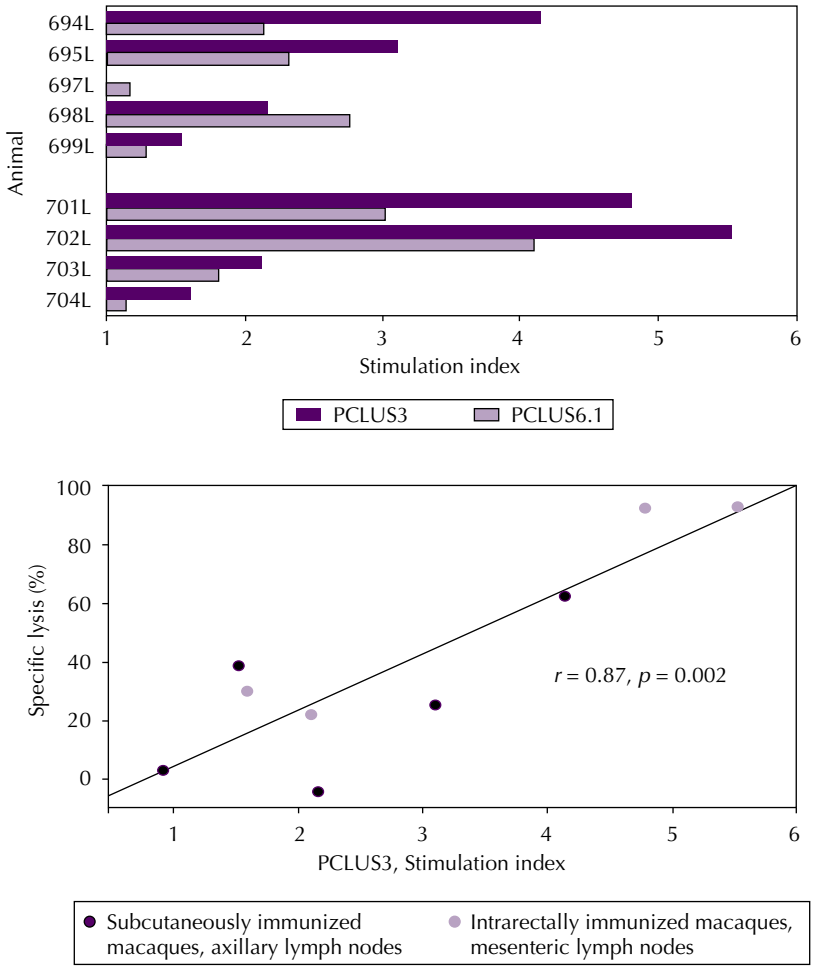
Tissue	Intrarectal vaccine	Subcutaneous vaccine	Intrarectal adjuvant only
Mesenteric lymph nodes	4/5	2/4	0/3
Colon	3/4	Not tested	0/3
Peripheral blood mononuclear cells	3/4	2/4	0/3
Axillary lymph nodes	4/5	4/4	0/3

Note: Both intrarectal and subcutaneous immunization of macaques with peptide vaccine induce CTLs in various tissues, compared to unimmunized controls. Intrarectal (mucosal) immunization seems to induce CTLs in a broader range of sites in a larger fraction of the animals.

Source: Based on data (with permission) from Belyakov IM, Hel Z, Kelsall B, Kuznetsov VA, Ahlers JD, Nacsa J, *et al.* Mucosal AIDS vaccine reduces disease and viral load in gut reservoir and blood after mucosal infection of macaques. *Nat Med* 2001;7:1320–1326.

nodes. All the animals in the subcutaneously immunized group had a good response locally in the axillary draining lymph nodes, but only two of four responded in other sites more distally. None of the animals in the control group, which did not receive the peptide, had a CTL response. The upper panel of Figure 6 shows that the T-cell helper response was variable. This is presumably because although these animals were all selected for the class I MHC molecule that presents the CTL epitope, they were outbred or varying for the class II MHC

FIGURE 6. Correlation of helper T-cell response with cytotoxic T lymphocyte (CTL) response in peptide-vaccinated macaques.



Note: Upper panel—T helper proliferative response to the helper epitopes in the vaccine in intrarectally immunized macaques (694L–699L) and subcutaneously immunized macaques (701L–704L). Lower panel—Correlation of T helper response to CTL response.

Source: Modified (with permission) from Belyakov IM, Hel Z, Kelsall B, Kuznetsov VA, Ahlers JD, Nacsa J, *et al.* Mucosal AIDS vaccine reduces disease and viral load in gut reservoir and blood after mucosal infection of macaques. *Nat Med* 2001;7:1320–1326.

molecule, such as HLA-DR and HLA-DQ, which determines the CD4 helper T cell response (*26*).

Of interest was the relationship between the helper response, shown in the lower panel of Figure 6 on the lower axis, and the CTL response, shown on the vertical axis. The good correlation ($r = 0.87$ and $p = 0.002$) shows that the ability to produce a good CTL response was very dependent on these variable helper responses, showing in primates what was seen in mice, i.e., that it is very important to be able to induce a very good CD4 helper T cell response to get an optimal CTL response.

The animals were then challenged intrarectally with pathogenic SHIV-Ku to determine

if they would be protected against mucosal transmission. As seen by the viral load in the blood (Figure 7), none of the groups were protected against mucosal transmission. However, the lack of protection allowed us to follow the course of infection over time. Measuring the number of viral RNA copies per ml of plasma over time showed that the intrarectally immunized group had a peak viral load as high as that of the other groups, but that it then dropped and fell below the limit of detection, remaining at that level at almost 200 days follow-up. In contrast, most of the animals in the control group and the subcutaneously immunized group had persistent viral loads (*26*). Thus, there was a difference in their ability to control the virus, which was also reflected in the intrarectally immunized group's maintenance of a more stable CD4 cell count and in the prevention of AIDS-related opportunistic infections.

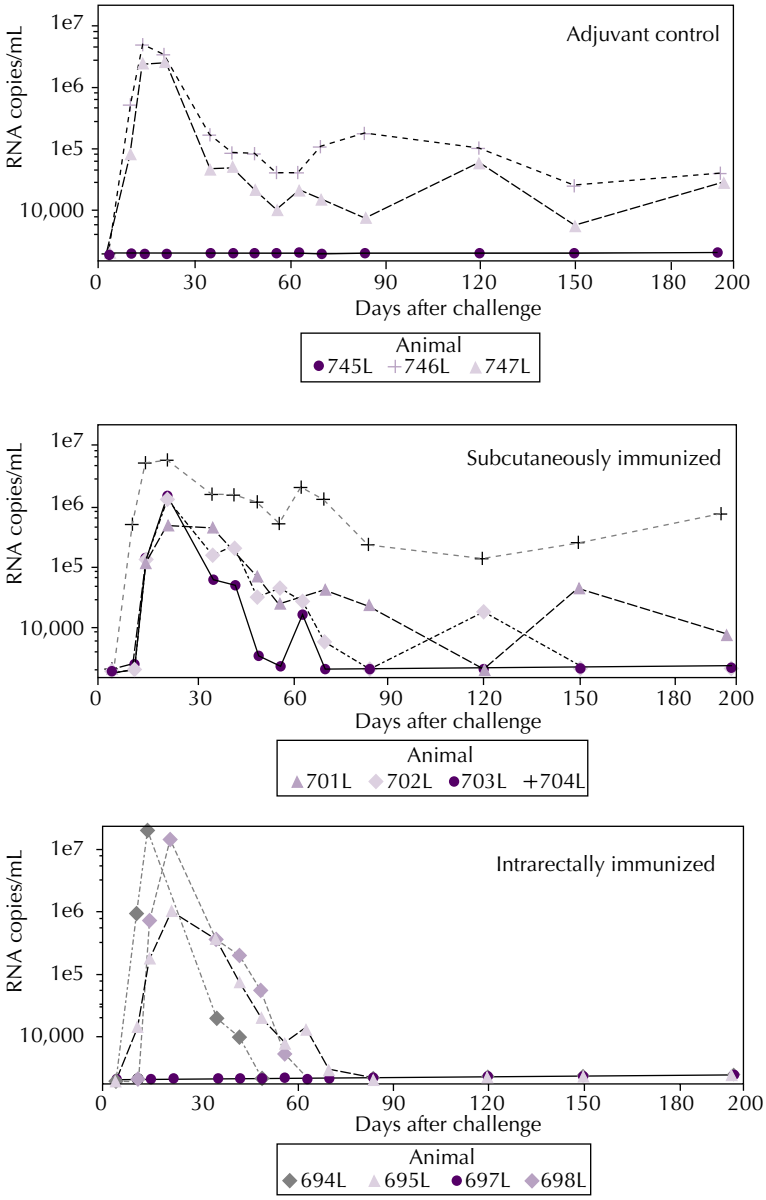
If this mucosal vaccine was not strong enough to protect against mucosal transmission of the virus, why did it reduce the viral load in the blood better than systemic immunization did? We knew that a major site for replication of these AIDS viruses is the gastrointestinal mucosa (*6*). As previously mentioned, there are more CD4 T cells, which can be the target of the virus, in the gut than in all the lymphoid organs combined. Therefore, if this was a major reservoir for virus replication that was seeding the bloodstream, and if mucosal immunization produced a higher level of CTLs in the gastrointestinal mucosa, then clearing this reservoir might remove the source of this virus, and therefore, indirectly reduce the viral load in the blood. To test this hypothesis, at around day 200, the animals were euthanized and necropsied. The upper panel of Figure 8 shows the CTLs in the colon sampled directly from the animals without any in vitro expansion, and the lower panel shows the virus load in the colon (similar data from jejunal samples not shown). As the upper panel shows, the intrarectally immunized group had the highest level of CTLs (except one animal, 697L, that seemed not to develop viremia and probably was not adequately infected, but which also showed no significant helper or CTL response); the control group produced almost no CTLs and the subcutaneously immunized group had lower levels than the intrarectally immunized group. The reverse was true of the viral load in the colon. All the intrarectally immunized animals were below the limit of detection, while the subcutaneously immunized animals and the control animals all had viral levels a log or two higher in the colon and in the jejunum (data not shown). Thus, these results support our working hypothesis, namely that inducing a higher level of CTLs in the colon was more effective at clearing the virus in the gastrointestinal sites that were seeding the bloodstream (*26*).

What seems to be happening is that in this major site of virus replication, subcutaneous immunization may induce CTLs in the blood, but not a good CTL response in the gut, and the high level of virus replication in the gut keeps seeding the bloodstream, with the consequent high viral load in the blood. On the other hand, inducing a lot of CTLs in the gut can clear this major site of virus replication and interrupt seeding of the blood. We thus conclude that, in addition to potentially preventing virus transmission across the mucosal barrier, which alone is a major justification for mucosal immunization, mucosal immunization and CTL induction is more effective than systemic immunization at controlling AIDS virus infection in a primate because it reduces the viral load in the major reservoir for viral replication, the gut mucosa (*16*).

Synergy of Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) and Interleukin (IL)-12 for Mucosal Vaccine Induction of CTLs and Protection against Mucosal Viral Transmission

To improve upon these results, we looked at the effective synergistic combinations of cytokines in enhancing that response (*27–31*). We

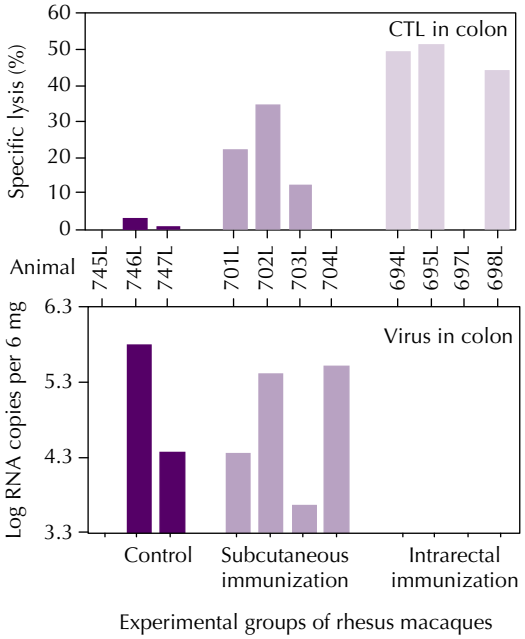
FIGURE 7. Plasma viral load in peptide vaccine-immunized and control macaques after intrarectal infection with pathogenic AIDS virus SHIV-Ku2.



Note: Control (upper panel), subcutaneously immunized (middle panel), and intrarectally immunized (lower panel) groups of macaques were challenged intrarectally with 10 rhesus infectious units of pathogenic SHIV-Ku2, expressing the HIV-1 IIIB envelope protein and the Gag and Pol proteins of SIVmac239. Plasma viral load (shown on a log scale) was monitored by nucleic acid sequence-based amplification over approximately 200 days.

Source: Modified (with permission) from Belyakov IM, Hel Z, Kelsall B, Kuznetsov VA, Ahlers JD, Nacsa J, *et al.* Mucosal AIDS vaccine reduces disease and viral load in gut reservoir and blood after mucosal infection of macaques. *Nat Med* 2001;7:1320–1326.

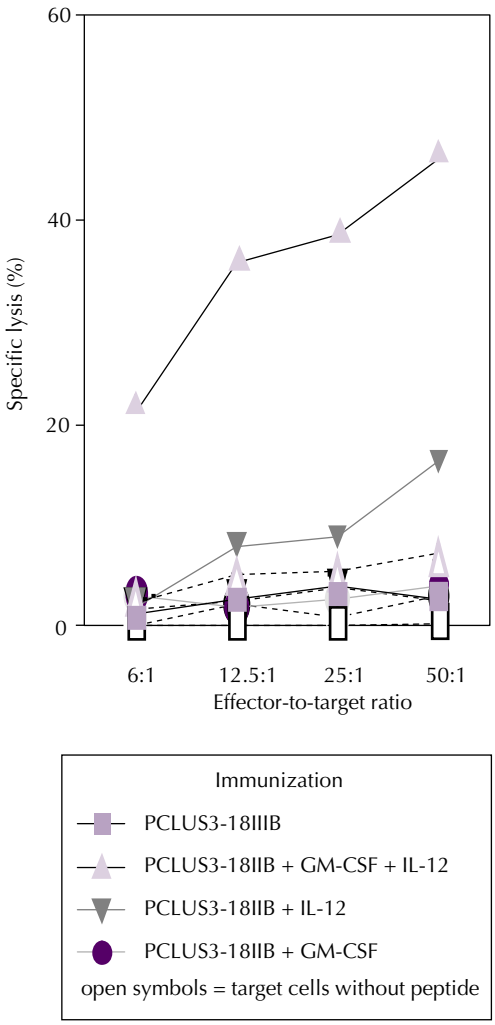
FIGURE 8. Ex vivo cytotoxic T lymphocyte (CTL) activity and viral load in the colon 200 days after intrarectal challenge of peptide-vaccine-immunized and control rhesus macaques with pathogenic SHIV-Ku2.



Note: Upper panel—CTL activity was measured directly ex vivo, without restimulation in culture, in lymphocytes isolated from colonic tissue specimens obtained at necropsy of the infected animals. Lower panel—Virus load in the same colonic tissue specimens was measured by nucleic acid sequence-based amplification (shown on a log scale). Similar results were obtained in specimens of jejunum (data not shown).

Source: Modified (with permission) from Belyakov IM, Hel Z, Kelsall B, Kuznetsov VA, Ahlers JD, Nacsa J, *et al.* Mucosal AIDS vaccine reduces disease and viral load in gut reservoir and blood after mucosal infection of macaques. *Nat Med* 2001;7: 1320–1326.

FIGURE 9. Synergy of granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin (IL)-12 in mucosal peptide vaccine induction of cytotoxic T lymphocytes (CTLs) in Peyer's patches after two intrarectal immunizations.

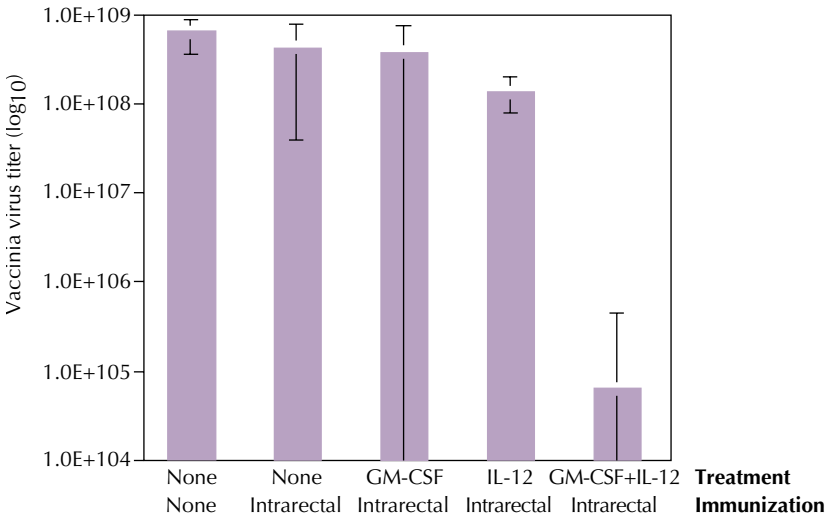


Note: BALB/c mice were immunized intrarectally with peptide vaccine from the HIV envelope protein, combined with GM-CSF and/or IL-12 in DOTAP, a cationic lipofection agent. After just two immunizations, a clear synergy was seen in the induction of CTLs in the Peyer's patches by the combination of GM-CSF and IL-12.

Source: Modified (with permission) from Belyakov IM, Ahlers JD, Clements JD, Strober W, Berzofsky JA. Interplay of cytokines and adjuvants in the regulation of mucosal and systemic HIV-specific cytotoxic T lymphocytes. *J Immunol* 2000;165:6454–6462.

found that granulocyte-macrophage colony-stimulating factor (GM-CSF) together with interleukin (IL)-12 was synergistic, inducing an optimal CTL response in mice after fewer immunizations, in this case, just two doses of vaccine (Figure 9), and that it afforded protection after just two immunizations (Figure 10) (*30*). The reduction in viral load is much more marked with this combination of cytokines after just two immunizations, compared to the

FIGURE 10. Protection against mucosal viral transmission conferred by mucosal treatment with granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin (IL)-12 administered with HIV peptide vaccine.



Note: BALB/c mice were immunized intrarectally with peptide vaccine from the HIV envelope protein, combined with GM-CSF and/or IL-12 in DOTAP, a cationic lipofection agent. After just two immunizations, mice were challenged intrarectally with a surrogate virus, HIV-1 gp160-expressing vaccinia virus. Six days later, virus titers were measured in the ovary, where the virus preferentially replicates (shown on a log scale). Clear synergy for protection against mucosal viral transmission was seen with the combination of GM-CSF and IL-12.

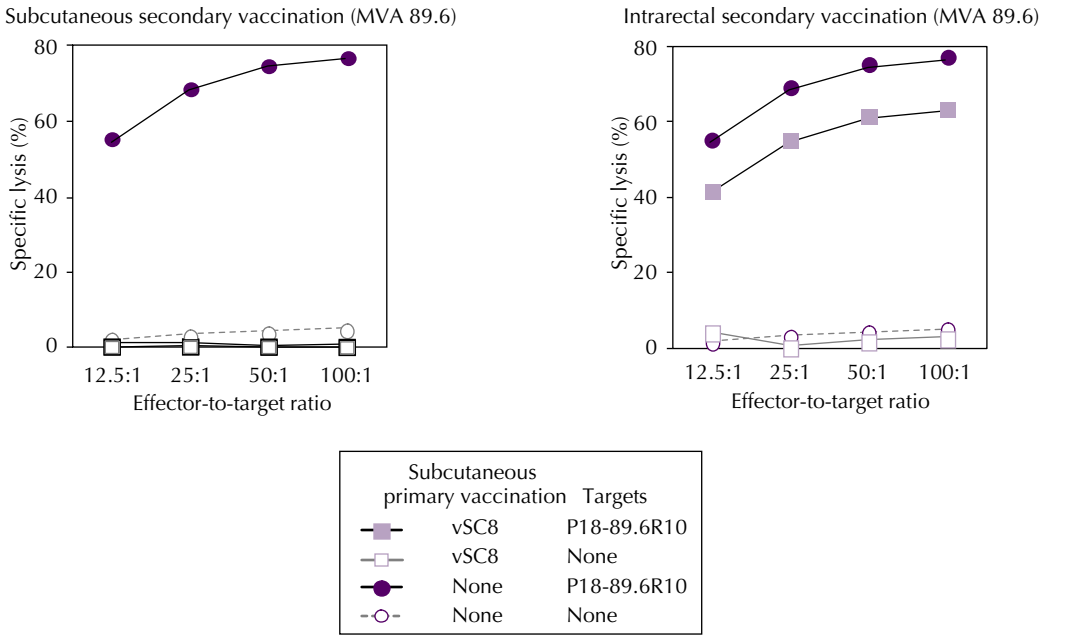
Source: Modified (with permission) from Belyakov IM, Ahlers JD, Clements JD, Strober W, Berzofsky JA. Interplay of cytokines and adjuvants in the regulation of mucosal and systemic HIV-specific cytotoxic T lymphocytes. *J Immunol* 2000;165:6454–6462.

four immunizations needed to achieve that much protection without the cytokines. These cytokines can be administered mucosally. In the beginning, it was not known if bacterial enzymes in a mucosal site would degrade cytokines, but we found that they can be administered with nothing more than DOTAP (a type of cationic lipofection agent) to protect them. Thus, we can use cytokines in the mucosal adjuvants to improve the vaccine response.

Overcoming Pre-existing Poxvirus Immunity for Use of Vaccinia Vector Vaccines by Taking Advantage of Asymmetry between Mucosal and Systemic Immunization

We also sought to determine whether we could take advantage of the asymmetrical responses prompted by mucosal versus subcutaneous immunization to accomplish yet another goal. Many people have devised vaccine vectors based on such poxviruses as vaccinia virus. However, most of the population born before 1970 has been immunized with vaccinia as a smallpox vaccine, and thus might be resistant to vaccinia-based vaccines due to prior immunity to the vector. If such vaccinees had received the smallpox vaccine by a route that conferred only systemic immunity (e.g., the subcutaneous route), as shown in mice (Figure 2), and no mucosal immunity, then the mucosal immune system might still be naïve and that might allow us to immunize those people mucosally. However, as seen in Figure 2, mucosal immunization induces a response not only in the mucosa, but also systemically, so we can take advantage of this asymmetry and

FIGURE 11. Splenic HIV-specific cytotoxic T lymphocyte (CTL) response to intrarectal vaccination with recombinant vaccinia in poxvirus-immune mice.



Note: Mucosal (intrarectal) vaccination overcomes the barrier to vaccinia vector immunization caused by pre-existing poxvirus immunity. BALB/c mice were either left naïve (circles) or immunized subcutaneously with a control vaccinia virus (vSC8), expressing only beta-galactosidase to generate pre-existing immunity to the vaccinia vector (squares). One month later, these mice were immunized with a vaccinia vector vaccine expressing HIV-1 gp160 envelope protein (MVA 89.6) either subcutaneously (upper panel) or intrarectally (lower panel). Three weeks after the second vaccination, spleen cells were harvested, stimulated one week in culture with specific peptide, and assayed for CTL activity against specific (closed symbols) or control (open symbols) targets. Only intrarectal immunization (lower panel) was successful in inducing an HIV envelope-specific CTL response in the mice that had prior immunity to vaccinia, whereas both subcutaneous and intrarectal immunization were successful in the naïve mice (solid black circles).

Source: Modified (with permission) from Belyakov IM, Moss B, Strober W, Berzofsky JA. Mucosal vaccination overcomes the barrier to recombinant vaccinia immunization caused by preexisting poxvirus immunity. *Proc Natl Acad Sci U S A* 1999;96:4512–4517.

the naïveté of the mucosal immune system after systemic immunization to immunize mucosally and still get a new systemic response.

To find out if this approach would work, we performed a fairly complex experiment. Mice that had first been immunized with the control vaccinia (so that they had vaccinia immunity) and then a vaccinia-based vaccine expressing the HIV envelope protein (MVA 89.6) via either the subcutaneous, intrarectal, or intranasal route were compared with mice that were naïve to vaccinia (32). We then measured the response to the HIV antigen that was carried by the second poxvirus vaccine (MVA 89.6).

As shown in the upper panel of Figure 11, the naïve mice given the vaccinia-based mucosal HIV vaccine via the subcutaneous route, shown in black, had a perfectly good CTL response, but the animals with prior immunity to vaccinia, shown in gray, did not show any response, just as people with prior immunity to vaccinia have a reduced CTL response to such vaccinia vector vaccines. The lower panel of Figure 11 shows that by administering the vaccinia vector vaccine via a mucosal route, in this case the intrarectal route, the naïve animals can be protected, and the animals with prior vaccinia immunity achieve almost the same level

of protection (32). Thus, this shows that we can circumvent prior vaccinia immunity by taking advantage of the asymmetrical response to the two routes of immunization and use a mucosal route. The same was true both for immunization with a replication-incompetent vaccinia (MVA 89.6) (Figure 12, upper panel) and for immunization with a replication-competent vaccinia (vPE 16) (Figure 12, lower panel). Although we were unable to immunize vaccinia-immune animals via a subcutaneous (e.g., systemic) route, we could immunize them either intranasally or intrarectally, though the intrarectal route was more effective (32).

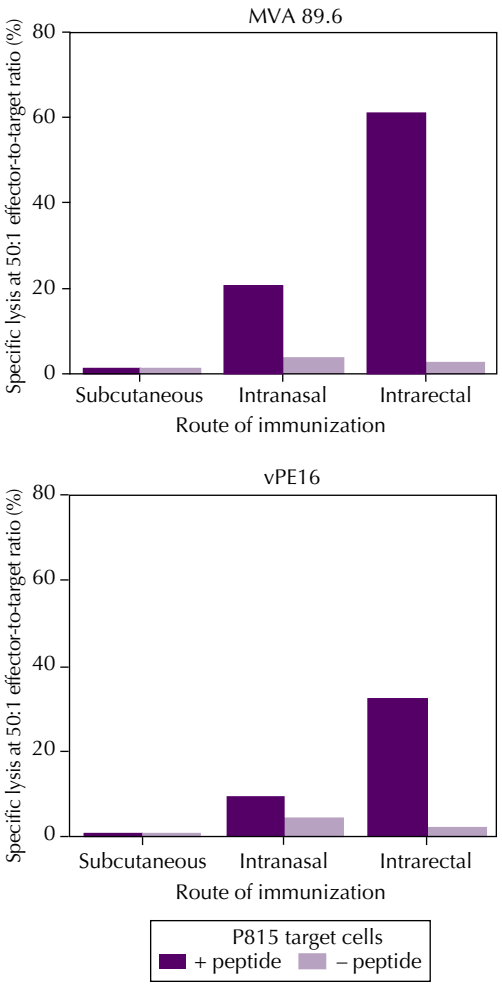
Conclusions

Natural transmission of HIV and many other viruses, including smallpox, occurs through mucosal surfaces; for CTLs to prevent mucosal transmission of the virus, they must be present in the mucosa at the site of transmission. Furthermore, the gut mucosa is a major site of AIDS virus replication, which can be controlled more effectively by CTLs in the gut mucosa than by systemic CTLs alone. Mucosal immunization induces both mucosal and systemic immunity, and thus affords dual protection, whereas subcutaneous immunization may induce only systemic immunity. Thus, mucosal delivery of an HIV, smallpox, or influenza vaccine may be the most effective route. In addition, mucosal immunization can also provide a way to circumvent pre-existing virus immunity to allow more effective use of poxvirus vector-based vaccines. Therefore, mucosal immunization has many advantages, such as overcoming pre-existing vector immunity, preventing viral transmission across the mucosal barrier, and clearing a major reservoir of virus replication. Thus, overall, mucosal delivery should be considered among the most effective immunization routes in designing vaccine strategies.

Acknowledgements

This work was carried out with many of the collaborators listed in the references.

FIGURE 12. Effectiveness of intrarectal versus intranasal immunization in overcoming pre-existing systemic immunity.



Note: Intrarectal vaccination is more effective than intranasal vaccination at overcoming the barrier to pre-existing poxvirus immunity. BALB/c mice were infected with control vaccinia virus vSC8 to induce pre-existing poxvirus immunity. One month later, they were vaccinated subcutaneously, intranasally, or intrarectally with HIV-1 envelope-expressing poxvirus vectors, either the replication-incompetent MVA 89.6 (upper panel) or the replication-competent vPE16 (lower panel). Three weeks later, cytotoxic T lymphocyte activity in the spleen was measured at a 50:1 effector-to-target ratio (E:T) against target cells with (dark bars) peptide or without (light bars). Similar results were obtained at E:T ratios of 25:1 and 12.5:1 (data not shown).

Source: Modified (with permission) from Belyakov IM, Moss B, Strober W, Berzofsky JA. Mucosal vaccination overcomes the barrier to recombinant vaccinia immunization caused by preexisting poxvirus immunity. *Proc Natl Acad Sci U S A* 1999;96: 4512–4517.

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MATERNAL IMMUNIZATION

W. Paul Glezen[1]

INTRODUCTION

Rather than addressing new technologies, this chapter will deal with strategies for using technologies already on hand that can be implemented today. Maternal immunization is an old strategy used for many years to combat neonatal and puerperal tetanus that can be adapted to prevent other serious diseases.

A recent paper in the *Journal of the American Medical Association* summarized the major causes of disability-adjusted life years (DALYs) for the world's population (*1*). Four of the top seven causes of global disease burden have the potential for amelioration by vaccines delivered during pregnancy (Table 1). Heading the list is (1) lower respiratory tract infections, followed by (3) perinatal conditions, (4) diarrheal diseases, and (7) vaccine-preventable diseases. These conditions account for over 10 million deaths per year and almost all occur in children < 5 years of age.

LOWER RESPIRATORY TRACT INFECTIONS

Lower respiratory tract illness (LRI) is the leading cause of DALYs and deserves consideration with some urgency attached. Most of the deaths occur in infants younger than 6 months of age, which makes these conditions prime candidates for prevention by passive immunity that might be enhanced by maternal immunization. Four main causes of LRI in children are influenza, respiratory syncytial virus (RSV), parainfluenza viruses, and pneumococcus. I will discuss approaches for prevention of each, beginning with influenza.

Influenza

Several studies have documented the impact of influenza on childhood LRI. A study from Vanderbilt University is particularly important, because it shows the risk of hospitalization for children who were otherwise healthy and without chronic underlying conditions (*2*). Furthermore, the investigators made every effort to exclude cases that might be attributed to RSV, which tends to circulate in midwinter along with influenza. The hospitalization rates attributable to influenza for children < 2 years of age are comparable to those for elderly, high-risk patients and certainly higher than those for older children with chronic underlying conditions such as asthma who are currently recommended to receive influenza vaccine. The Advisory Committee on Immunization Practices (*3*) and the Committee on Infectious Diseases of the American Academy of Pediatrics encourage vaccination of children 6 to 23 months of age; however, the greatest risk for hospitalization, as seen in Figure 1, is for infants under 6 months of age. Response to influenza vaccine in this vulnerable age group is unpredictable; therefore, vaccine is not encouraged for the group with highest risk of

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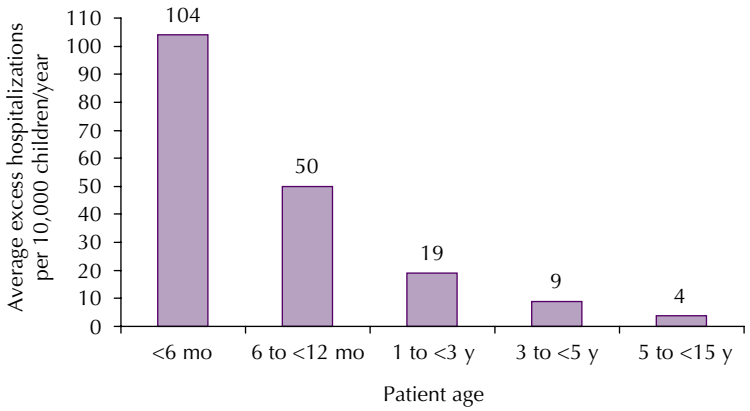
TABLE 1. Leading causes of Disability Adjusted Life Years (DALYs), worldwide, 1999.

Rank	Cause	DALYs (per 1,000)	Deaths (per 1,000)
1	Lower respiratory tract infections	96,682	3,963
2	Human immunodeficiency virus	89,819	2,673
3	Perinatal conditions	89,508	2,356
4	Diarrheal diseases	72,063	2,213
5	Depression, major unipolar	59,030	1
6	Ischemic heart disease	58,981	7,089
7	Vaccine-preventable diseases	54,638	1,554
8	Cerebrovascular diseases	49,856	5,544
9	Malaria	44,998	1,086
10	Nutritional deficiencies	44,539	493

Source: Adapted from Michaud CM, Murray CJ, Bloom BR. Burden of disease—implications for future research. *JAMA* 2001;285(5):535–539.

FIGURE 1. Excess hospitalizations per 10,000 children/year.[a]



[a] Values are weighted averages of annual excess hospitalizations for a population of 10,000 persons within the specified age group.

Source: Neuzil KM, Mellen BG, Wright PF, Mitchel EF Jr., Griffin MR. The impact of influenza on hospitalizations, outpatient visits, and courses of antibiotics in children. *N Engl J Med* 2000;342(4):225–231.

hospitalization and death. The only possibility for protection of young infants is passive immunity afforded by vaccination of their mothers during pregnancy.

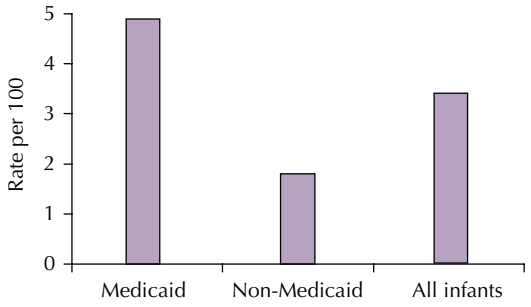
Influenza vaccine is recommended in the United States for women who will be in the second or third trimester of pregnancy during the influenza season (3). The primary indication for vaccination is the prevention of hospitalization of pregnant women for pneumonia. Studies have shown that women have an increasing risk for hospitalization as pregnancy progresses during the influenza season. At term, the risk is five times that during the first trimester of pregnancy. Observations during influenza pandemics have shown that pregnant women have high mortality due to fulminating pneumonia. A secondary benefit of maternal immunization would be the prevention of LRI due to influenza in the offspring during the first months of life (4). Studies have shown that infants born with maternal antibodies to the circulating influenza viruses are protected from LRI during the first months of life. The

age at time of culture-positive infection is directly related to the level of maternal antibody at birth. Influenza vaccine is well tolerated during the second and third trimester of pregnancy, and antibody is readily transmitted to the infant (*5*). The only part of the equation that is missing is direct evidence that maternal immunization prevents hospitalization of infants during the first months of life. Studies are in progress to seek that information.

RSV and Parainfluenza Viruses

From 1992 to 1996, the rate of hospitalization of infants during the annual RSV epidemic in Houston, Texas, averaged 2.4 per 100 (*6*). The rate was considerably higher for infants < 6 months of age, at 3.4 per 100 (Figure 2). About one-half of the infants were from low-income households, and their risk of hospitalization was 5.0 per 100, compared to only 1.8 per 100 for infants from middle-income groups. These rates are about twice the rates calculated 15 years earlier for the same population. The hospitalization rate for bronchiolitis increased similarly from 1980 to 1996 in the United States (*7*). The peak occurrence of admission to the hospital is in the second month of life. Therefore, it will be very difficult to actively immunize infants before exposure to RSV infection even if a vaccine is available. Passive immunization with RSV-specific monoclonal antibody is effective in reducing the risk of RSV hospitalization for infants born prematurely or with chronic lung disease (*8*). However, this prophylaxis requires monthly injections costing about US$ 5,000 for each child protected for the RSV season. This is not a practical solution for full-term infants.

FIGURE 2. Average rate of hospitalization for infants < 6 months of age during respiratory syncytial virus epidemics, Harris County (Houston), Texas, 1992–1996.



We are exploring an alternative strategy for protecting infants in their first months of life by boosting maternal antibodies with a subunit inactivated RSV vaccine. Previous studies had shown that infants endowed with high levels of naturally-acquired RSV antibodies were protected from serious infection during the first months of life (*9*). Boosting maternal levels by vaccination during pregnancy has the potential to protect all infants for the first 4–5 months of life, when they are at greatest risk for serious disease. A small controlled trial with PFP-2, the purified fusion protein of RSV, has been conducted among women in the third trimester of pregnancy (*10*). The vaccine was well-tolerated and no serious adverse events were attributed to the PFP-2, which is less reactogenic than inactivated influenza vaccine. Modest neutralizing antibody responses were measured in the women, and transmission of maternal IgG to infants was excellent. Infants were followed for one year for development and for all acute respiratory illnesses. Development of infants whose mothers received PFP-2 was the same as those whose mothers were given placebo; infants whose mothers received vaccine had fewer and milder acute respiratory illness during the RSV season than did infants whose mothers received the placebo. Titers generated by PFP-2 would extend protection for about one month. A more immunogenic vaccine is achievable and should attain a geometric mean serum neutralizing titer of 1:512. This would allow protection from severe disease for the first 4–5 months of life—the period of greatest risk. In addition to the increase in serum neutralizing titer, we observed a significant increase in antibodies in breast milk. Some studies have shown that breastfeeding decreases risk for serious LRI in infants.

Parainfluenza virus type 3 also infects infants at an early age, but causes LRI with less frequency than RSV (*11*). Maternal antibodies

may modify the severity of parainfluenza type 3 infection in early infancy, and a similar strategy of maternal immunization could be employed for this virus.

Streptococcus pneumoniae

Streptococcus pneumoniae is the most common cause of sepsis in infants younger than 3 months old (*12*). It also is an important cause of pneumonia. A protein conjugate vaccine is licensed for infants in the United States, but contains only seven serotypes that account for only about half of the systemic infections in infants worldwide. The conjugate vaccine is in short supply and is prohibitively expensive for utilization in developing countries. An alternative approach to prophylaxis is to administer the 23-valent pneumococcal polysaccharide vaccine to pregnant women. We have shown transfer of high levels of opsonizing antibodies to infants of vaccinated women (*13*). Furthermore, vaccination during pregnancy or lactation will generate specific IgA antibodies in breast milk. Under these circumstances, acquisition of nasal carriage of pneumococci may be delayed in infants, decreasing the risk of infection in the first months of life. The pneumococcal polysaccharide vaccine is considerably cheaper than conjugate vaccines, provides broader serotype coverage, and might allow active immunization of infants at an older age, thus reducing the number of doses needed to achieve protection. As new conjugate vaccines with 9 and 11 pneumococcal serotypes come on line, it will be important to explore the potential of maternal immunization to delay active immunization and reduce the number of doses necessary for protection.

Perinatal Conditions

Group B Streptococcus

Group B streptococci (GBS) are a common cause of neonatal sepsis and meningitis in early infancy (*14*). Early onset GBS disease occurs in the first week of life and is usually manifested by fulminating sepsis and death. It is commonly caused by serotypes Ia, III, and V. Pregnant women uniformly lack antibodies to these GBS strains. Prophylactic antibiotics have been used successfully to prevent these infections, but the methods for selecting women who require intrapartum treatment are not perfect, and many treatments are required to prevent each infection. Continued use of antibiotic prophylaxis will drive emergence of resistant GBS. Antibiotic prophylaxis during delivery does not prevent late onset disease that usually occurs within the first 4 weeks of life. Disease may be manifested by meningitis commonly caused by serotype III. Conjugate vaccines to five different GBS serotypes have been found to be safe and immunogenic in women of childbearing age (*15*). Dr. Carol Baker of Baylor College of Medicine has performed a pilot study of a GBS type III conjugate vaccine in pregnant women. The vaccine was well-tolerated and highly immunogenic. Studies are needed of combination vaccines with multiple GBS serotypes. In addition to causing amnionitis and urinary tract infections in pregnant women, GBS is also a common cause of sepsis in older adults with underlying conditions such as diabetes. GBS is second to pneumococci in this respect, and a GBS conjugate vaccine could have similar indications to those for the pneumococcal polysaccharide vaccine.

Tetanus

Maternal immunization has been demonstrated to reduce fatal perinatal events by reducing not only neonatal tetanus, but also puerperal tetanus contracted in the course of unsterile deliveries. As few as two doses of tetanus toxoid administered to women in the childbearing age has reduced neonatal mortality by 25% in developing countries such as Bangladesh (*16*). Infants with neonatal tetanus typically die four to ten days after birth.

Diarrheal Diseases

Rotavirus

Maternal immunization against rotavirus is being considered. Rotavirus is one of the most

important causes of severe gastroenteritis in infants. Most of the serious infections occur in the second semester of life, but the recently withdrawn rhesus reassortant vaccine caused intussusception when administered at < 6 months of age (*17*). Maternal immunization with a subunit vaccine such as the virus-like particles (VLPs) that might be administered orally could potentially allow infants to be actively immunized later with a safer vaccine.

Vaccine-Preventable Diseases

Pertussis

From 1998 to 2000, more than 5,000 reported cases of pertussis (one-third of all U.S. cases) occurred in infants under 6 months old (*18*). More than half of reported pneumonias and encephalopathies are included in these numbers, as are 80% of the hospitalizations and 90% of the deaths attributed to pertussis. The current schedule of primary immunization at 2, 4, and 6 months of age does not protect infants < 6 months of age. Furthermore, studies have shown that the most important risk factors for infant pertussis are having "an adolescent mother" and "a mother with cough for more than seven days." This would indicate that the mother is often the source of infection for the infant and that immunity from primary immunization wanes for adolescents and young adults. Because whole cell pertussis vaccines were reactogenic in older children, boosters were not recommended after 7 years of age; however, the currently used acellular pertussis vaccines are much less reactogenic and should allow booster immunization for older persons. Vaccination during pregnancy would be indicated if a woman presents for prenatal care without a prior booster. The acellular pertussis vaccine has been combined with the adult tetanus-diphtheria toxoid, Td, and could be administered on the same schedule as now recommended for Td.

Other Vaccines

Vaccination during pregnancy may be indicated for other vaccine-preventable diseases in certain circumstances (*19*). In areas of the world where sepsis and meningitis with *Haemophilus influenzae* type b (Hib) occur commonly before 6 months of age, maternal immunization with a conjugate Hib vaccine will provide protection between the time that naturally-acquired maternal antibodies wane and active immunity is achieved. Meningococcal polysaccharide vaccine, hepatitis A and B vaccine, yellow fever, polio, and rabies vaccines also may be indicated for pregnant women at risk for infection with these agents.

Indications for Maternal Immunization

In summary, this chapter has illustrated several indications for vaccination during pregnancy (Table 2).

- First, some agents produce life-threatening infections in the neonatal period before active immunization is possible. Early onset GBS sepsis is an example of such an infection. GBS conjugate vaccines have the potential to control this problem just as tetanus toxoid has been effective for preventing neonatal tetanus.
- Second, immunological immaturity prevents the development of active immunity for some viruses in the first months of life

TABLE 2. Factors favoring new strategies for disease control.

Factor	Examples
Life-threatening infection in neonatal period	GBS (as for tetanus)
Poor immune response in early infancy	RSV, measles, influenza
Exposure before effective active immunization	Pertussis
Tolerance in neonatal period	Whole cell pertussis, PRP-OMP
Cost of active immunization	Pneumococcal conjugate

(*19*). Passively acquired maternal antibody is important for amelioration of disease caused by influenza, RSV, and measles virus. Maternal immunization has the potential to extend the period of time that infants are protected against these viruses.

- Third, infants may be exposed to some agents before effective active immunization is achieved, as with pertussis. Acellular pertussis vaccines can be used to boost immunity among adolescent and adult household contacts of newborn infants, thus reducing the risk of exposure in the period before active immunization is adequate. If a woman presents for prenatal care without the appropriate booster, vaccination during pregnancy is indicated, as is currently recommended for Td.
- Fourth, whole-cell pertussis vaccine and the Hib vaccine, PRP-OMP, both generate tolerance if given to newborns; i.e., they not only fail to respond to the neonatal dose but have poor antibody responses to later doses. Maternally-derived passive immunity is safer than attempting active immunization of the neonate for most vaccines. Hepatitis B surface antigen is one notable exception.
- Fifth, and finally, some vaccines, such as the pneumococcal conjugate vaccine, are expensive and in short supply. Alternative approaches, such as maternal immunization with the pneumococcal polysaccharide vaccine to delay active immunization and reduce the number of doses of conjugate vaccine, should be considered.

Safety and Summary

Long experience with inactivated vaccines during pregnancy has established the safety of the procedure (*19*). Tetanus toxoid has been used safely throughout the world. Vaccines for influenza and polio have been shown to be safe after extensive use. In fact, non-reactogenic inactivated vaccines pose no known threat to the pregnant woman or to her fetus. Therefore, passive protection of infants by maternal vaccination during pregnancy is a strategy that should be included in the fight against infectious diseases. Important access for immunization occurs during routine prenatal care, and this opportunity for prevention should not be wasted. Finally, maternal immunization has the potential to protect both mother and infant at a time when both are vulnerable to infection.

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DNA VACCINES: A REVIEW

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INTRODUCTION

The DNA vaccines are simple rings of DNA containing a gene encoding an antigen, and a promoter/terminator to make the gene express in mammalian cells. They are a promising new approach for generating all types of desired immunity—cytolytic T lymphocytes (CTL), T helper cells, and antibodies—whilst being a technology that has the potential for global usage in terms of manufacturing ease, broad population administration, and safety. This review gives an overview of the mechanisms, preclinical and clinical efficacy of DNA vaccines, and points out the limitations of the first generation of such vaccines, and some of the promising second-generation developments. This technology is also being utilized in the field of proteomics as a tool to elucidate the function of genes. The breadth of applications for DNA vaccines thus ranges from prophylactic vaccines to immunotherapy for infectious diseases, cancer, and autoimmune and allergic diseases.

A well-known Chinese adage states, "Give a man a fish and you feed him for a day. Teach a man to fish, and you feed him for a lifetime." Whilst this has often been utilized in designing social assistance programs, it is also the secret behind the incredible success of vaccines as a medical invention. Indeed, vaccines are considered amongst the most, if not *the* most, effective medical development because they have successfully eliminated an entire wild-type disease from the planet (smallpox) with a second disease about to be eradicated (polio). The secret behind this success lies to a large extent in the ability of vaccines to teach the body to respond to the wild-type pathogen, rather than directly treating the disease, as therapeutics such as antibiotics do.

NEED FOR NEW VACCINES

A number of diseases have not yet been conquered by vaccines. Millions of people, including millions of children, die each year from infectious diseases for which there is no effective vaccine. They include newly emergent diseases such as HIV/AIDS and ancient scourges such as malaria. Additionally, immunotherapeutic vaccines for certain diseases such as cancer are critically needed. It has been felt that the inability of previously existing technologies to develop the required vaccines is because of the different types of immune responses that have to be generated for certain diseases including the unique pathophysiological characteristics of those diseases. In addition, issues such as the manufacturing requirements for certain current vaccines make the

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older vaccines less attractive technologies for a global scale. More recently, a new impetus has been added to the generation of new vaccines: bioterrorism. The threat of the misuse of infectious agents has created an urgency to develop new vaccines that have an increased safety profile and which can be easily administered to large populations.

Immunological Issues for Vaccines

New efforts to develop vaccines emphasize inducing CD8+ cytolytic T lymphocyte (CTL) responses and antibodies because of the increasing recognition of the role and need for CTL in such vaccines. Likewise, efforts are being taken to develop vaccines that can induce specific types of T helper responses, Th1 or Th2. The traditional methods for developing vaccines are given in Box 1, which compares their characteristics with DNA vaccines. Examples of a live attenuated viral vaccine include vaccines for measles, mumps, and rubella which are given as a combined vaccine. This vaccine is extremely effective, protecting at least 95% of children from all three diseases. The efficacy of the vaccine in the U.S. is shown by the decrease from 500,000 reported cases of measles per year before the licensure of the measles vaccine in 1963 (*1*) to only 86 cases reported in 1999 (including children who had not been immunized). Recombinant protein vaccines are also quite efficacious with an example being the licensed recombinant hepatitis B vaccines that have been shown to protect at least 95% of recipients. Although viral vectors and DNA vaccines have comparative attributes as given in Box 1, they are only in early stages of clinical development. Thus there are no examples of these types of vaccines as licensed products.

Figure 1 illustrates, in a simplified form, the intracellular and intercellular interactions required for an antigen to result in the generation of both cytotoxic and helper T-cell responses, and antibody generation. The reason that recombinant protein or inactivated virus vaccines cannot generate the desired CTL response is that generally such a vaccine is taken up by an antigen-processing cell into the endolysosomal system, degrades into peptides, and then associates with major histocompatibility complex (MHC) Class II molecules. These peptide/MHC complexes stimulate Th cells rather than the cytolytic T cells. In order to generate the CTLs, protein synthesized within a virally-infected cell enters a cellular processing pathway from the cytoplasm that results in peptides associating with MHC Class I molecules. These in turn are recognized by the appropriate cytolytic T cells that then can be activated to kill the infected cell (*2*). Thus, if one could deliver a gene encoding an antigen into a cell (as a virus does during infection), the protein (in this case an antigen) following synthesis would be in the cytoplasm,

BOX 1. Comparison of vaccine technologies.

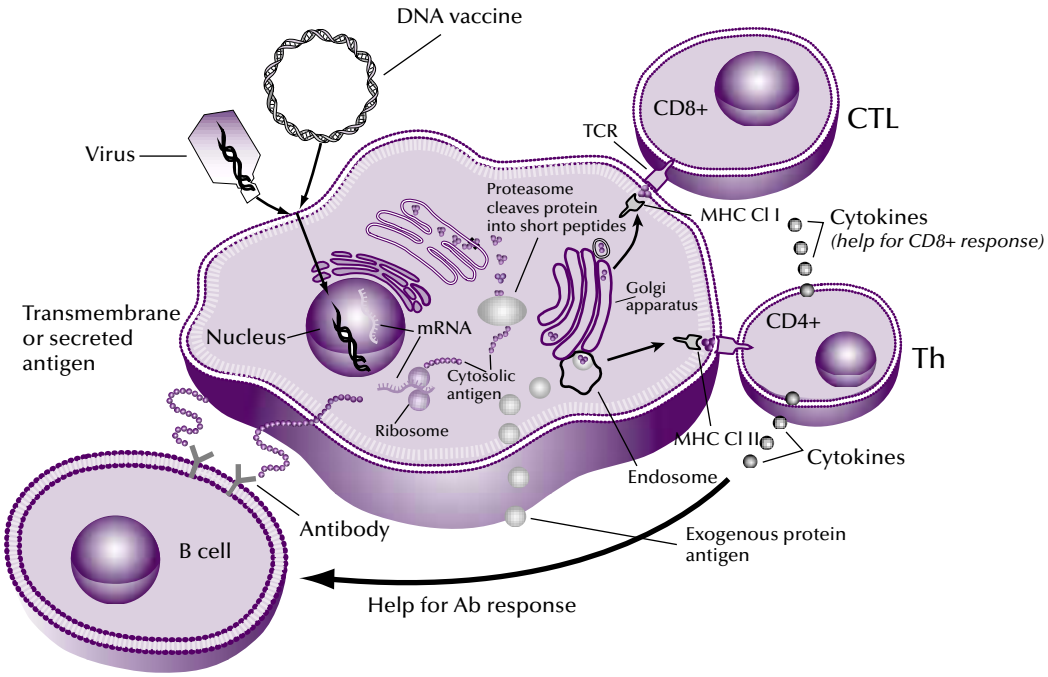
Live attenuated viruses
- Highly effective
- Potential risk for certain ones
- Manufacturing challenge

Recombinant proteins
- Potent antibody response
- Effective
- Non-native forms at times
- Do not induce cytolytic T lymphocytes (CTL)

Viral vectors
- Potential risk
- Resistance/pre-existing antibody
- Inflammation

DNA vaccines
- Need for increased potency
- Designer immune responses (e.g., type of helper T cell)
- Specificity: avoid deleterious or diversional antigens
- Relative stability
- Safety
- Generic manufacturing
- Cost advantage

FIGURE 1. Depiction of the mechanisms of generation of antigen-specific humoral and cellular responses.



Note: Professional antigen presenting cells take up an exogenous antigen (e.g., a protein outside of the cell) into its endolysosomal degradation pathway. The protein is degraded to peptides that associate with major histocompatibility complex (MHC) Class II molecules that then are exhibited on the surface of the cell. Specific helper T cells (CD4+ T cells) recognize this antigen peptide/MHC Class II molecule complex and are activated to produce "help" in the form of cytokines. These cytokines have myriad activities including, depending upon the cytokine, helping B cells activate into antibody-producing cells, and helping cytolytic T lymphocyte responses. Activation of cytolytic T lymphocytes (CD8+ T cells) generally is dependent upon an antigen-processing pathway reserved for intracytoplasmic proteins that are degraded into peptides that associate with newly synthesized MHC Class I molecules. These complexes, when presented on the surface of antigen presenting cells in conjunction with co-stimulatory molecules, result in the activation of the proper CD8+ T cells. For antibody responses, B cells recognize and respond to antigens that are either present extracellularly, or exposed extracellularly by being transmembrane proteins.

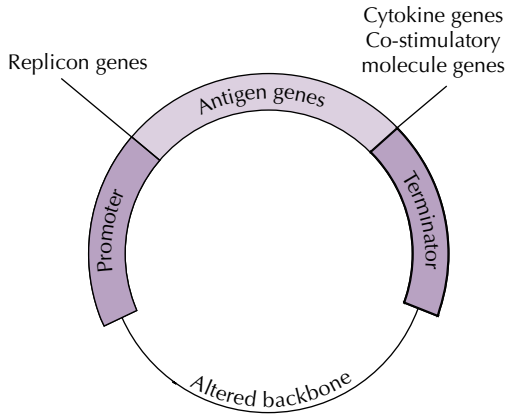
where some of it would enter the intracellular processing pathway resulting in the presentation of its relevant peptides on MHC Class I molecules for the stimulation of CTL.

The use of a live virus can be an effective means to accomplish this gene delivery with resultant CTL response. However, for certain viruses such as HIV, the use of a live virus, even attenuated, is considered too risky (3). As AIDS is currently a fatal disease, there is a possibility that the attenuated virus vaccine strain could revert to the wild-type or virulent strain as can occur for the oral poliovirus vaccine. In addition, certain live viruses have developed specific mechanisms to elude or downregulate the ensuing immune response. Many new technologies have been explored to specifically stimulate this MHC Class I-restricted CTL response without the concerns and limitations inherent in a live attenuated virus vaccine.

Characteristics of DNA Vaccines

Viruses have highly evolved structures and mechanisms that enable them to introduce their genetic material into infected cells. Therefore, despite emerging evidence in the 1980s, it was not until a 1990 publication by Felgner

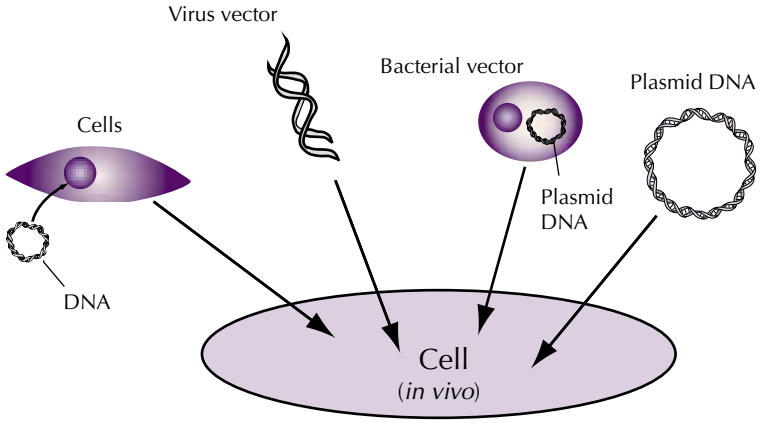
FIGURE 2. A schematic representation of a DNA vaccine.



Note: DNA vaccines are bacterially derived plasmids containing a gene encoding the desired antigen. Expression is driven by a promoter active in mammalian cells (generally a strong viral promoter), a transcription terminator, and often an antibiotic resistance gene that facilitates the selection of the plasmid during production in bacteria. Sites for increasing the potency of DNA vaccines are shown. For example, additional genes encoding cytokines or co-stimulatory molecules can be added to the gene for the antigen. Genes encoding a viral replicase have been shown to increase the potency of DNA vaccines. Alterations to the plasmid can also result in increased protein production, leading to increased immune responses.

and colleagues that the ability of simple plasmids of DNA (circular rings of DNA that exist extrachromosomally in bacteria) to directly enter mammalian cells when injected *in vivo* with ensuing synthesis of the protein they encoded (*4*) was accepted. The plasmid required no formulation or alteration other than a promoter active in mammalian rather than bacterial cells (see Figure 2). DNA plasmids as gene delivery vehicles have a number of advantages over other systems (Figure 3) which involve removal of cells from an individual in order to transfect them *in vitro* prior to reimplantation of the transfected cells, or which require the manipulation of viruses and bacteria (which are themselves pathogenic, immunogenic, or both)—a process significantly more complicated than manipulating and producing plasmids. But there were some concerns regarding their suitability and capability as vaccines. One of them arose from the original observation by Felgner and colleagues that the *in vivo* transfection of cells was still an inefficient process (*4*). Moreover, the cell type that took up the DNA and produced the encoded protein most efficiently were muscle cells, a cell type that under

FIGURE 3. Various methods of gene delivery.



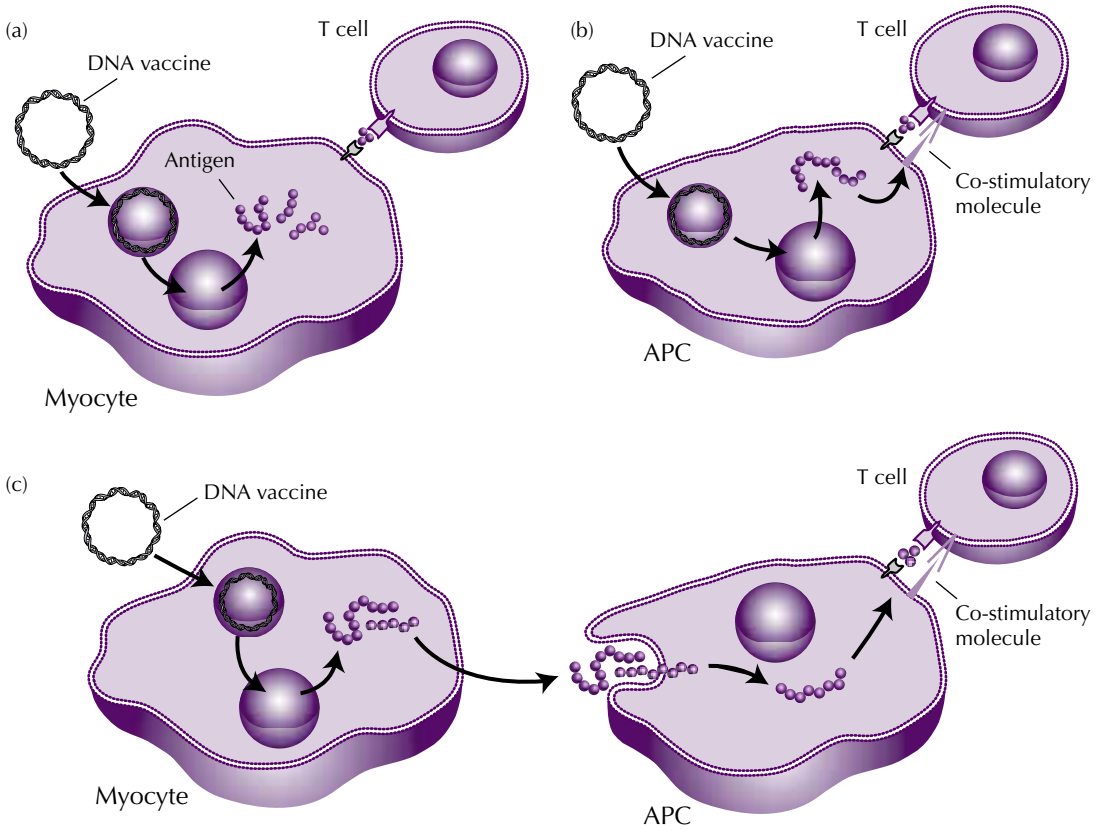
Note: Cells may be removed from the host, transfected *in vitro,* then re-implanted. Alternatively, a virus or bacteria can be modified such that it is no longer virulent, may be unable to replicate, and contains a gene encoding the desired antigen (and sometimes other viral/bacterial vector proteins). DNA vaccines are the simplest approach consisting of a plasmid encoding only the antigen.

normal conditions is not involved in the generation of immune responses.

Antigen Presentation Following DNA Vaccination

As shown in Figure 4(b), in order for a cell—which has synthesized an antigen—to successfully present antigen to a naive T cell resulting in the activation of the cell, interaction between other molecules on the surfaces of the T cell and the antigen-presenting cell (known collectively as co-stimulatory molecules) must occur in addition to the recognition of the antigen/MHC Class I complex by the T-cell receptor. Muscle cells are not professional antigen-presenting cells, and thus lack the co-stimulatory molecules (Figure 4a). Generally if a naive T cell encounters a cell bearing the correct antigen–MHC Class I complex in the absence of the co-stimulatory molecules, then the T cell becomes unresponsive to the antigen in the future, rather than activated. Thus, despite the ability of muscle cells to take up plasmid

FIGURE 4. Potential cellular interactions whereby DNA vaccines result in the stimulation of CD8+ cytolytic T lymphocytes (CTL).



Note: Although muscle cells take up DNA and produce protein more than other cell types when DNA is injected *in vivo,* muscle cells usually lack the co-stimulatory molecules needed as part of the CTL activation process (a). The mechanism for activation of CTL following DNA immunization may involve direct transduction of professional antigen-presenting cells (b). Another mechanism that has been demonstrated to occur is cross-priming wherein the muscle cell is transfected, produces the protein antigen, but then the antigen in some form is transferred to a professional antigen-presenting cell which then is directly responsible for activating the CTL (c).

DNA and synthesize the encoded antigen, it was not known whether the use of plasmid DNA would be effective for generating the desired CTL responses.

Initial Demonstration of Capability of DNA Vaccines

The initial publication by my colleagues and me (*5*) regarding the ability of plasmid DNA to result in the generation of CD8+ MHC Class I-restricted CTL following *in vivo* immunization with plasmid encoding an influenza protein, and the ability of this CTL response to protect mice subsequently given an otherwise lethal challenge with influenza, was thus considered to be a surprising demonstration of the capabilities of this approach. Subsequent work demonstrated that whilst the myocytes were transfected and produced antigen, the actual activation of T cells occurred because of cross-priming of professional antigen-presenting cells (*6–9*) (Figure 4c) and potentially the direct transfection of antigen-presenting cells (Figure 4b).

The protection observed in our initial work was cross-strain, that is, the mice were protected from challenge with a strain of influenza that was of a different subtype from the strain from which the gene for the viral protein had been cloned. Influenza, like HIV, mutates easily and hence can easily escape the antibody-based immune responses induced by the existing influenza vaccines. Antibodies are generally most effective when directed against surface or envelope structures and some of these can easily mutate without adversely affecting the robustness of the virus. CTL responses can be directed at epitopes from any protein of the virus regardless of its location in the virus. As some of the internal or functional proteins would thus provide epitopes for CTL, a major strategy of vaccine development has been to develop CTL responses against conserved viral proteins in order to develop vaccines that would be effective against a broader range of strains of a virus. Hence the demonstration that a DNA vaccine could induce protection that was effective against a very different strain of virus (a different subtype of influenza, and one that arose 34 years later) than the strain from which the gene was cloned opened the door for widespread development of DNA vaccines.

Preclinical Efficacy of DNA Vaccines

A large number of scientists and clinicians worldwide have now demonstrated the preclinical immunogenicity and/or efficacy of DNA vaccines in disease models of infectious diseases, cancer, allergy, and autoimmune diseases (Box 2) (*10–12*). In the category of infectious diseases, the models have included viral, bacterial, and parasitic diseases. The protection has been mediated by differing immune responses depending upon both the disease and the antigen. That is, CTL, antibodies, and different types of Th responses have been generated. The role of the type of T cell that helps in modulating immune responses is felt to be particularly important for the autoimmunity and allergic disease models.

Clinical Trials of DNA Vaccines

The compelling preclinical results propelled DNA vaccines into clinical trials for a number

BOX 2. Findings of DNA vaccine clinical trials.

Well-tolerated, safe
- No integration of DNA
- No autoimmunity
- No tolerance

Antibody responses
- Even in HIV-infected patients who did not make specific antibody with HIV infection (cytolytic T lymphocytes)
- CTL responses
- In naive patients
- Even in HIV-infected patients who did not make specific CTL with HIV infection

Th (helper T cells) responses

of diseases: HIV (both as a prophylactic and an immunotherapeutic vaccine), malaria, influenza, hepatitis B, and cancer. Whilst safety was demonstrated (*13–15*), and immune responses (both humoral (*13, 14, 16–18*) and cellular (*16–20*)) were generated, overall, the potency has been disappointing. Whilst most of the trials have utilized DNA vaccines injected intramuscularly, the hepatitis B DNA vaccine has been clinically tested by coating the DNA onto gold beads which are then propelled into the epidermis with a "gene gun." The so-called "gene gun" was actually the first means whereby, in an animal model, DNA was shown to be capable of *in vivo* delivery of a gene resulting in the generation of an antibody response (*21*). This device propels gold beads coated with DNA directly into the epidermal cells and immune responses have been demonstrated in a variety of systems (*22, 23*). In clinical trials with a gene gun, all vaccinees immunized with DNA encoding a hepatitis B antigen seroconverted, even those who had not responded to the licensed recombinant protein vaccine (*17, 24*).

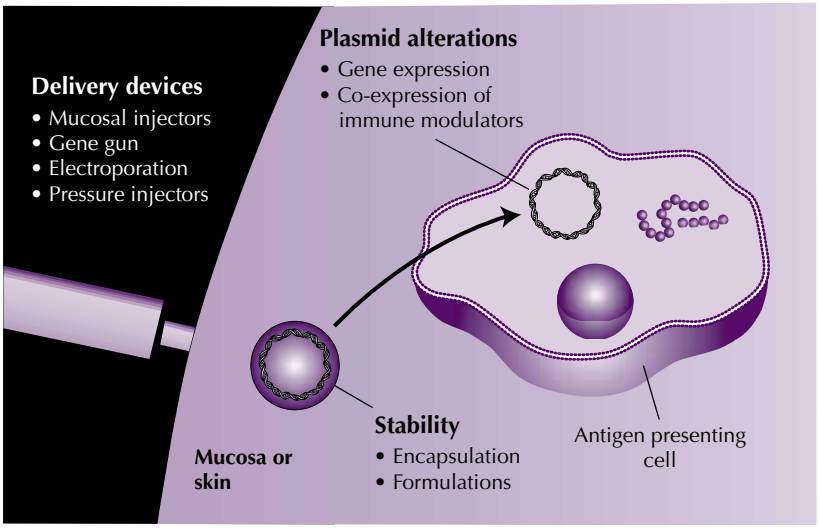
Interestingly, certain HIV-infected patients responded to HIV DNA vaccines with antibody (*16*) or CTL (*25*) responses against antigens to which they had not previously responded, which was not performed previously, despite living with high levels of antigen (virus), because of their infection. This underscores an observation that will be discussed below—that different gene delivery systems (whether natural infection, DNA plasmid, or other viral vector) result in different immune responses. This provides encouragement for the eventual success of developing vaccines against diseases such as HIV where natural infection—which had always been considered the gold standard for any vaccine—may not routinely induce immune responses adequate to clear the infection or provide protection against subsequent infection with a different strain. These results also set the stage for additional clinical trials for HIV and malaria where the DNA portion is intended to be the first component followed by a viral vector such as adenovirus or poxvirus encoding the same antigen genes.

Second Generation DNA Vaccines

A variety of approaches are under evaluation to increase the potency of DNA vaccines (see Figure 5) whilst still retaining their attractive features. Some of these are based upon devices to increase the transfection of cells or to target the DNA to specific sites, whilst also providing a means to avoid the traditional syringe (to facilitate global administration). These include propulsion devices targeting either the mucosa or benefiting from the transfection of Langerhans' cells in the skin. A mucosal jet injector device has been utilized in a clinical trial of an HIV DNA vaccine (*26, 27*). The advantage of targeting the mucosa is that most pathogens enter the body via the mucosa, so that a vaccine administered mucosally may generate better mucosal (versus only systemic) immune responses. Electroporation devices are being evaluated that greatly increase the uptake of DNA into cells and expression of encoded protein (*28, 29*) by delivering small amounts of electric current *in vivo* to briefly cause the formation of holes in cells locally in order to permit more of the injected DNA to enter the cells.

Encapsulating DNA inside or onto entities such as microparticles (*30, 31*), or into bacteria (*32, 33*) is another means of either protecting the DNA from degradation and/or enhancing its uptake into antigen-presenting cells. Adjuvants such as aluminium salts likewise have been shown to increase the potency of the DNA vaccines (*34*). Interestingly, the DNA itself has been shown to play a role in the immunogenicity of DNA vaccines (*35–37*). This is because the bacterial DNA sequences result in the plasmid having a different methylation pattern than mammalian DNA. These sequences then activate the innate immune system, resulting in a greater antigen-specific immunity than would occur otherwise. However, to date, it is not yet known exactly as to how to manipulate the backbone sequences of the plasmid to fully exploit these observations. Addition of genes encoding cytokines or costimulatory molecules (*38, 39*) is also a promising means to augment the potency of immune

FIGURE 5. Second generation DNA vaccines.



Note: DNA vaccines with increased potency that are under development include vaccine delivery devices that inject the vaccine into the mucosa or epidermis without the use of needles. Alternatively, the uptake of the DNA into cells can be increased by the addition of small bursts of electric current by a process known as electroporation. The DNA can be encapsulated into microparticles to protect the DNA from degradation and to facilitate the uptake of the DNA by antigen presenting cells. The cellular mechanisms for transfection, DNA expression, and antigen processing also provide targets for increasing potency.

responses or increase the protection observed in preclinical challenge models, or to alter the profile of the immune responses (such as the type of T-cell help).

Mixed Modality Vaccines

A very promising strategy that is entering clinical trials is to combine DNA vaccines with other gene-delivery systems. Interestingly, it has been observed in a variety of preclinical systems that if DNA encoding an antigen is given as a prime, followed by another gene-based vector system (such as a recombinant poxvirus or adenovirus) encoding the same antigen, the immune responses and protection are significantly greater than if either vector is utilized for both the prime and boost, or if the order of administration is reversed (*40–42*). Whilst the mechanisms for this increased potency remain to be established, the approach is being applied for HIV and malaria vaccines in clinical trials.

Other Applications of DNA Vaccines

The DNA vaccines, or simply plasmids, have also found utility as a research tool. For example, whilst the field of genomics has revolutionized science with the elucidation of whole genomes of pathogens and living beings, one of the limitations has been to translate the sequence information into functional knowledge. Knocking out specific genes in mice strains is certainly a useful approach, but cumbersome. Expressing the genes as proteins *in vivo* or *in vitro* can be carried out with viral vector delivery systems, but again, these are relatively time-consuming to make. Plasmid DNA can easily be utilized *in vitro* or *in vivo*.

For pathogen genes, it is possible to develop DNA vaccine libraries (*43*) and use them to determine which genes encode protective antigens without even knowing what the gene encodes or the function of its corresponding protein. DNA vaccines have also been utilized to make polyclonal and monoclonal antibodies. This has enabled antibody production as a reagent without the need to purify the antigen, or to recombinantly produce and then purify the antigen in order to immunize for developing the antibodies.

Conclusion

The DNA vaccines thus, in the decade since the initial demonstration of their efficacy, have rapidly advanced in clinical trials, with second generation formulations, delivery devices, and mixed modality approaches holding great promise for new vaccines and immunotherapeutics. At the same time, they are being utilized as research tools to help mine the vast amount of genetic information that has arisen from the field of genomics. The hope is that the fundamental simplicity of DNA vaccines combined with the sophisticated understanding of immune mechanisms and molecular biological manipulations will result in a platform technology useful for a variety of diseases (Box 3).

BOX 3. Applications of DNA vaccines under development.

Clinical applications
- Infectious diseases
  - Vaccines
  - Therapy
- Cancer
  - Vaccines
  - Therapy
- Autoimmune diseases
- Allergy

Technology toolbox
- Functional genomics
  - Antigen identification
  - Proteomics
- Reagent generation
  - Polyclonal antibodies
  - Monoclonal antibodies

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ORAL VACCINES DERIVED FROM TRANSGENIC PLANTS

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SUMMARY

Plant-made pharmaceuticals (PMPs) are organic molecules or recombinant proteins produced in plants and used for human or animal health. Subunit vaccines are a category of PMPs that have been validated in various studies, including human clinical trials. Current efforts at product formulation use food processing technology to convert transgenic plant materials to dried samples that can be delivered in unit doses with assurance of product uniformity and quality. Research findings gathered to date indicate that plant-derived vaccines offer product advantages which include: oral delivery, heat stability, lower cost for manufacture of active ingredient, and suitability of manufacturing technology for use in developing countries.

BACKGROUND

Advances in molecular biology and plant biotechnology have allowed the production of vaccines, antibodies, and other human and veterinary therapeutics to be attempted via gene expression in transgenic plants. The earliest publication relating to the use of this concept for vaccine manufacture occurred in 1990 in an international patent filing (*1*). Less than two years later, the first peer-reviewed publication in this field described the expression of hepatitis B surface antigen in transgenic potato (*2*). Numerous additional publications have since been issued describing subunit antigens from many pathogens (*3–6*). A quite comprehensive list of vaccine antigens produced in plants has been provided (*7*).

I have worked collaboratively with a team of scientists over the last decade in an evaluation of the value of plants for vaccine production. We identify four major milestones as hallmarks of our studies in gaining "proof of concept" for the concept (*8–18*).

- First, insertion of genes encoding antigenic proteins of human pathogens resulted in successful expression and assembly of multicomponent structures within plant cells. These structures, which mimic the native immunogens, include "virus-like particles" (VLPs) for the hepatitis B surface antigen (HBsAg), Norwalk virus capsid protein (NV capsid), and oligomeric B subunit of the heat labile enterotoxin of *E. coli* (LT-B) either by itself or in association with the enzymatically active A subunit to form a holotoxin (LT). (Similar studies also have been completed for cholera toxin, CT.) Other than introduction of the genes encoding the antigens with an appropriate DNA vector modified to optimize gene expression, no

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further cellular engineering of the plant cells was required to obtain immunogens resembling the native pathogen proteins. Subsequent studies, which are continuing in several laboratories around the world, are verifying these findings for other antigenic proteins from human and animal pathogens.

- Second, oral immunogenicity of HBsAg, LT-B, and NV capsid was demonstrated by providing plant material expressing these antigens directly to animals as feed. While two of these are from enteric pathogens, which might be anticipated to contain mucosally active immunogens, hepatitis B is not an enteric pathogen and is usually not thought to invade the body via the gut. The emerging results portend success with different types of antigens through oral immunization, albeit with very significantly higher levels of immunogen than would be required for injection.
- Third, in phase 1 human clinical trials, LT-B and NV capsid were found to stimulate both humoral and mucosal immune responses (as evidenced by serum and mucosal antibody responses) and HBsAg gave a strong boosting response in volunteers who had previously received the yeast-derived, injected commercial vaccine. Although the immune response to NV capsid was modest in amplitude, it was achieved with unprocessed plant tissues (raw potato) with no adjuvants, buffers, or additives; in all human clinical trials, the immunogens were active simply when the plant sample was eaten.
- Fourth, in unpublished studies, we have found that standard food industry freeze-drying technology can be used for multiple plant tissues (including tomato, potato, and carrot) to yield heat-stable, antigen-containing powders. Freeze-dried tomato powder containing NV capsid and LT-B has been found to be immunogenic in preclinical trials, and studies of other antigens are under way. Different batch samples can be blended to give uniform doses of antigen and can be stored at room temperature without antigen loss.

The next major milestone in development of plant-based vaccines will be animal and human clinical trials to show effectiveness of plant-derived vaccines in establishing protective immunity.

Over the last decade, the opportunity to engineer plants for production of subunit vaccines was embraced by researchers and noncommercial funding agencies as a new paradigm for vaccine manufacture and delivery. I estimate that more than 40 laboratory teams worldwide have explored the utility of plant-based antigen production using genes encoding at least two dozen different antigens derived from infectious disease agents. At the outset, the idea of delivering oral vaccines to recipients in developing countries via consumption of plant material was characterized as "edible vaccines." As the concept has evolved to embrace a goal of obtaining licensed products under strict biologics regulations, our research focus has shifted to production of refined plant products which will be administered as formulated unit dose materials. This chapter will emphasize the significant potential for plant-expressed antigens as vaccines, the tools of molecular biology that have been used to drive immunogenic subunit proteins in plant tissues, and recent efforts to use food processing technology to yield dried powders derived from transgenic plant tissue that can be used for unit dose vaccine formulations.

The Need for New Vaccine Manufacturing Technology

According to the World Health Organization (WHO), infectious diseases account for approximately 25% of all deaths worldwide, 45% of deaths in low-income countries, and 63% of deaths in children worldwide. It has been estimated that approximately 30 million children born each year are not adequately immunized by modern standards. WHO has stated, "The majority of deaths from infectious dis-

eases can be prevented with existing, cost-effective strategies." Such strategies include expanded utilization of available and emerging vaccine technologies. The development and introduction of new vaccines for the poor in developing countries faces many challenges. The vaccines must address the need for lower costs, oral activity, heat stability, and mucosal effectiveness, and they must include combination vaccines and those that protect against diseases that occur predominantly in developing countries. A consideration of these factors follows.

Lower Costs

The costs of future human vaccines are projected to be considerably higher than current vaccine production costs. Several factors drive these projections.

Regulatory hurdles in developed countries, particularly for construction of production facilities and for final quality control, have increased dramatically in recent years. Almost all new vaccines are first produced in these developed countries, requiring that the vaccine candidates be tested in the countries of origin according to their high regulatory standards. (Developing a candidate vaccine first in a developing country is a new strategy being pursued in a few cases.)

Largely because of the dramatically higher costs of meeting the regulatory hurdles, intellectual property rights have taken on crucial importance. Many new vaccines are produced with proprietary methodologies and patent holders vigorously prosecute their rights or conceal know-how because of its inherent intellectual property value.

In large multinational pharmaceutical companies, vaccines must compete for R&D resources against other products with high profit potential, such as those against heart disease and cancer. Thus, when these vaccines enter the market they must generate comparable returns on investment.

This combination of factors and the resulting higher costs of new vaccines have caused concern about the potential availability of these vaccines to the poor. Traditionally, governments and international and national assistance agencies have had to pay only pennies per dose for vaccines. New vaccines, such as that against *Haemophilus influenzae* type b, cost $2 or more per dose, or about 10- to 20-fold more.

Oral Activity

Orally active vaccines are sought because they obviate the need for injection equipment with its associated costs and risks of unsafe injection. The procurement, distribution, use, and disposal of syringes and needles present continuing impediments to the delivery of vaccines. Of great concern is the high risk of unsafe injection caused by reuse, poor sterilization, and misuse. Oral activity also is important because it permits vaccines to be delivered by a wider range of service providers, and requires production and formulation regulations that may be less rigorous than those governing injected products.

Heat Stability

Heat stability is prized because it reduces the need for expensive cold-chain systems. Maintenance of the cold chain and ensuring its reach in remote areas are proving to be daunting challenges for ensuring continued high levels of coverage for existing and new vaccines.

Mucosal Effectiveness

Mucosal effectiveness is important because it is seen as the most powerful means to prevent diseases that are caused by infections at the mucosal membranes.

Combination Vaccines

Combinations are highly valued because they reduce the need for multiple injections or administrations. The early operation of the Global Fund for Children's Vaccines through GAVI (the Global Alliance for Vaccines and Immunization) has shown that developing countries accord very high priority to combination vaccines.

Diseases Occurring Predominantly in Developing Countries

Most modern vaccine research relies extensively on collaboration with large pharmaceutical firms or biotechnology companies in developed countries. As a result, priority setting is invariably affected by the companies' need to serve their markets, which has led to a neglect of several important diseases that affect people in developing countries.

With the exception of combination vaccines, there has been little progress in addressing the challenges listed above. Cost of production continues to increase. Little research is under way to prepare orally active vaccines. GAVI has identified the use of sugar-glass technologies to improve heat stability of existing vaccines, but this technology can only increase the cost of the vaccine. Numerous combination vaccines are under development but they represent no savings and, in some cases, the cost of the combined vaccine is more than the sum of the cost of the separate vaccines. Although some research is being conducted on mucosal delivery of vaccines, most of the work is at an elementary level.

Vaccine Biomanufacturing

Conventional vaccine technology often depends upon purification of the immunogenic entity from mammalian cell cultures or tissues, yeast, fertilized eggs, or bacterial fermentation systems. The resulting product usually requires refrigeration during delivery to final point of use, which adds significant costs to the vaccination program. Dried plant extracts containing subunit vaccines may provide a solution in an ambient temperature-stable product, equivalent in storage and transport characteristics to dehydrated food products. In addition, the production of antigens in plants may improve product safety by removing animal cell-related contaminants.

Oral subunit (non-replicating) vaccines have not yet achieved commercial success using any means of manufacture. Factors which make oral delivery of immunogenic proteins difficult include the likelihood that the proteins will be subjected to degradation in the gut and that some immunogens may be minimally recognized at mucosal immune effector sites in the gut. As a result, higher concentrations of immunogen are likely to be required in oral vs. parenteral delivery. Although this is a potential limitation, the use of plants as a protein biomanufacturing system offers advantages in that the cost of obtaining the end product is comparatively low. In addition, accumulating empirical evidence suggests that encapsulation of the immunogenic protein within the plant cell matrix during administration provides protection from enzymatic degradation by gastric enzymes.

The best candidates for oral subunit vaccine production by transgenic plants are those proteins which are primary antigens in the case of natural infection, and which aggregate in forms that are recognized at mucosal sites where an immune response is triggered. These include viral surface proteins that co-assemble to form virus like particles (VLPs), and bacterial toxins which naturally aggregate to form mucosally targeted multimeric complexes. In addition, in several laboratories there are also ongoing efforts to produce a variety of fusion proteins that target immunoresponsive mucosal sites (*19*, *20*).

What Plants Will Be Best for Biomanufacture of Vaccines?

Early studies of plant-derived subunit vaccines utilized transformation systems for tobacco (*2*). The toxicity of tobacco precluded its use for oral delivery, but allowed immunogenicity studies of partially purified extracts (*8*). Most of the work my colleagues and I have done has focused on the use of potato (*9–13*), tomato (*14*), carrot (unpublished data), and tobacco cell suspension cultures which have diminished toxic alkaloid content (unpublished data). Potato and tomato were originally chosen due to their wide use in global diets and the availability of reliable transformation systems, as well as because of the comparably short time that elapses from genetic transfor-

mation to obtaining fruit or tubers for bioassay. The duration of this process can be reduced to as little as four months to obtain samples sufficient for preclinical feeding trials, whereas other systems (particularly monocotyledonous plants) require significantly longer periods to obtain prototype materials. We also have experimented with banana as a novel production system, but have had to deal with a time frame of three or more years to obtain fruit-specific expression of antigenic protein (*15*). This makes banana a technically difficult candidate for vaccine production.

Unit Dose Plant-derived Oral Vaccine Technology

The ability to produce oral vaccines in transgenic plants and also minimize the cost of product delivery will very likely be related to our ability to derive stable and concentrated products from otherwise perishable tissues. The major concern is the variability in expression, accumulation, and stability of antigens within tissues of the same plant or clones thereof. While variability of expression among plants or even in tissues of a single plant has been observed and will continue to be anticipated, this must be overcome to achieve uniform doses for delivery. The use of freshly harvested produce such as fruit, tubers, roots, foliage, or any other plant tissue is limited due to the relatively short shelf life of these biological materials. We have, therefore, focused our efforts on finding inexpensive sample stabilization technology, primarily by drying plant materials to yield batch quantities of temperature stable material that can be stored, shipped, and administered at ambient temperatures. Our primary strategy is to utilize one or more food processing technologies to reduce freshly harvested, antigen-containing plant tissues to a stable formulation, whereby the protein of interest is encapsulated within the preserved plant cell in a dehydrated state. The resulting material allows us to address issues of homogeneity, stability, concentration of antigen, and addition of adjuvants.

The fate of plant-derived vaccines will depend on the ability to provide a consistent dose, with appropriate clinical data to support the minimal and maximal doses to be applied. Batch manufacturing and quality assurance can only be validated if the vaccine material provides consistent antigen concentration within an acceptable range. An advantage of applying food processing techniques is the concentration of protein achieved. Material mass can be reduced by as much as 94% (11% of the initial weight for potato, 6% for tomato). Additionally, the physical qualities of this powdered material conveniently allow formulation for oral intake by palatable methods, such as in gelatin capsules or as reconstituted into liquid. We have evaluated several processed formulations expressing model antigens of interest for ambient stability of the desired protein at regular periods up to 12 months. Pharmaceutical preparations from either potato or tomato were stable at room temperature and equivalent to identical samples stored under dry conditions at –80°C (unpublished data). This indicates that efficient dehydration is an effective means of providing a protective state for antigens, which remain encapsulated within plant cells and partly disrupted cell debris.

Our ongoing studies utilizing food processing techniques allow us to conclude that standardized and ambient-stable material can be derived from any perishable plant source. The processing stages provide opportunities for the addition of excipients such as adjuvants, buffers, antioxidants, or other protein stabilizers, to easily create a final formulation as desired for administration or storage. Mixing batches of dry material could easily produce multivalent or multicomponent vaccines.

Development and Licensure of Plant-derived Vaccines

Depending on what methodology is used, conventional pharmaceutical development of a single product is estimated to have an average

price tag between US$ 110 million and US$ 800 million, and to take 12 to 15 years for licensure. Development of conventional vaccines may be somewhat less costly, although new recombinant DNA-derived subunit vaccines produced in fermentation-based systems are likely to be similar to protein pharmaceuticals in development costs. To date, research in the production of transgenic plants as a technology base of oral vaccines has little more than a decade of developmental history, and may have a combined investment of under US$ 30 million (divided among commercial and noncommercial sources) spread across more than 40 research groups worldwide. At present, none of the major pharmaceutical companies has a development effort directed to plant-derived vaccines for infectious diseases. The reasons relate to:

- doubts about the potential for significant return on investment;
- uncertainties in the regulatory processes for licensure;
- limited human clinical trial data for establishing required dosages, timing of delivery, and evaluation of possible adverse immunological effects; and
- a lack of personnel in pharmaceutical companies with needed plant biology research and development expertise.

Given this, the development of vaccines using plants as a biomanufacturing system is a classic example of a situation in which reliance on market forces fails in the development of needed health products. The public sector and the nonprofit sector will be essential to provide leadership and investment support to unlock the potential of plant-derived vaccines.

A principal justification for public sector promotion of plant-based vaccines is the significant characteristics of this technology for serving as the preferred technology base to manufacture vaccines against rare and neglected diseases. Developing new pharmaceuticals for these diseases is not a high priority for most current commercial ventures due to the low profit margins.

Confronting Genetically-Modified-Food Issues

Plant production of vaccines is a technology that has the potential to produce great transformations. As often occurs with such developments, however, the use of a similar technology for agricultural biotechnology has stimulated significant public debate, mainly focused on genetically modified foods. Knowledgeable public debate is valuable, but the debates over genetically modified foods, for example, have not always been based upon scientific considerations. As a result, these debates have become polarized.

Because plant-derived pharmaceuticals are not intended for use as food products, the crops that produce them must have special stewardship to ensure crop genetic containment. In addition, the proteins they produce will have to be separated and purified in processing plants solely devoted to that purpose. Protocols setting good manufacturing practices must be redefined for pharmaceutical plant materials, as well as for processing and handling practices that utilize the raw product. The use of crop species that currently are part of the food supply to produce oral vaccines will mandate crop stewardship (genetic separation from the food supply) as an essential parameter for any production of these materials. Such containment can be provided by appropriate greenhouse facilities, or by significant geographic isolation from related crops. Transfer of technology for manufacture in locations such as developing countries will require equal standards for crop stewardship to ensure integrity of the product, and of the technology as a whole. As pharmaceutical materials, all such tissues will be highly controlled and could not be released like other transgenic plants used for modern agricultural commodity production. It is likely that global health organizations such as PAHO and WHO will play an essential role in the process of transferring the technology on a global scale as new regulatory frameworks are implemented.

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NEW ADJUVANTS

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Over the past few decades, major new breakthroughs in the understanding of molecular biology have allowed the field of vaccine development to move from more of a trial-and-error based, observational science to rational vaccine design. New generations of technologies have emerged, all of which are of interest but none of which has yet yielded products that are effective in public health. This chapter discusses three of the more than 25 adjuvant systems that have been developed at GlaxoSmithKline (GSK) since 1990.

First and foremost, an adjuvant's objective is to enhance the immune response to a given vaccine antigen, particularly to a purified- or recombinant-produced single antigen. In several instances, adjuvants could help boost immune responses to particular subtypes of a given pathogen. They can enhance antibody responses or cell-mediated responses, or certain subtypes of cell-mediated responses. Most importantly, adjuvants could also allow the targeting of vaccines to high-risk populations, such as the elderly and cancer patients. They have also opened the way to the potential development of therapeutic vaccines that can be used to treat patients who are chronically infected, particularly with pathogens that bias the immune response, such as HIV, hepatitis B or C, *Mycobacterium tuberculosis*, and others. Because of the many aims that vaccines can have, different adjuvants may be needed to meet particular needs.

GSK has designed various adjuvant systems based on a combination of "immunostimulants" and "vehicles." Immunostimulants are molecules that exhibit immunomodulating properties. Vehicles can be inert, such as aluminium salts (also known as alum), or have immune system activation properties due to their particular nature, such as stable oil-and-water emulsions or liposomal structures. Three of the many adjuvant systems that have been assessed are discussed in this chapter.

The first immunostimulant, monophosphoryl lipid A (MPL) (*1*), is derived from the cell wall of *Salmonella minnesota*. It is a detoxified form of lipopolysaccharide, which is known to activate monocytes. The second, QS21 (*2*), is a purified fraction of Quil A (extracted from the bark of the *Quillaria saponaria* tree) and has membrane-interacting properties that may impact antigen presentation properties. The third is a DNA sequence enriched with CpG motifs.

The first, and simplest, adjuvant system described is adjuvant system 4 (AS04), which combines a traditionally used adjuvant—aluminium salt—with MPL. This system is primarily used for a series of vaccines that are in phase 2 and phase 3 clinical trials; these vaccines primarily target adolescents, and focus particularly on sexually transmitted infections.

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The second system is adjuvant system number 2 (AS02), which combines an oil-and-water emulsion with MPL and QS21. MPL and QS21 have been shown to be synergistic in inducing antibodies as well as in enhancing TH1 and cytotoxic T-cell response against exogenous protein. This adjuvant system is very powerful and is being used in a number of vaccines against highly complex pathogens, such as *Plasmodium falciparum*, HIV, and *Mycobacterium tuberculosis*.

The third system, presently called ASX, is the most powerful and is being used in the development of therapeutic cancer vaccines, among others therapeutic vaccines. Each of these adjuvant systems will be exemplified with vaccine candidates currently being evaluated at the preclinical or clinical level.

The first adjuvant system, AS04, was used in the development of the GSK herpes simplex vaccine (*4*). The vaccine is based on the recombinant glycoprotein of the virus envelope—glycoprotein D—combined with AS04. This adjuvant system was selected because it enhances antibody responses and biases the immune response towards the TH1-type T-cell response. In a mouse experiment that compared this vaccine with one based on glycoprotein D with aluminium salt only, there is an enhancement of antibody responses and a change in the isotypic ratio that is produced, which indicates a TH1 T-cell response (Figure 1). This is confirmed by the induction of a high cell-mediated immune response with high interferon gamma (INF-γ) production (the marker of the TH1 T-cell response) and low interleukin-5 (IL-5) production (the marker of the TH2 cell response) (Figure 2).

This novel adjuvant system also allows for an enhanced protective immune response in the genital herpes guinea pig model, in which female guinea pigs are infected in the genital tract and actually develop disease. In this model, very good protection is obtained with the formulated vaccine (Figure 3).

Two phase 3 trials of the aforementioned herpes simplex vaccine with AS04 were designed very similarly to phase 3 trials conducted with another vaccine by Chiron at the same time. The Chiron vaccine used a TH2-inducing adjuvant called MF59. It is in a stable oil-and-water emulsion with glycoprotein D, combined with a second glycoprotein from the virus envelope.

In the trial of the GSK herpes simplex vaccine, the vaccine conferred absolutely no protection in the men immunized; however, it produced very significant levels of protective antibodies in women (Figure 4). This surprising outcome was reproduced in a completely independent clinical trial conducted in Canada by GSK Biologicals. Unfortunately, these trials were designed based on the assumption that they would be effective in both men and women; gender-specific protection was not expected. GSK is therefore conducting a very large phase 3 trial in the United States in collaboration with the National Institutes of Health (NIH) that aims to demonstrate efficacy in females.

The second vaccine, AS02, is a vaccine against *Plasmodium falciparum*. After a mosquito injects the parasites (in sporozoite form) into the human host, they have a few seconds to reach the liver. There, the sporozoites infect the hepatic cells, where they develop into merozoites. The merozoites then rupture the hepatic cells and enter the bloodstream, invading the erythrocytes and initiating the blood stage that is responsible for the clinical disease.

The immune response that the vaccine seeks to induce is to block the parasites from reaching the liver, which is very difficult because of the very short period of time available to do so. Most importantly, this vaccine, and in particular the adjuvant system, aims to induce a T-cell response that can either block or kill infected hepatocytes so that the blood stage does not occur.

The vaccine antigen was designed to take advantage of GSK's experience with hepatitis B surface antigen (HBsAg). HBsAg was fused with both B-cell and T-cell epitopes of *Plasmodium falciparum*'s circumsporozoite protein (CSP), the major outer surface protein of the

parasite. The primary particles look very much like hepatitis B and are produced as recombinant protein, or particles in yeast; they are already in industrial production.

A U.S. Food and Drug Administration-approved trial has been conducted in collaboration with the Walter Reed Army Institute of Research (*5*). This trial compared three adjuvant formulations of the GSK vaccine antigen, RTS,S: AS04, AS02 (both previously described), and AS03 (an oil-in-water adjuvant system).

In this trial, human volunteers were vaccinated with one of the vaccine candidates and then challenged with *P. falciparum* via the bite of infected mosquitoes. All three vaccine adjuvant formulations elicited an antibody response, with AS02 and AS03 eliciting the highest responses. The AS04 and AS03 formulations only marginally protected those subjects that received them (one of eight, and two of seven, respectively), while the AS02 formulation conferred very clear protection in the subjects immunized with it (six of seven), indicating that protection was not related solely to antibody titers.

The RTS,S/AS02 vaccine has been tested in a field trial in Uganda and is undergoing field trials in Mozambique. In field trial conditions in young adults, the vaccine candidate yielded very promising results, although protection lasted only three or four months. GSK is currently working on improvements to the vaccine to enhance the duration of protection and is testing it in toddlers and infants, a population at high risk that suffers up to 2 million deaths per year. This work is being done with the collaboration and support of the Malaria Vaccine Initiative.

AS02 is also being used in the development of GSK's HIV vaccine. Monkeys were immunized with a combination of envelope and nonstructural or regulatory proteins of a particular strain of HIV virus. The animals (four per group) were challenged with a partially heterologous strain of a chimeric virus, SHIV (simian-human immunodeficiency virus). The objective of those formulations was to induce T-cell responses that could manage the viral load and help the monkeys control, and potentially clear their infection. In the control group, one of the four monkeys spontaneously managed its viral load; the other three had high viral loads (Figure 5). Interestingly, the group using AS02 (also known as AS02A) and a combination of envelope-like protein exhibited very clear control of the viral load over a period of two-and-a-half years since the experiment was started.

Only the animals in the group vaccinated with the full combination (gp120/Neftat/SIV nef/AS02A) controlled their CD4 response (Figure 6). This experiment was repeated twice. In the first instance, the same results were obtained; in the second, using primates of a different origin, less interpretable results were seen. A clinical trial with human volunteers is currently being conducted in collaboration with NIH to advance the concept.

The only group of animals that survived more than 120 weeks was group 2, which was vaccinated with the gp120/Neftat/SIVnef/AS02A adjuvant formulation and antigenic mix, suggesting that in this model, only the full combination of antigens and adjuvant used were effective (Figure 7).

The last adjuvant system to be discussed is ASX. The objective in the development of this formulation was to maximize every possible immune response that can be induced in order to help achieve therapeutic immunization for various chronic infectious diseases or for cancer. A mouse model was developed for breast, prostate, or lung cancer antigen. The animals were immunized with antigens in various adjuvant formulations and the immune response induction and resistance to tumor cell challenge were observed.

A comparison of the AS02 formulation of the vaccine containing breast cancer antigen with three other adjuvant systems (AS01:MPL/QS21-based, AS07:CpG, and ASX) showed that all the systems are very potent at inducing antibody responses and very good lymphoproliferative responses (Figure 8). Those responses are further enhanced with the ASX system,

which strongly enhanced INF-γ production and decreased IL-5 production.

Mean tumor growth observed after the mice were immunized four times and then challenged with breast-tumor cells showed that the ASX formulation controlled tumor growth better than the AS02 formulation did (Figure 9). ASX with tumor-specific antigens from lung, breast, and prostate cancer is currently being taken into clinical trials to establish their potential in the field.

In conclusion, GSK has developed various adjuvant systems; this chapter discussed three of these systems. Some have a safety profile that is completely comparable with that of aluminium salt, and GSK is developing them for use in young populations (at this stage either adults or adolescents) to develop vaccines against sexually transmitted infections, such as herpes simplex virus (which is in phase 3 trials) human papillomavirus, hepatitis B virus, and others.

GSK has also developed the more powerful AS02 adjuvant system, which has a slightly enhanced local reactogenicity profile, but is fully compatible for use in young populations, including infants, as approved by the U.S. Food and Drug Administration

All trials that have been conducted, such as those in Africa for malaria, are run under investigational new drug regulations. We are very hopeful that these trials will result in the development of vaccines against diseases where we have all failed up to now, such as for malaria or HIV, which are highly relevant to the developing world. Finally, GSK hopes that ASX will be a helpful tool to establish vaccines against cancer and other diseases.

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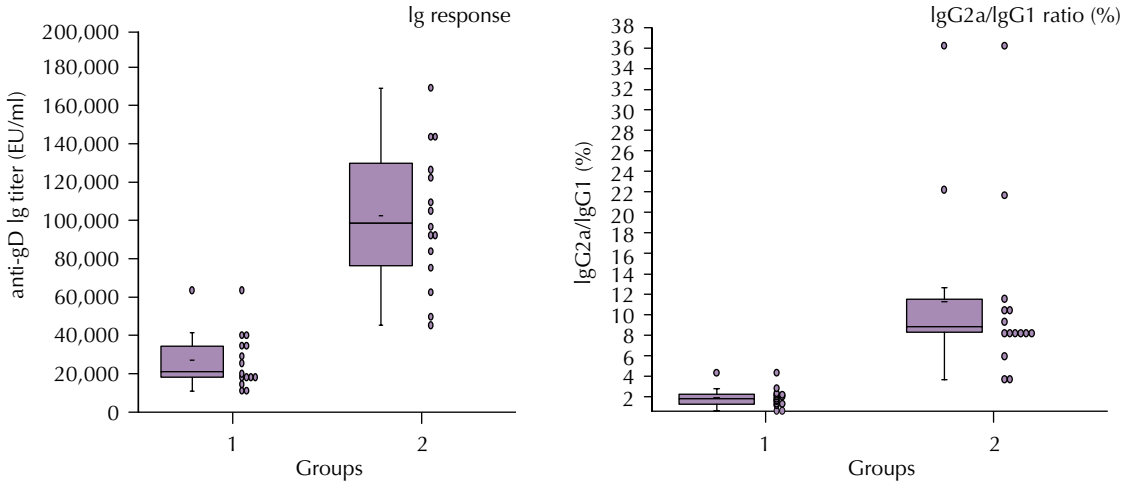
FIGURE 1. Comparison of antibody responses and isotypic ratios produced in mice by glycoprotein D-based herpes simplex vaccines with different adjuvant systems (aluminium salt versus aluminium salt with MPL [AS04]).



Groups: (1) gD/Alum; (2) gD/AS04

FIGURE 2. Comparison of cell-mediated immune responses and interferon-γ and interleukin-5 production in mice immunized with glycoprotein D-based herpes simplex vaccines with different adjuvant systems (aluminium salt versus aluminium salt with MPL [AS04]).

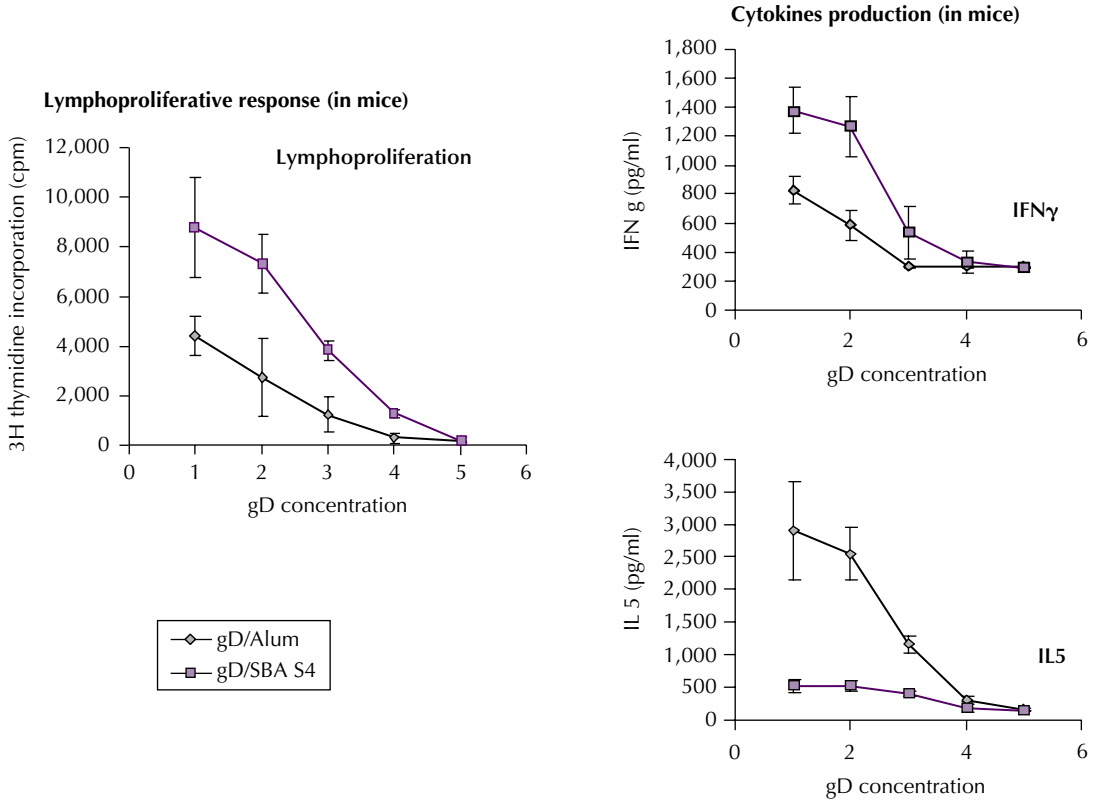
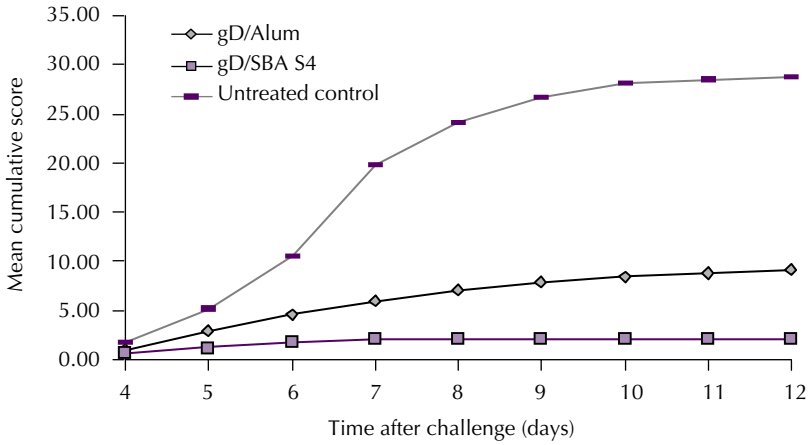


FIGURE 3. Comparison of protection[a] conferred by glycoprotein D-based herpes simplex vaccines with different adjuvant systems (aluminium salt versus aluminium salt with MPL [AS04]) in guinea pigs.



[a] Protection is measured through the decrease of the mean cumulative titers of genital lesions observed during the course of the full experiment.

FIGURE 4. Comparison of protection conferred by herpes simplex virus (HSV) vaccine in men and women.

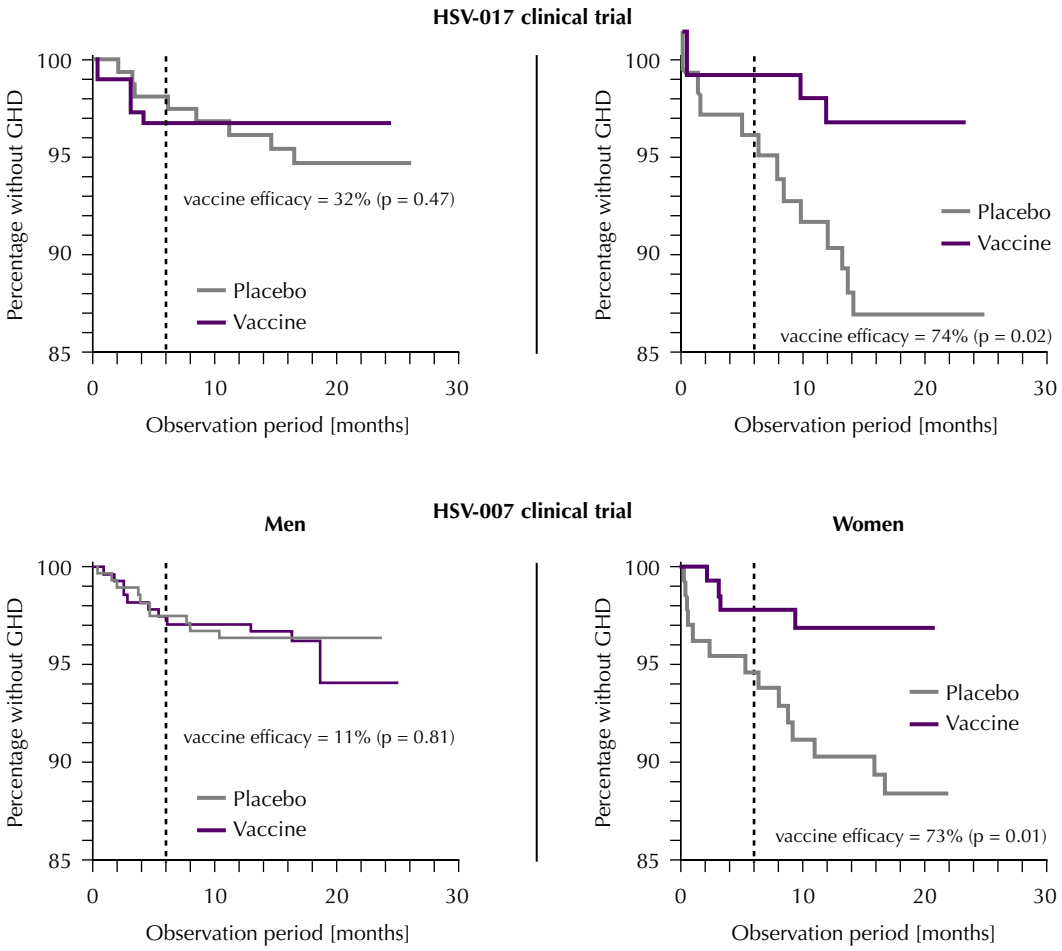


FIGURE 5. Control of plasma virus load after immunization with various human and simian immunodeficiency virus vaccine formulations (AS02A, AS06 CpG-based formulation).

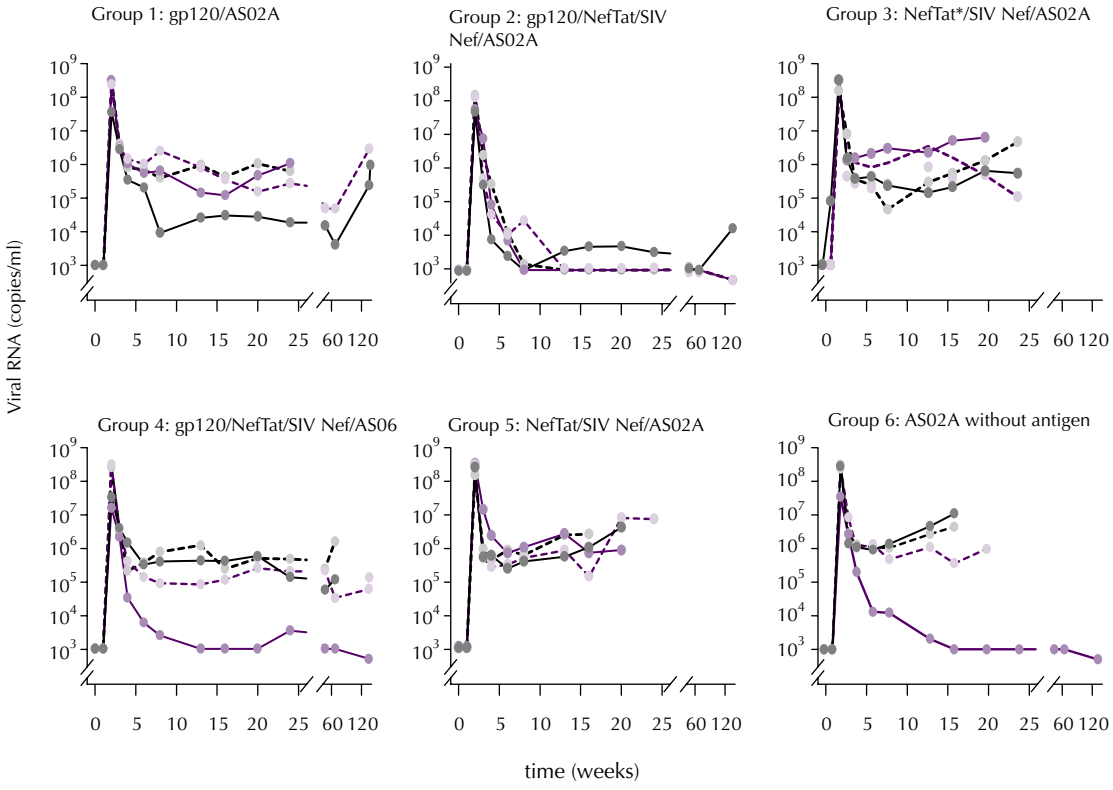


FIGURE 6. CD4+ counts after immunization with various human and simian immunodeficiency virus vaccine formulations.

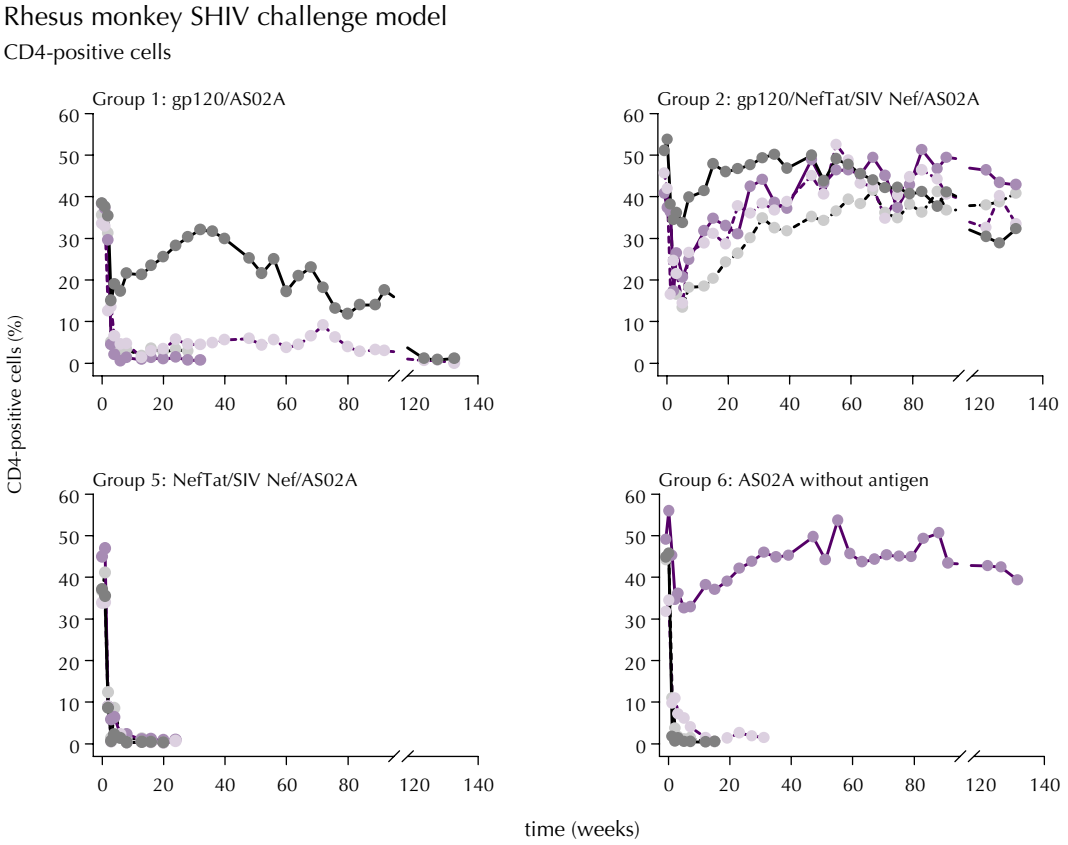


FIGURE 7. Survival of animals immunized with various human and simian immunodeficiency virus vaccine formulations after simian-human immunodeficiency virus challenge.

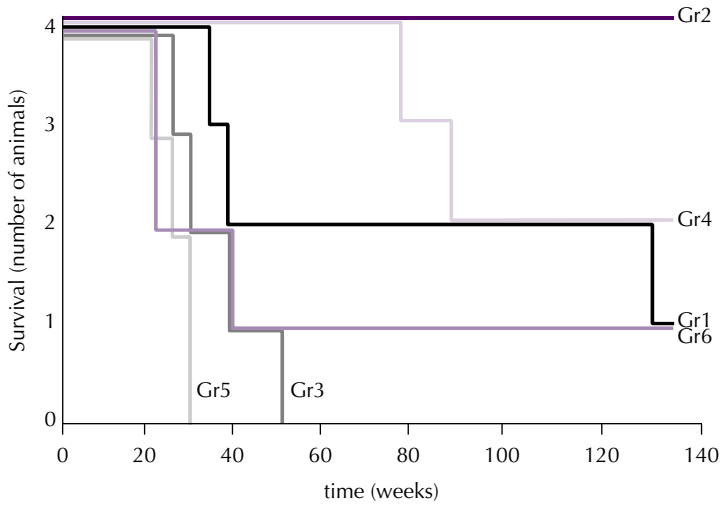


FIGURE 8. Immune responses of mice vaccinated with breast, lung, or prostate cancer antigen with differing adjuvant formulations and subsequently challenged with the respective tumor cells.

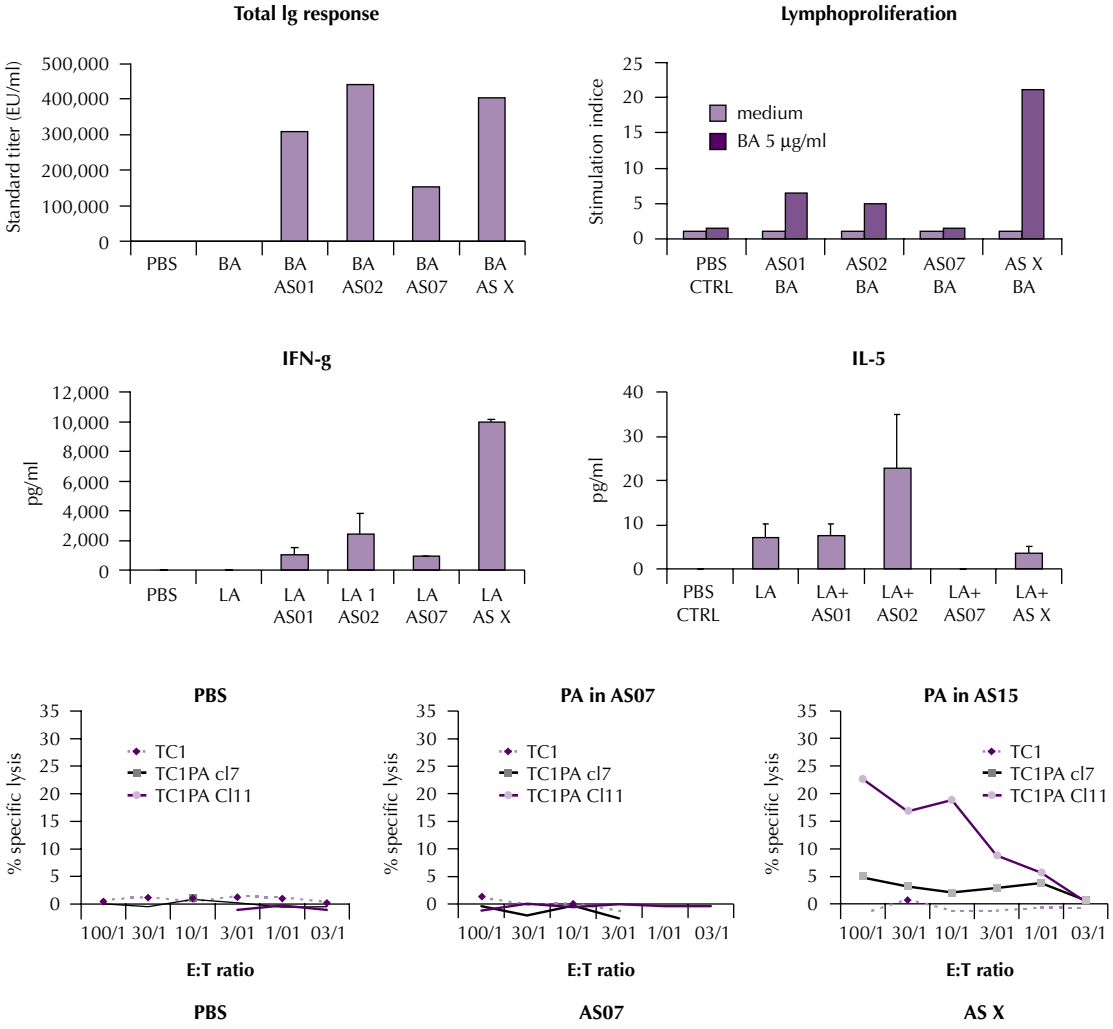
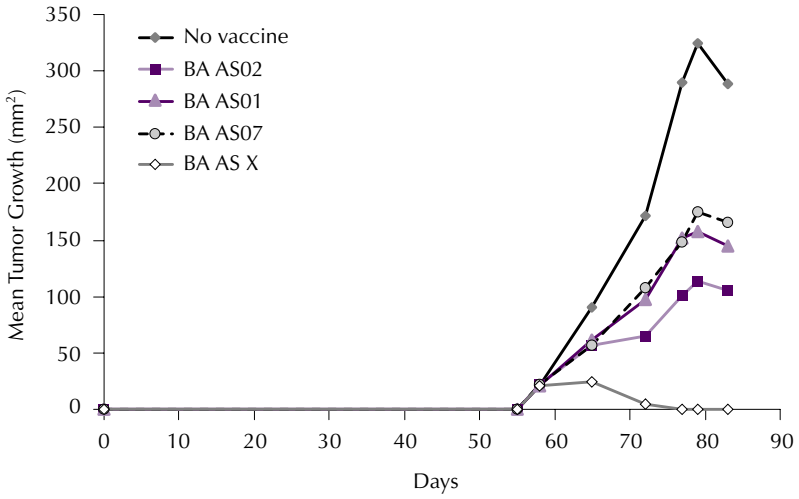


FIGURE 9. Mean breast tumor growth in mice immunized with differenct adjuvant formulations of breast cancer vaccine, following challenge with breast tumor cells.



THE POWDERJECT PARTICLE-MEDIATED EPIDERMAL DELIVERY OF DNA VACCINES: A NEW TECHNOLOGY

John Beadle[1]

INTRODUCTION

This chapter will provide basic background on PowderJect's particle-mediated epidermal delivery (PMED) of DNA vaccines, a technological advance that many individuals may not be familiar with. A description of the PowderJect hepatitis B project, which is a collaboration between PowderJect and GlaxoSmithKline, will follow, as a case study to demonstrate what has been achieved with this technology in the clinic to date. In the final section, the future opportunities for this technology and the use of genetic adjuvants with the PowderJect system will be explored.

THE POWDERJECT TECHNOLOGY

The epidermis, which acts as a barrier between the body and the exterior environment, is highly adapted to deal with external insults. Indeed, any insult that enters the body in the normal course of life that is not either inhaled or ingested will have to pass through the epidermis. The epidermis has thus structurally and functionally evolved into a highly efficient immunological organ that is very rich in professional antigen-presenting cells (APCs). With a traditional needle and syringe approach, epidermal APCs are not accessible, because the epidermis is a very thin structure relative to the size of even the smallest gauge needle. Injecting a DNA vaccine intramuscularly, subcutaneously, or even intradermally would thus bypass this important immunocompetent organ. Furthermore, in the case of DNA vaccines, the DNA needs to be targeted intracellularly in order to produce an effect, since the DNA first needs to be intracellularly transcribed and then translated into proteins before it can be processed and presented by the APCs. Needle and syringe administration cannot deliver DNA vaccines directly into APCs, and thus must rely upon passive uptake of extracellular DNA, either directly into local cells or via the lymphatic system. This has significant implications for the amounts of DNA that need to be administered by needle and syringe in order to produce an immunological effect. Therefore, an ideal DNA vaccine delivery system would deliver the DNA directly into the APCs of the epidermis. In essence, this is what the PowderJect delivery system does.

The PowderJect delivery system can be divided into two components that need to be optimized. The first is a gas-powered device for delivering powdered vaccines to the epidermis at high velocities. The second is a particulate formulation of the vaccine that is of the correct size and density to penetrate the viable epider-

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mis. In the case of PowderJect DNA vaccines, this formulation is composed of microscopic gold particles coated with a DNA plasmid.

In the original, reusable, experimental PowderJect XR1 device (so called because the "X" stands for "external gas source" and the "R" for "reusable"), depressing the trigger actuates the solenoid, and a burst of high-pressure gas is allowed to pass through the cylindrical cartridge. The cartridge is coated with a layer of DNA-coated gold particles. The high-velocity gas stream passing through the cartridge entrains the gold particles and accelerates them down the nozzle to near-supersonic speeds. The gas stream is vented through spaces at the end of the nozzle, but the density of the gold particles gives them a momentum that carries them into the viable epidermis. In the XR1 device the DNA cartridge is replaced after each administration, and the large external gas supply makes this a reusable device that might, for example, be a useful modality for mass immunization campaigns.

In a PowderJect ND device (the "N" denotes an internal gas source, and the "D" stands for "disposable device"), the gold is contained in a small capsule that has a thin membrane on either side. The gas source in the ND device is a small microcylinder of helium gas. When the button is actuated, the seal of the gas microcylinder is broken and the gas pressure builds up very rapidly in the rear chamber. The membranes of the capsule burst at a predetermined gas pressure, and the gold particles are entrained in the gas stream, accelerating to near supersonic speed down the device nozzle. The bursting of the membranes causes a popping sound, so a silencer has been added to the ND device to minimize the sound heard by the patient. (As for the XR1 device the gas is vented, in this case through the silencer, but the momentum of the gold particles is such that they will be delivered into the viable epidermis.)

PowderJect's gold DNA plasmid-coated particles have a mean diameter between 1 to 3 microns. The particles must possess the correct size and density characteristics to ensure a momentum that will carry them through the stratum corneum and into the viable epidermis, which is rich in APCs. Optimization of the PowderJect system has involved a series of what might be called *pharmacoballistic* studies, in which the gas-driving pressure, particle size, density, and payload have been varied in order to achieve a configuration that ensures consistent delivery through the stratum corneum and into the viable epidermis. We now typically use 2 μm of DNA on 1 mg of gold per dose. This is an exceptionally small dosage of DNA compared to the amounts required by needle and syringe administration.

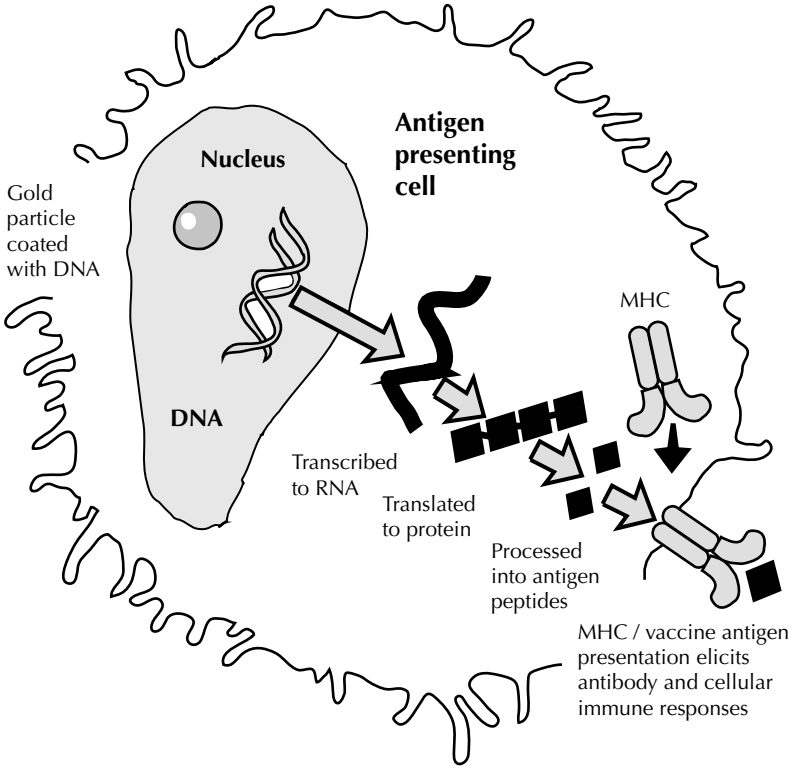
Because the gold particles are so small, they are able to penetrate into the cells of the viable epidermis, and some particles will penetrate directly into or alongside the nucleus of an APC. When the DNA elutes off of the gold, it is already intracellular or even intranuclear and ready for cellular transcription, translation, and antigen processing as shown in Figure 1. By imitating the intracellular processing of antigen, the PowderJect PMED DNA system is thus able to stimulate both humoral and cell-mediated immunity (CMI). An ability to stimulate significant CMI is important in the quest for therapeutic vaccines for the treatment of chronic infectious diseases, such as HIV or viral hepatitis, but also in fields that are entirely new for vaccines, such as oncology and allergies.

In addition to its density characteristics, elemental gold is also inert. Furthermore, because of the very rapid turnover of cells in the epidermis, gold particles are very quickly eliminated from the body, and after a few days it is no longer possible to detect gold in the epidermis.

The PowderJect Hepatitis B Project

In this section, the hepatitis B study project—the most advanced of the DNA vaccine programs to date—will be highlighted as a case study for what has been achieved with the PowderJect DNA vaccine system so far. All of the studies presented in this section have used the prophylactic hepatitis B expression vector

FIGURE 1. Mechanism of action for DNA on gold vaccine.



pWRG7128, which encodes the entire hepatitis B surface antigen (HbsAg), and all studies have used the XR1 device.

There have been five studies conducted to date, the data from which three will be presented here. Study 1A was what could be described as a *pharmacoballistic* study, and has previously been reported by Tacket and colleagues (*1*). Escalating gas-driving pressures were used in order to assess local tolerability. The results of this study show that with the correct pressure and payload it is possible to achieve a well-tolerated administration of gold particles. Typically there is some local erythema that starts within a few minutes and lasts for three to five days. In general terms, in this and subsequent studies, PMED has been shown to be a very well-tolerated form of DNA vaccination. There have been a few cases of transient hyperpigmentation that resolved after 10 to 60 days.

Study 1B was a dose range-finding study, looking at humoral and CMI immunological endpoints using various DNA dose levels in 12 volunteers. This study has been reported previously by Roy and colleagues (2). The volunteers were all naive for hepatitis B vaccination. The amount of DNA delivered was varied by altering the payload of DNA-coated gold particles per administration and the number of administrations at each time point (Table 1). All three groups received dosing on days 0, 56, and 112. The maximum cumulative dose of DNA was thus 3 μg for group 1, 6 μg for group 2, and 12 μg for group 3.

Table 2 shows that seroprotective humoral antibodies were achieved after the second

TABLE 1. Dosing schedule for study 1B.

Group number	Number of subjects	Nominal dose level	Dosing days	Deliveries per dosing	Maximum cumulative dose
1	4	0.5 μg DNA 500 μg gold	0 56 112	2	3 μg DNA on 3 mg gold
2	4	1.0 μg DNA 500 μg gold	0 56 112	2	6 μg DNA on 3 mg gold
3	4	1.0 μg DNA 500 μg gold	0 56 112	4	12 μg DNA on 6 mg gold

TABLE 2. Seroprotection rates for study 1B following each administration.

	Seroprotection rate		
Group	Post prime	Post boost 1	Post boost 2
1	0/4	1/4	4/4
2	0/4	0/4	4/4
3	0/4	1/4	4/4

booster dose in all three groups although the absolute levels achieved are not equivalent to those which would normally be seen with conventional vaccination.

Table 3 summarizes the CMI responses seen in study 1B. From these results a very high CMI response rate is seen in these previously naive subjects, with 8/8 evaluable subjects showing T-cell responses after the second booster.

For clinical study 1C, preliminary data have previously been reported by Poland et al. (3). The design of the trial is very interesting in that it investigates the use of DNA vaccine in individuals who have either failed to respond to conventional vaccines or whose titers have waned subsequent to conventional vaccination. The three groups examined were Nonresponders (previously received a three-shot vaccination course but have not seroconverted), Highly Nonresponsive (previously received 6–9 vaccinations but have not seroconverted), and Waning Titer (completed 2–4 vaccinations with a titer > 100 mIU/ml; waning defined as either < 10 mIU/ml or "negative," or < 50% of their previous levels). The preliminary data show that 28 days after receiving a single administration of the PowderJect vaccine 2/6 (33%) of the Highly Nonresponsive subjects, and 4/4 (100%) of the Nonresponders, had seroconverted.

To summarize the clinical results to date, DNA PMED vaccination with an HbsAg-encoding plasmid has elicited both humoral and CMI immune responses at doses that are in the region of 20 to 2,500-fold less than that required for administration with needle and syringe intramuscularly (2). This is an efficient, effective, and well-tolerated form of vaccination.

Genetic Adjuvantation

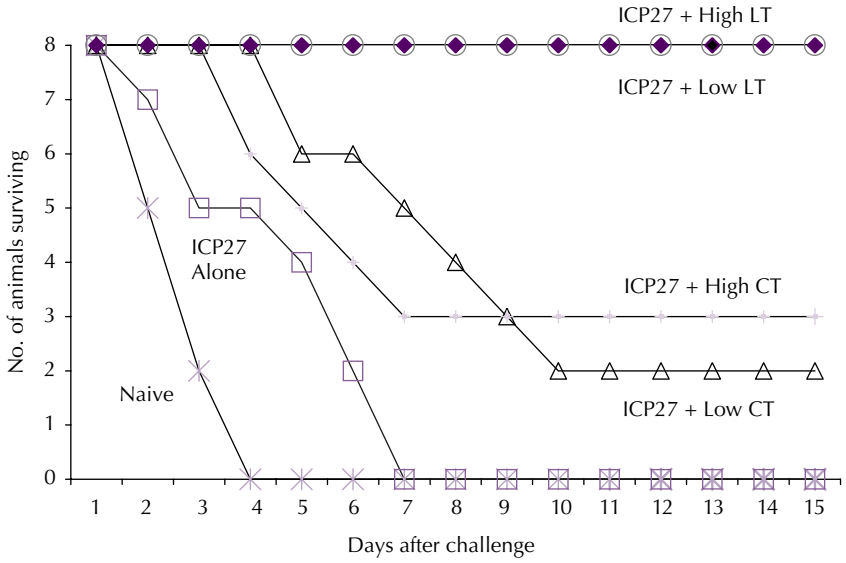
As we continue to explore the PowderJect technology we are discovering yet other interesting potential applications and modifications for PMED DNA vaccination. While none of these has yet reached the clinic, there are many possibilities for the future. Perhaps the most exciting of these is the use of genetic adjuvants. In the same way that DNA vaccines use DNA encoding the desired antigen in order to elicit humoral and cell-mediated immunity, we can also combine this with DNA encoding for an adjuvant to enhance and modify the immune response.

Preclinical data already exist to demonstrate the use of genetic adjuvant plasmids that encode for subunits of bacterial enterotoxins. Figure 2 illustrates data from a mouse challenge

TABLE 3. CMI response rates for study 1B following each administration.

Immune response	Methodology		Response rates		
			Post prime	Post boost 1	Post boost 2
T-helper cell responses	ELISPOT measuring relative frequencies of T cells secreting either IFN-g (Th1) or IL-5 (Th2), following *in vitro* stimulation with purified HbsAg protein for three days; determined in 12 volunteers	γIFN (Type 1) T-helper cell responses	3/12	7/12	7/12
		IL-5 (Type 2) T-helper cell responses	0/12	0/12	3/12
MHC I-restricted T-cell responses	ELISPOT for IFN-g secreting HbsAg peptide-specific cells in the eight volunteers determined to be HLA-A2		1/8	1/7	8/8
Cytotoxic T-cell responses	Cytotoxicity assays (chromium release) against HbsAg-peptide-pulsed target cells in the two volunteers determined to be HLA-A2.1		—	—	2/2

FIGURE 2. Effect of CT and LT vectors on protection against HSV-2 challenge in mice.



model for HSV2. In this model, naive mice nasally challenged are all dead within four days. Using an experimental HSV2 plasmid called ICP27 administered with the PowderJect device, survival can be enhanced but all mice are still dead by day 7. Administering the ICP27 plasmid in combination with a plasmid-encoding cholera toxin (CT) further enhances survival such that at 15 days post-challenge either two or three mice out of eight are alive, de-

pending upon the dose level of CT plasmid used. Most impressively, the coadministration of ICP27 plasmid with a plasmid encoding for the *E. coli* heat labile toxin (LT) eliminates animal death during the 15-day follow-up period, regardless of the dose level of LT plasmid used.

These initial data, which have subsequently been supported using larger mammalian species and alternative plasmid systems, demonstrate that potent genetic adjuvantation using the PowderJect PMED system is feasible. Furthermore, it has been demonstrated that genetic adjuvantation has the potential to enhance and modify both humoral and CMI immune responses. This discovery opens up further opportunities for this technology's use for both prophylactic and therapeutic vaccines.

Conclusion

In summary, the PowderJect PMED DNA vaccine delivery system is ideally suited for DNA vaccines. It targets DNA to the intracellular compartment of epidermal antigen-presenting cells. It is very well tolerated, and it is able to produce both humoral and CMI immunity with doses of DNA several orders of magnitude lower than with conventional needle and syringe administration. Genetic adjuvants can further enhance the immunogenicity of DNA vaccines. The PowderJect system opens the door for exciting new prophylactic and therapeutic vaccines that may be targeted not only at infectious diseases but also at other diseases such as cancer and allergies.

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Part VI

Vaccines and Bioterrorism

SMALLPOX VACCINE

Donald A. Henderson[1]

INTRODUCTION

Twenty-five years ago, the final chapter for smallpox appeared to have been written. To be sustained, smallpox had to be transmitted from person to person in a continuing chain of infection, because there is no animal reservoir. Thus, when Ali Maalin became infected in Merka, Somalia, on October 26, 1977, and no further cases were found, he was presumed to be the last case in a chain of virus transmission extending back at least 3,000 years (*1*). In May 1980, the World Health Assembly certified smallpox eradication and recommended that routine vaccination be stopped. All countries did so by 1983. Vaccine production also stopped. My colleagues Frank Fenner, Isao Arita, Zdeno Jezek, and Ivan Ladnyi, and I wrote *Smallpox and Its Eradication*, expecting that the book would be primarily of archival interest and was destined for historical obscurity (*1*). With the newly perceived threat that smallpox might be used as a biological agent, however, the book is now out of print and smallpox is back on the world's agenda.

THE THREAT OF SMALLPOX AS A BIOLOGICAL WEAPON

Dr. Ken Alibek, formerly deputy director of the Soviet bioweapons program, wrote in *Biohazard* (*2*), "On May 8, 1980, WHO announced that smallpox had been eradicated from the planet. . . . Soon after WHO's announcement, smallpox was included in a list of viral and bacterial weapons targeted for improvement in the (Soviet) 1981–1985 Five-Year Plan. . . . Where other governments saw a medical victory, the Kremlin perceived a military opportunity . . . the Soviet military command issued an order to maintain an annual stockpile of 20 tons."

The site where the smallpox virus was produced in such large quantities was Sergiyev Posad, about 45 miles northeast of Moscow (*2*). It was, and still is, a secret facility under the Ministry of Defense. The site for research on methods for large scale production of smallpox virus was the VECTOR laboratory in Novosibirsk, which continues, even now, to do smallpox virus research. We suspect that smallpox is retained in as many as two to four other sites in Russia. Meanwhile, the former Soviet Union's economic problems have led many scientists to leave those laboratories. Some have come to the United States, some went to Europe, and some have spent time in countries such as Iraq, Iran, and Syria. Thus, it is possible that today the smallpox virus might be present in laboratories of several countries. Meanwhile, routine vaccination was stopped in the United States in 1972 and worldwide in 1983. At this point, there is a very large susceptible population, such as has not existed before in history. Because *variola major*, the Asian form of smallpox, carries with it a 30% case-

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fatality rate, there is cause for serious concern should it be released. If smallpox were to recur, only two actions are possible to deter and stop it—the isolation of patients and vaccination. There are no effective antiviral drugs or other therapies.

Smallpox Vaccine in History

It was in 1796 that Edward Jenner, an English country physician, performed the first vaccination, taking material from the hand of a milkmaid, Sara Nelms, and inoculating it into the arm of a boy, James Phipps (*3*). Some six weeks later, he inoculated James with smallpox virus but the boy exhibited no illness. Jenner then took material from the pustules of vaccinees and vaccinated others, thereby demonstrating that cowpox virus could be passed from person to person. It was a dramatic discovery, widely acknowledged to be one of the most important medical discoveries of all time. Until late in the 19th century, smallpox vaccination continued to be propagated by arm-to-arm transfer.

Toward the end of the 19th century, vaccinia virus, as it came to be known, began to be grown on the flank of a calf (*4*). The animal was first shaved and then washed; the flank was extensively scarified; and then vaccinia virus was applied. After one week, the animal was sacrificed; the pustular material was scraped off, centrifuged, and packaged. It was obviously not a sterile product, but it served the world well in providing protection against smallpox and, ultimately, in eradicating the disease. So far as we were able to determine, there were no serious complications resulting from the presence of the few persistent non-pathogenic bacteria that remained in the vaccine.

The first international standards for smallpox vaccine were established in 1959 (*3*, *5*). They called for 7.5 logs of virus per ml and a bacterial count of less than 1,000 non-pathogenic bacteria. By 1965, the minimum titer was raised to 8.0 logs of virus, in recognition of the fact that most vaccinations in the course of the eradication program would be performed in tropical and subtropical areas where refrigeration is problematical (*6*). A higher titer product provided greater assurance that the vaccine would be potent at the time of delivery, even if storage conditions were less than optimal. As time progressed and production methods improved, the bacterial count usually found in the vaccine declined to less than 10 organisms per ml. Meanwhile, beginning in the 1950s, a number of dose-ranging studies were done to ascertain the optimal virus concentration for use. They showed clearly that vaccine with a titer of 8.0 logs contained from 10 to 50 times more virus than was required to obtain a satisfactory take among previously unvaccinated persons. Such studies were repeated this past year, which showed yet again that the vaccine could be satisfactorily diluted and still produce satisfactory takes (*7*). With a 10-fold dilution of the vaccine, however, a point is reached on the curve where any further loss in titer is apt to result in increasing failures of vaccination, especially among those who have been vaccinated previously.

In 1967, when the global program began, there were a total of some 64 laboratories engaged in producing vaccine, using more than 20 different vaccine strains (*8*). The New York City Board of Health (NYCBOH) strain was the one used in the United States and in most of the laboratories of the Americas. The Lister strain (from the Elstree Laboratory, United Kingdom) was used in several European countries. These two strains appeared to be equally protective and engendered fewer serious vaccination reactions than did other vaccinia strains. In 1968, the World Health Organization asked the National Institute of Health in the Netherlands to produce batches of the Lister strain for distribution to production laboratories around the world. By 1971, that strain had been adopted for use by 39 laboratories.

An important step taken in the 1960s was the adoption of the seed-lot system for producing vaccine. Before this step was taken, the normal procedure was to take a small amount of vaccinia that was harvested from a calf and use it to inoculate the next animal. Under the

seed-lot system, the laboratory prepared a large batch of vaccine—a seed lot—and stored it under refrigeration. When new batches of vaccine were to be prepared, an aliquot of the seed lot was used for inoculating the calves. With this approach, there was little likelihood that the characteristics of the vaccine would change as a result of passaging the virus. That the virus did change in character after repeated passages was recognized by the producers. Thus, they would periodically test the vaccine by vaccinating a group of children. If the vaccination response was considered to be deficient, the vaccinia strain was then passed through intermediate hosts (e.g., rabbit testis to human and back to calves). With continuing passage of the virus, the vaccine strains in the different laboratories, even if bearing identical strain names, could have quite different characteristics both in terms of safety and efficacy.

In 1985, vaccine production stopped. Indeed, in the course of WHO surveys it was determined that by 2000 there were no existing vaccine production laboratories anywhere in the world. WHO surveys in the late 1990s revealed that there were between 60 million and 80 million doses of vaccine in storage worldwide, although how much was still fully potent was not known (WHO, unpublished report). The United States had 15 million doses.

The quantity of available vaccine was insufficient to deal with more than a limited outbreak. A further problem was the availability of bifurcated needles. The bifurcated needle had been invented by a Wyeth Laboratory scientist and had been utilized in eradication programs throughout the developing world (*1*). There were only a few hundred thousand needles in storage, and the original manufacturer had stopped producing them. The importance of the needles related both to their efficacy as a vaccination instrument and the fact that they required much less vaccine than did traditional techniques. The smallpox vaccine was packaged in vials as a freeze-dried product which, after reconstitution, contained 0.25 ml. Using traditional methods, a drop was transferred to the skin and several pressures or scratches were made through the drop. A vial provided about 25 doses. The bifurcated needle used only one-fourth as much vaccine. When it was dipped into the vaccine and withdrawn, vaccine was held by capillarity between its two tines and it was used to make 15 rapid punctures over a small area. The vial provided enough vaccine for 100 doses and the estimates of the quantities of vaccine being held in storage assumed that the bifurcated needles would be used. Thus, without the special needles, the number of vaccinations that could be performed would be far fewer than the quantities of vaccine reported to be in storage.

Preparations for Response in the United States of America

Following the events of September 11, there was considerable concern about the possibility of a terrorist attack utilizing a biological weapon. Smallpox was the most feared of all possible agents. Vaccine, needles, and Vaccinia Immune Globulin to treat possible complications of vaccination were urgently needed. Of greatest concern was the vaccine.

There was confidence that the 15 million doses of calf-lymph vaccine, in storage since 1978, could be diluted five-fold, and this was verified in special studies. Still, given the fact that nearly half the United States population had never been vaccinated and that immunity was waning in others, this was far from enough even for the United States, were it to be seriously threatened by a developing epidemic. Moreover, with the possible spread of smallpox internationally, the occurrence of smallpox anywhere on earth had to be recognized as a threat to all nations; few countries possessed smallpox vaccine.

A decision was made by Tommy Thompson, Secretary of Health and Human Services, that enough vaccine should be procured to secure the equivalent of a dose for everyone in the country. It was decided that additional supplies of vaccine would have to be produced using modern techniques of tissue cell culture rather than calves. However, a vaccine grown in a to-

tally different manner must be considered as a new vaccine, and its properties have to be fully evaluated. Normally, five or more years are required to equip and validate new production facilities, develop and test the vaccine, and fulfill all of the requirements for licensing a new product. Heroic efforts are in progress to greatly accelerate this effort. A team under the direction of Dr. Phillip Russell and comprised of senior scientists from the country's National Institutes of Health (NIH), Centers for Disease Control and Prevention (CDC), and the Food and Drug Administration (FDA) are working with the manufacturer in a priority effort to have more than 200 million doses of the vaccine licensed in 2004. So far, they are on target. The vaccine is being grown on Vero cells using virus which is seven passages from the NYCBOH strain that is currently in use.

Meanwhile, the Aventis Pasteur Company discovered that they had in storage a large supply of calf-lymph vaccine that had been produced in 1958. That has now been tested and found by the manufacturer and by FDA to be potent. Tests are in progress to validate efficacy and safety in human trials and to ascertain that this vaccine could, if required, be diluted five-fold. We believe that it could. Because the vaccine was produced so many years ago, the decision has been made to retain it in the stock pile for emergency use.

Meanwhile, a manufacturer is now producing bifurcated needles in large numbers, and supplies of Vaccinia Immune Globulin have begun to be processed.

Because the threat of smallpox being released is a problem that will be with us for the indefinite future, it was decided to undertake special studies of vaccines that would be less likely to cause serious adverse reactions but would be as protective against smallpox as the NYCBOH strain. Ordinarily, this would be a time consuming effort because the development of an entirely new and different vaccine requires many years of work. Fortunately, there are two experimental vaccines, developed more than 30 years ago, that have undergone limited testing and could well prove useful.

The first, developed in Germany, is called MVA (Modified Vaccinia Ankara) (*7*, *9*). It is a non-replicating strain that has been used as a vehicle in a number of recombinant HIV vaccine studies. Because it does not multiply after inoculation, it presumably would be safe to administer to those with immune deficiency disorders or eczema vaccinatum. The second vaccine, called Lc16m8, was developed in Japan in the 1970s (*7*, *9*, *10*). It is a live vaccine, attenuated by multiple low temperature passages, and has been administered to some 50,000 Japanese children before routine vaccination programs ceased. It produces much less fever and less marked cutaneous reactions than does the NYCBOH strain, but induces equivalent antibody levels. Studies of both vaccines by NIH, FDA, and manufacturers are now in progress.

Vaccination Policy

When it became apparent in the spring of 2002 that by autumn there would be more than enough vaccine to meet emergency needs, options were considered as to making vaccine more widely available prior to the occurrence of a smallpox outbreak. In deciding policy, it has been necessary to weigh the uncertain risk of a possible use of smallpox as a biological weapon, the frequency of adverse reactions following vaccination, and the capability of the health system to respond rapidly and effectively should smallpox virus be disseminated. The alternative possible policies cover a broad spectrum:

- Vaccinate no one;
- Vaccinate only those at high risk—such as health care workers, first responders, truck drivers, and other essential personnel;
- Vaccinate anyone wishing to be vaccinated, either with a recommendation to vaccinate or with a recommendation not to vaccinate;
- Have compulsory vaccination.

The first and last options have been discarded. The question now under consideration

is who should be offered vaccination. Medical and public-health-care workers who are most likely to be exposed in the health care setting at the outset of an outbreak are those at greatest risk, and might constitute as many as 500,000 persons. Beyond this group, questions persist as to whether all essential workers should be vaccinated and, if so, which are the groups that would be considered "essential." There is an additional question as to whether, once there are sufficient quantities of vaccine for emergency purposes and the new tissue cell culture vaccine is licensed, this vaccine should be offered to all who desire it. This has to be a societal decision rather than an individual one, because of the risk that the vaccinee might transmit the virus to others.

If the frequency of expected adverse reactions were comparable to those following influenza vaccine, the decision would be much easier. Unfortunately, smallpox vaccine is much more reactogenic than any other vaccine product on the market. There are three less serious complications—generalized vaccinia, accidental inoculation among vaccinees and contacts, and rash and fever. In addition, three infrequent but serious adverse events represent potentially life-threatening complications (*11–13*). The first is progressive vaccinia which occurs among those whose immune systems are not functioning properly, such as those with AIDS and those who are receiving immunosuppressant drugs because of an organ transplant or cancer. In such individuals, the virus continues to grow and spread. The second is eczema vaccinatum, which may occur in individuals who have had eczema or atopic dermatitis at some time in their lives. They may experience serious illness because of the widespread growth of the virus in those areas affected by eczema. The third and last condition is post-vaccinal encephalitis, a serious neurological problem, but one that is seen only in primary vaccinees. There are no known predisposing factors, and Vaccinia Immune Globulin, although of benefit for cutaneous complications, is of no value. Based on studies that documented vaccination complications during the 1960s, at least 25 serious adverse events would be expected among each 1 million vaccinees, of which one to four would prove fatal. Were only a portion of our population to be vaccinated—say 100 million persons—this would translate into 100 to 400 deaths and 2,500 persons with serious complications potentially requiring hospitalization.

The difficulty in reaching decisions on vaccination policy is that although the risk of complications is at least partially calculable, the likelihood of smallpox being dispersed as a weapon is most uncertain. It would most likely be dispersed as an aerosol, either as a powder, like anthrax, or as a spray (*14*). As we know, smallpox in ton quantities was produced and stored in the former Soviet Union, and the principal site of its former manufacture is still a secret facility (*2*). The former Soviet Union's then-Deputy Minister of Health admitted this year that in the 1970s they had tested smallpox as a spray in the open air.

How quickly could we respond in the case of an attack? Vaccine and bifurcated needles in large supply are packed and available to be delivered within 12 hours to any city in this country. Educational materials to aid in diagnosis are up on several web sites. Laboratory diagnosis today requires that specimens be delivered to the CDC, but diagnostic materials are being developed so that any of more than 100 designated state and federal laboratories can confirm the diagnosis. All hospitals have been asked to have one or more isolation rooms in their emergency wards, so that suspect patients with rash and fever can be examined safely. All metropolitan areas have been asked to develop plans for accommodating, if necessary, 500 patients in negative pressure settings that would prevent aerosol transmission. Finally, health departments have been asked to develop plans that would permit vaccine to be made available to a large number in the population in the first seven days after an attack. So far, only a few areas have yet achieved these objectives.

Our principal strategy, called surveillance and containment, would rely on early identifi-

cation and isolation of patients, vaccination of those who were in contact with the patient subsequent to his development of fever, vaccination of the household members of the contacts, and vaccination of all in a hospital who might have been exposed to smallpox cases. This is the strategy that worked well during the eradication program. It worked so well as it did because of the fact that smallpox does not spread rapidly or easily; the smallpox patient usually is so acutely ill that he or she takes to bed before infection can be transmitted to others; and that smallpox vaccine, even when given three to four days after a person is infected with the virus, will protect against a fatal outcome and may prevent the disease altogether.

The risk of smallpox must be taken seriously. We need to recognize that smallpox, anywhere in the world, represents a threat to every country. Its control and elimination would represent an international emergency to which all countries would need to contribute in order to again rid the world of the disease as soon as possible.

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ANTHRAX

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INTRODUCTION

The current interest in vaccination against anthrax is solely because of the possible threat of using *Bacillus anthracis*, the causative agent, as a bioterrorist weapon. Naturally occurring disease, particularly inhalational anthrax, is extraordinarily rare, with fewer than 30 cases reported in the United States in the 20th century. In 1990 during the Gulf War, and again in 1998, for the very first time in human history a decision was made to vaccinate a human population, not against naturally occurring disease, but against the threat of using a microorganism to intentionally cause disease. That threat was realized in the fall of 2001, with the anthrax outbreak due to letters containing anthrax spores. That event changed the face of public health and medicine and altered our own personal lives. A congressional study indicated that the intentional release of about 100 kg from a single airplane flying over Washington, D.C., under ideal meteorological conditions could cause from 1 to 3 million deaths. While these are only estimates, it is safe to say that the consequences of such a release would be catastrophic.

Inhalational anthrax is a disease that first came to the attention of the medical community in the 19th century, in association with the developing industrial revolution in Europe. What was once an important occupational pulmonary disease is now, unfortunately, a concern because of bioterrorism.

The disease's most dramatic clinico-pathological findings are a widening of the mediastinum associated with relatively clear lungs and often bilateral pleural effusions. On a CAT scan of the chest, the massively enlarged lemon-sized lymph nodes are striking. Pathologically, the nodes are hemorrhagic and necrotic, and the disease is actually a lymphadenitis and mediastinitis.

Anthrax is associated with the origins of infectious diseases and vaccinology. It was the first disease for which a microbial etiology was definitely determined by Robert Koch in 1877, when he demonstrated the life cycle of *B. anthracis* from its persistence in the environment as a dormant spore to its germination and transformation to a bacillus and finally back to the spore. Some years later, Louis Pasteur developed a live-attenuated vaccine against anthrax. This was one of the first bacterial live vaccines.

The organism is a gram-positive, nonhemolytic, non-motile spore-forming bacillus. The characteristics of the spore, including its dormancy, its prolonged persistence once produced, and its infectivity by the aerosol route, are what make *B. anthracis* one of the most likely bioterrorist agents. There are three known virulence factors: an anti-phagocytic poly-glutamic acid capsule, and two toxins, so named because of their biological effects. Lethal toxin is lethal to experimental animals

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and the edema toxin produces edema when inoculated into the skin. The virulence determinants are encoded on plasmids, as is the case with many bacterial pathogens.

Pathogenesis

Our knowledge of the pathogenesis is based on work reported many years ago, that only now is being re-examined by more modern molecular techniques. The spore, which is the infectious form, enters through a break in the skin, through the gastrointestinal tract, or through the lung, which is the route of concern from a bioterrorism perspective. In the lung it is taken up by an alveolar macrophage, which transports it to the regional draining lymph node. While some spores may be killed, others germinate to the bacillus that then escapes or is released from the macrophage. The infection spreads from node to node within the mediastinum and then destroys and breaks out from the node causing mediastinitis. The nature of the *in vivo* germinant(s) is under intensive investigation. The germination is nonsynchronous, which is important from the perspective of therapeutics, because some spores may remain dormant in the host for extended periods. After the bacillus escapes from the phagocytes, it is encapsulated and resistant to subsequent phagocytosis. There is local production of the two toxins leading to edema and necrosis. The toxins are thought to act early in the infectious process, perhaps both intra- and extracellularly to interfere with cells involved with innate immunity. Terminally, the organism and the toxin reach high levels in the bloodstream. Death in inhalational anthrax is likely due to lymphatic and vascular obstruction in association with hemorrhagic pleural effusions and toxemia. In reference to vaccines, it is important to keep in mind where in the infectious process—from spore uptake to germination, to the bacillus, to the toxin interaction with host cells—the vaccine might work.

The toxins are binary toxins comprised of two different proteins. The central player is a protein called protective antigen (PA), first discovered to be a protective immunogen before we knew anything about the toxins. It was only later that the importance of this PA as a central player in the toxins began to be apparent. The PA binds to eukaryotic cell receptors, which were recently identified. It is then cleaved by a cell protease and serves as a receptor for binding to the cell surface, either edema factor or lethal factor, the enzymatic components of the edema and lethal toxins, respectively. These toxin complexes are then transported into the cell cytosol, where they exert their toxic effects. Edema factor raises cyclic AMP within cells, likely causing edema and interfering with neutrophil and perhaps other cell functions; lethal factor, a protease, lyses macrophages and likely affects other cell types. Antibodies to PA neutralize toxin activity, perhaps acting at several stages of the intoxication process.

Vaccines

The initial approach to anthrax vaccines began at the end of the 19th century, with live attenuated vaccines developed by Pasteur in France and Greenfield in England. This was followed some 20 years later by the development of an acellular *in vivo*-expressed antigen vaccine, an interesting concept from an historical point of view. The Pasteur vaccine was most likely a mixture of attenuated, encapsulated but nontoxigenic organisms with fully virulent organisms that produced both toxins and capsule. The lack of either toxins or capsule attenuates the organism. Max Sterne in the late 1930s identified a non-encapsulated, toxigenic strain that has been used since then throughout the world to effectively control anthrax in domesticated and wild animals. In Russia, a similar live, attenuated human vaccine was developed, which is still in use today. The success with early protein vaccines eventually led to the use of *in vitro*-produced PA as a vaccine resulting in licensure in the United States in 1970 and in the United Kingdom in 1979.

The current vaccine in the United States is called AVA, anthrax vaccine adsorbed, or Bio-

thrax. It is composed of the sterile culture supernatant from an attenuated, non-encapsulated, toxigenic strain that is adsorbed to aluminum hydroxide. It is composed primarily of PA. The vaccine schedule consists of three initial doses at 0, 2, and 4 weeks, followed by doses at 6, 12, and 18 months. The United Kingdom makes a similar vaccine, and a live attenuated vaccine is used in countries of the former Soviet Union and in China. The evidence for the efficacy of the United States vaccine is limited because of the extreme rarity of the disease. A similar but less potent vaccine than the current licensed vaccine was tested in wool mill workers in New Hampshire in the 1950s. These are the only human data that exist. One cutaneous case occurred in the vaccinated group, versus 13 cutaneous and two inhalational cases in the placebo group. This vaccine resulted in an efficacy of 93%. There were insufficient numbers of inhalational anthrax cases, when analyzed separately, to show statistically significant protection. However, in addition to these two cases in the placebo group, there were three additional cases in the mill workers who did not choose to participate in the vaccine trial. Vaccine efficacy has also been evaluated in various animal models. In guinea pigs, the vaccine is poorly protective against an aerosol challenge with approximately 25% survival. However, in both the rabbit and the nonhuman primate models the vaccine is highly efficacious, with > 90% survival against lethal challenge.

The demonstration of efficacy in the best animal models, together with identification of immunological correlates of protection, will be critical for licensure of any new vaccine against diseases like anthrax that cannot be tested directly because of the rarity of the disease and the inability to perform volunteer challenge studies. In the rabbit aerosol model of AVA-induced protection, antibodies to PA measured by ELISA or lethal toxin neutralization correlate with immunity. Toxin-neutralizing antibody is measured in an *in vitro* assay by the ability of antibodies to protect macrophages against cytolysis induced by lethal toxin. Antibodies to PA are produced after vaccination with AVA or purified PA that block binding of PA to the cell receptors and binding of lethal factor to PA on the cell surface, thus reacting with both functional binding domains of the PA molecule. Somewhat surprisingly, antibodies to PA also inhibit spore germination and enhance their phagocytosis. Thus, vaccines containing PA, both AVA and purified PA, appear to induce antibodies that act on both the toxin and the organism itself.

New approaches to vaccines have focused mainly on PA. The most advanced of these efforts uses recombinant PA (rPA), produced in various expression systems, combined with aluminum hydroxide as an adjuvant. Other efforts include the use of mutants of PA, as well as mutants of the enzymatic domains of the lethal and edema factors, in an attempt to determine whether lethal or edema factors may contribute to the immunity induced by PA alone. Furthermore, many new adjuvants are being studied, as are alternate delivery systems, including vaccination by the oral, transcutaneous, and aerosol routes. DNA, nonreplicating viral particles, and adenovirus are among other delivery systems being evaluated. Most importantly, additional efforts are directed to identify new antigens that might contribute to immunity. Spore antigens have been demonstrated to be protective in some animals and work on the capsule is also under way. The recent sequencing of the *B. anthracis* genome has intensified efforts to identify new virulence determinants and vaccine candidates.

The most mature of the vaccine candidates, rPA, has been shown to have a high degree of efficacy in both the rabbit and nonhuman primate models of inhalational anthrax, with an overall survival > 90%. This includes survival of 28 of 29 nonhuman primates that received a single dose of the rPA vaccine. These results in animal studies have led to the initiation of two phase 1 human safety trials. One trial, conducted in conjunction with the National Institute of Allergies and Infectious Diseases and the Department of Defense's Joint Vaccine Acquisition Program at the University of Mary-

land, uses rPA produced in an avirulent strain of *B. anthracis*. The other, in conjunction with the Henry Jackson Foundation at Walter Reed Army Institute of Research and Dynport Vaccine Company, uses rPA produced in *E. coli*.

Conclusion

In summary, anthrax has long been considered to be one of the most likely bioterrorism agents, and we are fortunate to have a licensed vaccine. Furthermore, we are well along in the development of a new rPA vaccine that should be in phase 1 human trials within the next few months. However, there remains much to learn about the mechanism of immunity, particularly the correlates of immunity that will be necessary for the licensure of any new vaccine, as it is highly unlikely that it can be tested directly in humans. The advances in the genomics of *B. anthracis* may also lead to the identification of potential new virulence factors and vaccine candidates.

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VACCINES AGAINST VIRAL HEMORRHAGIC FEVERS

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INTRODUCTION

Although there are many agents that could be used in bioterrorism, only a few are considered highly problematic. Consequently, it makes sense to focus on those threat agents that can cause mass casualties (see Box 1 for a list). Among these are agents that can be efficiently dispersed as aerosols and/or that are highly contagious, such as smallpox and anthrax. Smallpox and anthrax are the most readily adapted as biological weapons; the rest require additional skills to be turned into true weapons of mass destruction.

Viral hemorrhagic fevers, too, are among those agents of greatest concern. Viral hemorrhagic fevers (see Box 2) illustrate some of the problems inherent in the development of any biodefense vaccine. Given the fact that there are several virus families involved, multiple vaccines will need to be developed. Furthermore, it is important to consider that all vaccines are inherently dangerous. For most civilian populations, therapeutic drugs would usually provide a more feasible approach than preventive vaccines—a point well illustrated by the recent debate about the use of smallpox vaccine. That said, drugs are not risk-free either, nor are there drugs available for all these agents.

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Moreover, these viruses are not simply biothreat agents; some are significant emergent pathogens. In the last five years, the Centers for Disease Control and Prevention (CDC) has dealt with many biosafety level-4 (BSL-4) pathogens (see Box 3). Every year, it seemed, brought either a new virus—such as Hendravirus or Nipavirus—or an old virus that was behaving in an unexpected way—such as the Andes virus that became the first hantavirus to be transmitted from person to person or the Rift Valley fever virus that moved out of its African home and into the Arabian Peninsula. All of them are aerosol-infectious, as are all BSL-4 agents. Thus, they are biothreats—they have potential for being used as weapons of mass destruction.

So, there are at least two good reasons to make vaccines against these agents: their role as emergent pathogens and their potential to be used as bioterrorism weapons. And there is a third reason that is perhaps not so evident if one does not work in a laboratory—vaccines need to be developed to protect laboratory staff working with these viruses. Yellow fever, for example, was studied in the United States of America and in the Soviet Union in aerosol infections. During the Rockefeller Foundation era, in the early 1930s, yellow fever caused many cases and deaths among laboratory workers. Before vaccination, of 55 laboratory personnel working with the virus, 16 fell ill and 5 died. After vaccination, among 189 lab

BOX 1. Bioterrorism agents of greatest concern.

Bacteria:	**Viruses:**
Anthrax	Smallpox
Plague	Monkeypox
Tularemia	Arenaviruses
Typhus and other critical rickettsiae	Filoviruses
Glanders	Rift Valley fever
	Tick-borne flaviviruses
	Alphaviruses (VEE)
	Nipah virus

BOX 2. Viral hemorrhagic fevers.

Arenaviridae
- Lassa fever
- South American hemorrhagic fever (Argentine, Bolivian, etc.)

Bunyaviridae
- Phlebovirus, Rift Valley fever
- Nairovirus, Crimean Congo hemorrhagic fever
- Hantavirus, hemorrhagic fever with renal syndrome and hantavirus pulmonary syndrome

Filovirus
- Marburg hemorrhagic fever
- Ebola hemorrhagic fever

Flavivirus
- Yellow fever
- Dengue hemorrhagic fever (not a biothreat)
- Kyasanur Forest disease and Omsk hemorrhagic fever

workers, none fell ill and none died. After May 1931, the International Health Division vaccinated its staff with 17D yellow fever vaccine.

Clearly, even if there are vaccines against some of these agents that are perhaps not sufficiently well tested for safety to be used in the general population, they can still represent a very real advantage for researchers.

This chapter will look at a case study of vaccine development for the South American hemorrhagic fevers and then touch briefly on Rift Valley fever vaccines.

Argentine Hemorrhagic Fever Vaccine

The Junin virus, which causes Argentine hemorrhagic fever, first appeared in the 1950s. It spread through Argentina's pampas, in areas where several million people live, and severely affected the country's economy. The disease is focal in global terms, but not so for Argentina, because of its enormous consequences for the country's foreign exchange. There were hundreds, sometimes thousands, of cases of Argentine hemorrhagic fever each year. The disease's mortality rate is between 15% and 30%, and it is a debilitating disease that requires survivors one or two months to recuperate.

Several of the usual obstacles seen in vaccine development also are present in an effort to develop a vaccine for Argentine hemorrhagic fever—a lack of constituency, high financial and legal risks, and difficulties in translating preclinical research into products for human use. In addition, this is a hazardous virus. Furthermore, although this is not as extensively discussed, there is little scientific in-

BOX 3. Biosafety Level-4 (BSL-4) viruses that the Centers for Disease Control and Prevention dealt with in 1995–2000.

1995	Hendra virus	Australians discover new paramyxovirus
1995	Ebola virus	Zaire and Gabon epidemics, Zaire species
1996	Ebola virus	Exported to South Africa
1996	Andes virus	Person-to-person transmission hantavirus
1997	Hantavirus pulmonary syndrome	Total in South America reaches 229 recognized cases
1997	Rift Valley fever	East African epidemic associated with ENSO (El Niño phase of the Southern Oscillation)
1997–1998	Nipah virus	Discovered in Malaysia; 289 cases, 37% fatality rate
1998	Smallpox	Role in bioterrorism?
1998–1999	Hantavirus pulmonary syndrome	ENSO-associated in southwestern United States (rodents, human cases)
1999	Marburg virus	Active in the Democratic Republic of Congo; multiple genotypes
1999	Crimean-Congo hemorrhagic fever	Reports of activity in Central Asia, Russia
2000	Ebola	Ugandan epidemic, Sudan species
2000	Rift Valley fever	Epidemic in Yemen, Saudi Arabia

terest and scant funding for finding new ways to advance the development of this vaccine.

There also are obstacles that are specific to the development of an Argentine hemorrhagic fever vaccine. These include the lethality of the parent virus and its neurovirulence and transmissibility, as well as the problem with persistent infections inherent to all arenaviruses. It turns out, however, that the virulence and aerosol infectiousness of the parent virus was a hidden advantage in developing a vaccine against Argentine hemorrhagic fever. After the virus was initially isolated, many people were becoming ill in the endemic area, and several laboratory deaths occurred. A researcher in Argentina used a virus that had been attenuated in guinea pigs to inoculate some of the people in his laboratory—something that couldn't have happened today, because that virus would not have met acceptability criteria. Nevertheless, those inoculated in the laboratory suffered only mild fever and thrombocytopenia, and they developed good neutralizing antibody responses. This experiment resulted in the availability of a virus strain against which to calibrate wild type viruses for attenuation.

And there were other advantages that helped move the development of this vaccine along. There were good animal models in place, young mice, guinea pigs, and macaques. In addition, and of paramount importance was the Government of Argentina's active interest in developing this vaccine. On the one hand, it created a public health laboratory—the Instituto Nacional de Enfermedades Virales Humanas Dr. Julio Maiztegui (INEVH); on the other, it established a vaccine manufacturing facility (*1*). The Argentine virologist who came to work at the United States Army Medical Research Institute of Infectious Diseases (USAMRIID), Julio Barrera-Oro, was absolutely critical to the effort. Barrera-Oro had trained with Joseph L. Melnick, a classical vaccinologist such as we rarely see these days. PAHO's input and support also proved key. At that time, the United States Army maintained an infrastructure that could provide certified cell banks that met vaccine requirements and a manufacturing plant. PAHO provided, and

continues to provide, expertise in this regard. The Organization's support and international connections were particularly invaluable for executing some of the later phases. Neil Halsey and his colleagues at Johns Hopkins University were mobilized to help USAMRIID, PAHO, and especially INEVH in field-testing the vaccine. A treatment with immune plasma was used to treat humans with acute Argentine hemorrhagic fever. Although not ideal, at least it provided something that could be employed should there be a reversion to the virulence in the initial human testing. And finally, unlike anthrax, this disease occurs annually at a defined geographical place with a good public health infrastructure available.

The vaccine was developed and began to be inoculated in human volunteers. The antibody response was bad, less than 1% that conferred by the natural disease. The simulation index over about a year's time in some of these volunteers showed good evidence for cellular immunity, however (*2*). In addition, with a more sensitive neutralization test almost everyone showed some antibody to the vaccine (*3*). Then, a randomized, double-blind, placebo controlled field trial was launched, and it was found that the vaccine was, indeed, effective (*4*). Subsequently it was administered to those at highest risk of the disease.

The vaccine began to be used intensively around 1990, and there was a clear decline in the number of cases. Over time, after the uneventful vaccination of about 250,000 persons, the number of cases fell to about a 100 per year, instead of 600 to 800. Even now, ten-plus years later, only about one vaccinated person per year becomes ill, so the vaccine appears to be long lasting. There is much more that needs to be done in the development of this vaccine, but we do have at least one effective, conventional, live-attenuated vaccine. The vaccine has shown no serious side effects in some 250,000 adults, and it has been helpful in controlling the disease, although there are certainly many more subgroups that need to be studied (*5*). The vaccine has been manufactured in Pergamino, Argentina, in prototypic lots.

Unfortunately, while INEVH, PAHO, and others involved in the vaccine development effort concentrated on building manufacturing capacity, other critical elements were overlooked. For example, there is no experienced regulatory agency to be able to evaluate and license this vaccine, a problem that is being dealt with today.

There are three other arenaviruses that are highly virulent pathogens for humans. Junin vaccine protects against the closest relative, Machupo virus (*6*), which causes hemorrhagic fever in Bolivia, but not against Sabia virus from Brazil or Guanarito virus from Venezuela.

Rift Valley Fever Vaccine

Rift Valley fever (RVF), another viral hemorrhagic fever, is an African disease that is endemic, with intermittent epidemics, throughout sub-Saharan Africa. Epidemics are driven by climate. Heavy rainfall results in large numbers of cases and huge economic losses: the disease kills sheep and cattle, causes abortion in infected animals, and increases the possibility that the disease could be introduced outside its endemic area. Epidemiologists are beginning to be able to predict epidemics, so they can be prevented by vaccination.

RVF virus presents an easy target for vaccine, in that neutralizing antibody appears to be sufficient to deal with the disease. Vaccines are needed for both humans and animals, however. Moreover, RVF virus belongs to that group of mosquito-borne viruses that have the capability of moving around worldwide (Table 1).

These viruses either have a vector that traces man's path (*Aedes aegypti* with yellow fever or dengue would be the best examples) or that can use multiple vectors (Venezuelan equine encephalitis virus or Rift Valley fever virus are good examples). They also can use vertebrate species that are present in many different areas as amplifiers. Rift Valley fever has already spread to Egypt more than once, and it is now

TABLE 1. Arbovirus diseases that can move around worldwide, their vectors, and their vertebrate amplifiers.

Disease	Vector	Amplifier
Yellow fever	*Aedes aegypti*	Human
Dengue	*Aedes aegypti*	Human
Chikungunya	*Aedes aegypti*	Human
West Nile fever	*Culex pipiens*	Birds
Venezuelan equine encephalitis	Multiple	Horses
Rift Valley fever	Multiple	Sheep, cattle

in the Arabian Peninsula. Based on laboratory studies, it is believed that North and South American mosquitoes would be able to transmit this virus quite efficiently.

What has been done in terms of vaccines? The United States Army developed an inactivated vaccine which has been used in several thousand people. It was used in the Swedish Defense Forces when the Egyptian epidemic occurred in 1977. It is not a great vaccine, but it is not a bad one (*7*). Unfortunately, there is not much of this vaccine around, and it cannot be made again. The attenuated strains do not make enough antigen for vaccine production, so wild type strains must be used, which require a special containment manufacturing facility. The U.S. Army used to have such a facility, but no longer does.

In the late 1980s, the U.S. Army developed a live-attenuated vaccine for humans. It is called MP-12 because it was passed 12 times in the presence of the mutagen5-fluorouracil and then amplified; eventually a strain was developed which had attenuating regions in all three of the viral RNA segments (*8*, *9*). It had reduced neurovirulence in rhesus monkeys (*10*); it was attenuated in multiple species including sheep, pregnant sheep, lambs, cattle, bovine fetuses, nonhuman primates, mice, rats, and hamsters. The vaccine induces neutralizing antibodies and protects from virulent virus challenge. Mosquito transmission occurs, but the viremia that occur in humans or other animals are not high enough to infect them orally. Mosquitoes must be fed a special artificial blood meal, or inoculated directly. It has been used in about 66 people, with no significant side effects. An intramuscular dose of 25,000 to 50,000 pock-forming units (PFUs) will give a high rate of seroconversion and long lasting neutralizing antibodies. Initial lots of MP-12 have been lying undisturbed in freezers for the last several years. It seems desirable for this vaccine to get out of its cold sleep and be used again to see if it is really a good vaccine. As it is known, a live-attenuated vaccine used in 66 people is nothing, and the next vaccinee can represent a disaster. This vaccine clearly needs additional work.

Vaccines for Public Health or for Biodefense?

Developing vaccines for biothreats will be a very different process than developing them for public health use. The following are some of the underpinnings regarding the development of vaccines for public health:

- knowledge of disease burden and disease pathogenesis;
- a large target population that makes efficacy and safety testing feasible;
- a large and experienced R&D community, including government laboratories—such as the National Institutes of Health (NIH) and the Centers for Disease Control and Prevention (CDC) in the United States—academic laboratories, the vaccine industry, and the biotechnology industry; and
- commercial incentives for investment.

Organizations such as PAHO and the CDC, for example, would identify the disease burden, and NIH would sponsor research on disease pathogenesis. There are large populations where viral vaccines can be tested; and there is adequate expertise and commercial incentives in many cases. In biodefense, on the other hand, it is much more difficult to make the risk-benefit equation congruent with the public health model.

The risk of smallpox is either zero or too high to accept. The policy issues are difficult. In addition, much less is known about these agents; and, of course, there are no economic incentives. The following list sets out some approaches to consider in deciding for which group of agents it would be possible to develop a vaccine, and for which, not:

- An understanding of the biology of each group of agents so that a rapid response is possible (consider lessons learned with HIV-1);
- Agent-specific strategies for the most important threats or determining whether there are any candidates already available (e.g., anthrax, smallpox, Rift Valley fever, Argentine hemorrhagic fever);
- Generic approaches for other problems (e.g., tick-borne flaviviruses);
- A realistic pathway to a usable remedy (lesson of TC-83, ribavirin, Argentine hemorrhagic fever vaccine).

HIV or Ebola provide lessons for the first item in the list. In terms of agent-specific strategies, anthrax and smallpox are clearly some of the most important threats, and there should be good vaccines for both. Rift Valley fever and Junin virus are significant threats (category A according to the United States government classification), and vaccines are close to being developed. More generic approaches for vaccines also should be considered, as should the area of drug development. For example, there are tick-borne flaviviruses that certainly constitute biothreat agents, such as Omsk hemorrhagic fever, Kyasanur forest disease, and tick-borne encephalitis. If broad-spectrum drugs against these threats can be developed, there would be an opportunity to intervene in natural disease to assess these drugs' capabilities for treating infected patients, and this also would give some insurance against biothreats.

There are several things that enter into the broader equation, but these experiences are not generally appreciated. For example, TC-83 is a live attenuated Venezuelan equine encephalitis vaccine developed in the 1950s and 1960s. It has been used in a few hundred people and it is not a good vaccine. It makes 10% of recipients sick and its percentage of seroconversion is not as good as it should be, yet it spares many laboratory workers and field workers from experiencing much more severe wild-type virus infections. So, even though it should not be used at the population level, the vaccine, in effect, has been very useful. Vaccine protection of laboratory workers has allowed faster and safer progress in Venezuelan equine encephalitis virus research, including the development of better vaccine candidates. Unfortunately, several vaccines that fall in this category are no longer available to researchers or are prohibitively expensive. To seriously approach the advance of research on viral hemorrhagic fevers and other hazardous viruses, these kinds of vaccines—particularly Venezuelan equine encephalitis and Rift Valley fever vaccines—should be made accessible to those at high risk, such as laboratory workers and veterinarians.

Antiviral drugs also play an important role in control of viral hemorrhagic fevers when vaccine solutions do not exist or are not practical to deploy. Ribavirin has been a boon in treating arenaviruses, for example. In addition to treating natural infections, it also is an effective therapy for laboratory workers and medical staff who may deal with infected patients. Perhaps the greatest need in this area is a drug for filovirus infections because there is no practical vaccine, effective drug, or other post-exposure prophylaxis to offer.

The story of the Junin virus vaccine illustrates some important principles. From the beginning, it was developed as an international venture—the United States' infrastructure coupled with Argentine know-how to produce an effective vaccine. Initial phase 1 and phase 2 testing in the U.S. led to phase 2 and phase 3 trials in Argentina, where the disease is endemic. Subsequent use of the vaccine in Argentina allowed for the development of an experience base among at-risk persons that

would enable the vaccine to be deployed in an emergency outside the endemic area (5). This sort of effort must be repeated, and the many manufacturing and regulatory obstacles must be overcome, if vaccine solutions are to be developed against these bioterrorist and emerging infectious disease threats.

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PART VII

REGULATORY AND SAFETY ISSUES

THE PUBLIC SECTOR PERSPECTIVE

Manfred Haase[1]

Since 1995, the European Agency for the Evaluation of Medicinal Products (EMEA) has been charged with developing regulatory and safety standards for drugs and vaccines. Balancing vaccine safety and availability has become an enormous challenge, not only for regulators but also for manufacturers.

Today, vaccines are very heavily regulated, with regulation being largely dominated by the World Health Organization (WHO), by the EMEA in Europe, and by the Food and Drug Administration (FDA) in the United States of America. In less developed countries, there is a tendency to accept the EMEA and FDA regulations and product assessments, though this approach may not take into consideration important local issues. It also does little to foster the development of independent regulatory authorities in developing countries. Regulation is a response to any actual risk involved with vaccination. Modern vaccines are safe, though not entirely without risks, since some people experience adverse events following immunization. Regulation is also a response to public pressure, based on historical events. The public does not accept failure, it does not excuse errors, and it is averse to risk. In some cases, the fear that vaccination may occasionally be followed by adverse reactions might become greater than the fear of the diseases that vaccines prevent. This situation raises the question, Is the vaccine sector overregulated? This question is valid and should be raised, especially in light of a recent editorial in *Nature Immunology* (*1*), which called attention to the world shortage of vaccines.

Could it be that the work of regulators has contributed to this shortage? Regulators globally pursue the same goals and are driven by the same concerns, that is to guarantee the quality, safety, immunogenicity, and efficacy of vaccines. This, in large measure, is achieved with the help of regulatory guidance, good manufacturing practices, official batch testing, post-marketing surveillance, and last but not least, with what we in the European Union call "notes for guidance" on quality and preclinical and clinical testing of vaccines.

There has been rapid progress in the field of vaccine development, which requires the regular review of regulatory guidelines. Traditional vaccines are improved upon by new production technologies, removal of preservatives, and replacement of human albumin. Technological progress offers the prospect of a new generation of vaccines, such as those involving new antigen combinations, nucleic acids, and live vectors. Several combination vaccines have been licensed in the European Union, but not in the U.S. These vaccines contain 7 to 10 different antigens and pose major challenges for regulatory authorities. What should regulators primarily bear in mind when new measures to improve vaccine safety are consid-

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ered? They should consider the impact on overall safety and their influence on efficacy and supply. For example, the replacement of thiomersal as a preservative requires a new benefit-risk assessment. Removing thiomersal requires a shift from multidose to single-dose vials, with important consequences for supply. It has been pointed out by the vaccine industry that the latter change would result in a 25% loss of product from accumulated overfill of single-dose vials.

The regulatory environment must favor new technological developments, yet maintain high quality standards that address all safety concerns and try to avoid differences in regulatory standards. It cannot be denied that this is an extremely difficult task. Regulatory measures should not become barriers, either to new vaccine development or to trade. As has been the case in the past, differences in regulatory standards may put one country in a more advantageous position. A challenging question for regulators is how to make existing regulatory structures function more efficiently in order to encourage the development of improved vaccines that meet public health needs and, at the same time, generate proper guidelines to safeguard the public interest. In the past, European regulators have tried to encourage the development of better vaccines by generating appropriate guidelines, such as the following:

- a note for guidance for pharmaceutical and biological aspects of combination vaccines,
- a note for guidance on preclinical pharmacological and toxicological testing of vaccines,
- a note for guidance on clinical evaluation of new vaccines, and
- several notes for guidance on requirements for batch release.[2]

The vaccine industry supported these efforts to further harmonize the technical requirements for the registration of vaccines in Europe, because the notes for guidance have benefited industry by reducing the time and resources invested in vaccine development, enabling the simultaneous launch in European countries of new vaccines, and facilitating market globalization. However, the industry has complained that it has often not been involved early enough during the development of the notes for guidance, and that the ensuing requirements entailed an extra burden for them. Closer collaboration between all interested parties—regulators, manufacturers, providers, researchers, and consumers—is needed to improve upon these processes. Guidelines and requirements are not the whole story; like all biological products, vaccines are the result of several years of investment, involving developmental work, formulations, stability studies, clinical trials, and registration. Therefore, the following measures to improve cooperation between manufacturers and regulatory authorities may contribute to improved vaccines in the future:

1. Incorporate regulatory considerations early in product life;
2. Identify potential regulatory issues early;
3. Organize informal meetings with key authorities;
4. Pose the right questions as early as possible during product development;
5. Address issues before investing too heavily in the overall project; and
6. Do not hesitate to change direction in the development process in order to address identified weaknesses or concerns.

Currently, regulatory requirements constitute a major constraint for vaccine manufacturers and suppliers. The use of preservatives that contain mercury, such as thiomersal, is a long-standing issue. How far can regulators go to minimize the risks of thiomersal before manufacturers abandon vaccine development and production? Although there is no evidence of harm caused by the level of mercury exposure from vaccines, regulatory authorities recommend the use of vaccines without mercury-

[2] These and other notes for guidance can be found on the EMEA website, at www.emea.eu.int.

containing preservatives. Also, the possible contamination of human blood plasma proteins, used as excipients in vaccines, with bloodborne viruses or the agent of Creutzfeldt-Jakob disease, has led to the recommendation to remove such stabilizers.

Where, in the regulatory environment, are additional potential or real problems? The differences in regulatory standards and requirements between regions highlight the need for further steps towards global harmonization. Incompatibilities among guidelines from Europe and North America, WHO and UNICEF requirements, and regional pharmacopoeia requirements are but a few examples. Such differences pose difficulties for manufacturers, and sometimes make it virtually impossible for them to follow guidelines. It would be useful to encourage more active collaboration among FDA, WHO, and EMEA as regulatory authorities. However, this goal cannot be easily achieved outside the scope of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). The need for global harmonization is obvious. Uniform requirements have already been adopted by ICH with regard to stability testing, viral safety evaluation, expression constructs in rDNA production cells, cell substrates, and specifications. This proves that although harmonization may be a dream, it can be achieved.

In conclusion, the following golden rule should be followed by vaccine regulators: introduction of measures, in particular those taken in association with theoretical risks, must be thoroughly weighed against the potential implications for the availability and acceptance of the product concerned.

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THE INDUSTRY PERSPECTIVE

Luis Barreto[1]

INTRODUCTION

Ever since Jenner developed a smallpox vaccine in the 18th century, the primary challenge to vaccine development has been technical. Vaccine technology has advanced steadily over the last hundred years, with the development of live attenuated vaccines, vaccines made from whole organisms, and protein/polysaccharide vaccines, increasing our ability to protect against several diseases and saving millions of lives. However, the vaccine industry today faces far more than just technical challenges.

- New technologies, such as protein conjugation, genetic engineering, bioinformatics, vector technology, and genomics, have made a dramatic number of vaccines available since the 1980s. These new technologies, however, also greatly increased the complexity of vaccine development and production.
- The industry is constantly challenged to find safer and less invasive delivery mechanisms. Advances in formulations for longer and more stable shelf life for vaccines, inherently fragile products due to their biological nature, are also being requested.
- In the past, vaccine development was mainly done by academics in research laboratories. The challenges of the diseases that concern us today rarely can be solved in a single lab. The vaccine industry is now a global entity. Vaccine development involves industry working with academic and biotechnology partners worldwide, increasing the intricacy of the development process.
- Issues concerning unequal access to medicines in the developed and developing worlds present challenges. Unprecedented private-public partnerships have been created to address these issues, and the vaccine industry is committed to seeking equitable solutions.

These issues all pose their particular challenges but, for the most part, are conceptually well understood. However, two of the strongest challenges facing industry, the growing antivaccine movement and its impact on the regulatory environment, are often poorly understood, despite their potential global implication for public health.

THE PRECAUTIONARY PRINCIPLE

Immunization is one of the most valuable weapons to combat the infectious diseases that have caused widespread death, illness, and disability or permanent disfigurement throughout history. Paradoxically, as immunization has progressively controlled or eliminated vaccine-preventable diseases, people's fear of immunization has risen. This phenom-

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enon can be explained, in part, by the successes of immunization programs. Parents in developed countries are no longer reminded of the pain and suffering, even death, associated with vaccine-preventable diseases. Instead, parents are bombarded, through the media, with messages about rare but serious adverse effects of vaccines in children. The growing anti-vaccine movement emphasizes vaccine safety issues, often raising scientifically unfounded concerns while ignoring volumes of data demonstrating a vaccine's real benefit. Furthermore, anti-vaccine activists fail to acknowledge that it is vaccines themselves that have reduced the occurrence of many diseases to virtually nil.

The growing fear of immunization leads to conflict between an individual's freedom of choice and the need to protect the community, and between people's right to information versus trust in the expertise of others. Through the Internet, sophisticated, well-organized anti-vaccine groups are increasingly disseminating myths and rumors about the safety of vaccines—considerable misinformation that is, nevertheless, well-packaged and persuasively delivered. Parents who formerly would have relied on the opinions of medical experts are increasingly turning to these dubious sources for information on immunization, and more and more fear that adverse events may be caused by vaccines. As even more parents object to immunization, the collective benefit of immunization will increasingly be compromised.

The *perceived* safety of vaccines and the resulting "precautionary principle" profoundly affect the regulatory environment today. A vocal minority, whose confidence in the safety of vaccines is dwindling, is ignoring the successes of immunization and focusing attention on perceived safety issues. In response, regulatory authorities operate under the "precautionary principle," striving to eliminate all risks, real or perceived.

> *The precautionary principle essentially states that where there is uncertainty as to the existence or extent of risks to human health, the [regulatory] institutions may take protective measures without having to wait until the reality and seriousness of those risks become apparent* (*1*).

The irony, of course, is that this attitudinal shift is occurring at a time when vaccines have never been safer, given the stringent regulatory demands and compliance involved in vaccine manufacturing.

Impact of the Precautionary Principle on the Regulatory Environment

The vaccine industry is responding to these issues and supports all measures to increase confidence in vaccine safety. The broad implications of the precautionary principle and its impact on regulatory policies, however, are not always obvious to regulatory agencies, let alone the public. The removal of thimerosal from vaccines exemplifies this fact.

Since mid-1999, policymakers have taken the position that thimerosal, a preservative, must not be included in vaccines. This direction was reinforced in 2001 in a statement of biological plausibility issued by the United States Institute of Medicine. Despite the fact that no reliable scientific data support this position, thimerosal has been removed from existing vaccines and is not being used as a preservative in new ones. The use of thimerosal in vaccines meant that health care providers could purchase and use convenient multidose vials without risking bacterial contamination of the vaccine each time they drew from a vial. If a vaccine does not contain a preservative such as thimerosal, only single-dose vials of the vaccine can be used. While the vaccine industry believes that the available scientific data indicate that this policy change was unnecessary, it also supports measures that will increase parental confidence and moved quickly to achieve that end.

Aventis Pasteur's decision to remove thimerosal from vaccines significantly affected supply for some time. The manufacturing

process itself had to be changed in order to assure aseptic filling of the single-dose vials. Production yields decreased significantly because it is necessary to overfill every vial to ensure that the provider can obtain a full dose. The cumulative effect of this overfill is substantially greater for single-dose vials than for multidose vials.

In addition, reformulating a vaccine, as was required in this case to convert from a preservative-containing vaccine to a preservative-free vaccine, requires passage through the regulatory approval process. Any change to a vaccine is a complex endeavor. The licensing of a reformulated product means that the manufacturer must establish new procedures, have the product validated, tested, and labeled, and take the necessary steps to get the product into the marketplace. The net effect is that, in the case of Aventis Pasteur, approximately two years were invested in a development effort to replace an existing product, and total output declined by approximately 25%. Obviously, these changes had significant impacts.

This case illustrates the cascade of events that can—and did—threaten the supply of a vital childhood vaccine, DTaP (diphtheria, tetanus, and acellular pertussis), following the decision to remove thimerosal from the product. The global implication of the removal of thimerosal from vaccines is that clients in the developing world would no longer have access to multidose vials, their format of choice for convenience and economic reasons.

The implications of the precautionary principle and its impact on regulatory policies can also be seen in another recent example—the call for the elimination of bovine-derived products sourced from non-approved countries.

All vaccine manufacturers strive to supply the safest and most effective products. However, these types of regulatory actions have consequences. Regulatory bodies must carefully weigh credible evidence so as to avoid decisions based on unreliable data, factor in the implications of their decisions on supply, and allow realistic time frames when considering such changes. Every independent regulatory action has dependent reactions, some of which are detrimental.

Another area in which the regulatory environment is changing is the demonstration of vaccine safety, which is driven by decreasing acceptance of risk. Decreasing risk acceptance worldwide is affecting the size of clinical trials. While clinical trials are essentially conducted according to the same criteria as before, the amount of data now required in order to prove vaccine safety has increased substantially. Clinical trials are longer and more resources are needed to run them, as is shown below:

- The number of subjects in vaccine safety trials has increased from 5,000 to 10,000, and more than 60,000 subjects are now involved in rotavirus trials to demonstrate the absence of a rare but possibly severe adverse event.
- Concomitant use studies of vaccines (e.g., pneumococcal vaccine given concurrently with other childhood vaccines) are now required prior to large-scale phase 3 studies, which increases development timelines.
- Agencies are beginning to request that concomitant vaccine studies be carried out with all vaccines currently on the market, a task that exponentially increases the complexity of the trial, considering the current and growing number of vaccines available today.
- Post-marketing surveillance requirements are also increasing. In the case of pediatric acellular pertussis vaccine, as many as 10,000 subjects may need to be followed up in the U.S. alone.
- U.S., European Union (EU), and Japanese regulatory bodies require separate clinical trials, and none of these bodies is satisfied with clinical trials carried out in other jurisdictions, even if done under current good clinical practices (cGCPs).

Rising Costs of Compliance

In the case of clinical trials, what is being tested has not changed, but how much data needed for validation has. Similarly, current

good manufacturing practices (cGMPs) have not changed significantly in the last five years, but meeting them has become a more demanding process.

cGMP regulations are broadly stated guidelines devoid of detailed regulatory requirements. These regulations are structured in this way so that they are dynamic and can be modified without going through the procedures mandated by law for revising regulations. By stating the regulations as guidelines, it is possible to modify them functionally, as technological advances, procedural changes, or industrial advances occur in what was previously called "best practices." The U.S. Food and Drug Administration (FDA) changed the name of the regulation to "current good manufacturing practices" to emphasize the fact that these standards are dynamic.

Today, cGMP compliance requires demonstration of control and reproducibility of *all* systems and processes throughout the full manufacturing cycle. Ensuring compliance with cGMP regulations has required vaccine manufacturers to stay current with technological advances and has necessitated a significant and ongoing investment in:

- Facilities. State-of-the-art plants and equipment are required in order to maintain cGMP compliance. Each improvement in processes or equipment becomes the new minimum standard, which requires constant upgrading. At its Canadian manufacturing facility, Aventis Pasteur just completed a CAN$ 23 million new quality control building to meet with compliance standards. This investment, however, does not add any new vaccine production capacity.
- Process validation (data collection and recordkeeping). The amount of paperwork entailed in such validation is already staggering and is growing. While electronic recordkeeping may eventually replace paper records, a whole new set of regulated processes have to be established, ranging from preservation of original document integrity, to archiving, to electronic signing, etc.
- Hiring and training of personnel. Employees are being hired who have the high levels of expertise needed to ensure that long-term cGMP quality standards are being met and sustained. The number of quality-related manufacturing personnel positions has increased at significantly higher rates than is the case with other positions in the workforce. Due to the current regulatory environment, since the mid-1900s, Aventis Pasteur has tripled its personnel in quality operations, medical affairs, and regulatory affairs.

Because of the costs to vaccine manufacturers of such investments, it is important that one international standard be established to avoid unnecessary duplication of efforts. Therefore, international harmonization and mutual recognition agreements (MRAs) or memoranda of understanding (MOUs) are becoming increasingly important to the vaccine industry.

The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) was established in 1990 to improve, through harmonization, the efficiency of the process for developing and registering new medicinal products, including biological products. One objective of the ICH is to develop MRAs or MOUs between countries regarding the equivalence of GMP compliance standards. The U.S. FDA, the EU, the Japanese Ministry of Health, and other regulatory authorities participate in the ICH.

The Pharmaceutical Inspection Convention Scheme consists of regulatory GMP inspectorates located all over the world that collaborate on and issue harmonized regulatory requirement documents. As a result, state-of-the-art industry practice has increased globally. In many cases, the most stringent global standard or industry practice has driven the minimum standards to higher levels. The effect of these new global standards has been a considerable increase in the cost of enhancing systems and processes in the industry's infrastructure. The following are the main cost drivers:

- The global harmonization process itself, which entails numerous costs related to modifying practices and processes in order to meet constantly changing state-of-the-art industry practices.
- Complex new technologies, used more and more in the vaccine industry, that require expensive validation techniques.
- The need for higher standards for sterility, characterization, procurement, and monitoring.
- E-documentation (the regulatory filing done by electronic means—an increasing requirement for several jurisdictions) is a huge and costly undertaking.
- Retrospective evaluation and validation of all legacy systems that support already licensed products.

The last point merits emphasis because retrospective evaluation has major implications for vaccine production. Products that have been manufactured and licensed for years, such as vaccines for measles and polio, must all be manufactured in a way that meets the changing GMP requirements, resulting in costly and lengthy process reviews. The vaccine industry accepts this, but pricing structures for these vaccines are based on "old" manufacturing guidelines that do not take these additional costs into account. Increasingly, the regulatory environment is making these already low-margin products unprofitable for the vaccine industry, dramatically reducing manufacturers' incentive to continue producing them.

In addition, escalating its enforcement, the FDA established Team Biologics in October 1999, a framework for partnership between its Office of Regulatory Affairs (ORA) and Center for Biologics Evaluation and Research (CBER), and put more emphasis on cGMP quality issues for all biologics companies, starting with blood products manufacturers and moving to vaccine manufacturers. The ORA and CBER have focused resources on inspection and compliance. Inspection teams have become larger, and the scope, depth, and duration of GMP inspections have increased, particularly those of validation programs.

The consequences of enforcement—warning letters, consent decrees, fines, and termination of operations—have intensified. Two warning letters were sent to vaccine manufacturers in 1999; by 2000, the number had increased to 10. Several companies received consent decrees and others were fined; one company ceased operating. Some of these enforcements have resulted, directly or indirectly, in product shortages.

The evolving compliance standards and the complexities of regulatory harmonization also add to the length and cost of an already complex vaccine development process. In the current environment, the process takes approximately 10 years or more. The costs of bringing new therapies to market have been estimated to have more than doubled in the past decade. Making the process even more convoluted will further increase the time and costs involved.

Sharing the Costs of Compliance

The vaccine industry has responded and will continue to respond to these changes and to meet the safety and compliance requirements of today's markets, including those in the developing world. All parties concerned must be realistic, though, about the necessary investments in time and money involved in operating a modern vaccine production facility. When making purchasing decisions, our public health officials have not always taken into account the investments the vaccine industry has made and must continue to make. Consequently, fewer manufacturers are willing to or capable of assuming the risks and costs involved in producing vaccines—a factor often cited to explain the dwindling number of vaccine manufacturers in the Western world. Since 1967, their number in the U.S. has fallen from 37 to 10, and the number of licensed vaccines has plummeted from 380 to 52 (*2*, *3*). During the last few years, well-publicized vaccine shortages, particularly of vaccines used routinely in children, have emphasized the decline

in the number of manufacturers. In developing countries, the availability of old and new technologies and the price of vaccines are already issues. Older generation vaccines, such as DTwP (diphtheria, tetanus, and whole-cell pertussis), which are preferred in the developing world, cost manufacturers increasingly more to produce, yet these incremental costs are not recognized. This could potentially affect millions of children in developing countries. All interested parties must work together and review the risks and benefits of the precautionary principle and prevent setting in motion a potentially dangerous chain of events:

The public demands greater vaccine safety

↓

Regulatory compliance increases

↓

The costs of manufacturing vaccines increase

↓

Governments are unwilling to pay the increased costs

↓

Private investors balk at investing in a high-risk, low-return business

↓

Vaccine supplies become short

↓

Public health is compromised

Conclusion

Safety requirements and regulatory demands are changing rapidly for every stage of vaccine and drug development. The vaccine industry, as always, will continue to respond to these issues, which are likely to increase as vaccine technology and analytical capabilities advance. It is essential, however, that all partners recognize the costs of development and production incurred by these changes in order to ensure that the supply of vaccines, for use in both the developed and the developing world, is not further compromised.

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THE CONSUMERS' PERSPECTIVE

David M. Salisbury[1]

INTRODUCTION

When considering the public's perspective on immunization, it is important to appreciate that it is influenced considerably by the environment created by all communications on immunization topics. This perspective changes over time and place. When certain diseases were endemic and vaccines against them subsequently became available, immunization coverage increased and the diseases disappeared. In those circumstances, the public perception was that the vaccine was of enormous benefit, for it saved lives and prevented disease. However, as a disease disappears, the fear of it also disappears and vaccine safety becomes a new concern (*1*). This chapter identifies the setting for communications on vaccines that affect the public perception, describes how that perception is influenced, and considers how one country—the United Kingdom—has dealt with intense pressure over vaccine safety.

INFLUENCES ON THE IMMUNIZATION COMMUNICATIONS ENVIRONMENT

Government agencies, vaccine manufacturers, and print, radio, or television media may provide communications on vaccines. With the latter, the way in which the communications appear is dependent on how the media choose to represent the story and is vulnerable to interpretation by journalists (*2*).

In the period 1990–2000, there was a progressive increase in the number of articles on vaccines in the main UK newspapers (Figure 1) (*3*). The proportion of these articles that focus on safety issues appears to be increasing in recent years. The United Kingdom's Department of Health analyzed the way in which newspapers reported stories on measles, mumps, and rubella (MMR) vaccine during 2001–2003. Overall, the stories were neutrally or negatively reported—even those that supported the vaccine.

Cookson identified the types of medical-scientific news that the media are interested in and report on (*3*). First come the stories that highlight the risk to readers, listeners, or viewers, or to their children. In the UK, these stories have concentrated on scares over the MMR vaccine and autism, thiomersal and autism, the risk of HIV and vaccines, and bovine spongiform encephalopathy ("mad cow" disease) and vaccines. In each of these circumstances, however, there is no or minimal scientific evidence that actually supports the concerns that are raised.

Second come stories in which there is a perceived benefit to the consumer, for example the introduction of a new vaccine that will prevent meningococcal infection.

Third are reports concerning the funding of national and international vaccine programs. Interestingly, the U.N. Children's Fund

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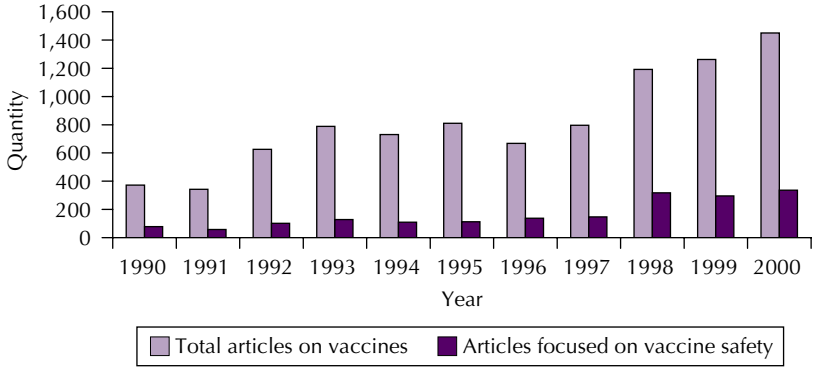
FIGURE 1. United Kingdom newspaper articles on vaccines, 1990–2000.



Source: *Financial Times,* Lexis-Nexis, cited in Cookson C. Benefit and risk of vaccination as seen by the general public and the media. *Vaccine* 2001;20(Suppl 1):S75–77.

(UNICEF), World Health Organization (WHO), Bill and Melinda Gates Foundation, and the Global Alliance for Vaccines and Immunization (GAVI) attract a great deal of media interest. This may be a consequence of those organizations having learned that they need to better market themselves so that the wider world is able to understand their roles.

The scientific aspects of vaccines themselves also get media attention. AIDS vaccines, DNA vaccines, asthma vaccines, edible vaccines, therapeutic vaccines, and Alzheimer's vaccines are all being covered regularly in the newspapers in the UK and elsewhere. However, technical issues, such as vaccine research and development, make up a small proportion of the news stories.

One aim of immunization programs is to provide vaccinations for all appropriate individuals, and since this may involve the whole population, immunization has the potential to affect the lives of all families and hence has widespread media appeal. This relevance for many people makes the subject newsworthy, irrespective of whether the news is good or bad. It also provides the potential for immunization news to spread beyond the community and even the country affected. For example, the safety concerns over pertussis vaccine and adverse neurological outcomes (*4*) spread from the UK, negatively affecting immunization programs in many countries (*5*). However, it is hard to predict which vaccine scares will disseminate widely. The widely reported fears of exacerbation or precipitation of demyelinating disease following immunization with hepatitis B vaccine in France (*6–8*) did not extend to other countries and did not have the same consequences for vaccine acceptance. More typical is that one country's fears over vaccine safety are reported in other countries and thus raise the public's level of fear of the vaccine there. For example, the fear of the contamination of tetanus toxoid in the Philippines was reported in Central America (*9*), the suggested but never confirmed link between MMR vaccine and autism started in the UK but affected vaccine acceptance in Scandinavia, and a reported link between measles/rubella vaccine in the UK appeared in Caribbean newspapers.

Investigating the Public's Perspective on Immunization

In the UK, there is considerable experience on this topic. Twice a year, a market research company interviews 1,000 mothers of children under 3 years of age. Interim sampling of

500 mothers—focusing entirely on MMR—has also been commissioned. The Department of Health has been commissioning this tracking research for at least a decade and has accumulated data from more than 20 of these surveys. Trained staff use computerized questionnaires to conduct the interviews, which last about one hour each and cover the parents' knowledge of vaccines and the diseases against which they protect, their experiences with the immunization program, awareness of advertising on immunization, and the sources they use to inform themselves about immunization. Since the tracking studies began, Internet access among those surveyed has increased from none to approximately 60%.

The sample selected is representative of all population groups. The sampling can be adapted so that the number can be increased, for example, from ethnic communities. The core questions asked in each of these surveys can be augmented so that additional questions can be added. Thus, the public's views on forthcoming vaccines can be investigated or current concerns can be more fully explored. The cost is around UK£ 85,000 (approximately US$ 135,000) for each full study, and about two-thirds that amount for an interim sampling.

The information generated by these surveys is used to inform the Department of Health's communication strategy regarding the ways in which information is provided to parents and health professionals. Thus, the perceptions and prejudices of those managing the program do not define the communication strategy; instead, it is based on what the public knows and wants to know. The aims of this research are straightforward: to provide information for strategic planning on mothers' knowledge of immunization, to define parents' attitudes towards immunization, and to explore their experiences with immunization. This research is also used to monitor the Department of Health's paid advertising. The research allows differences in knowledge, attitudes, and experience to be analyzed by key subgroups, such as different ethnic communities. In addition, all of the above is examined in the light of continued publicity surrounding childhood immunization, especially the problems with adverse publicity about the safety of MMR.

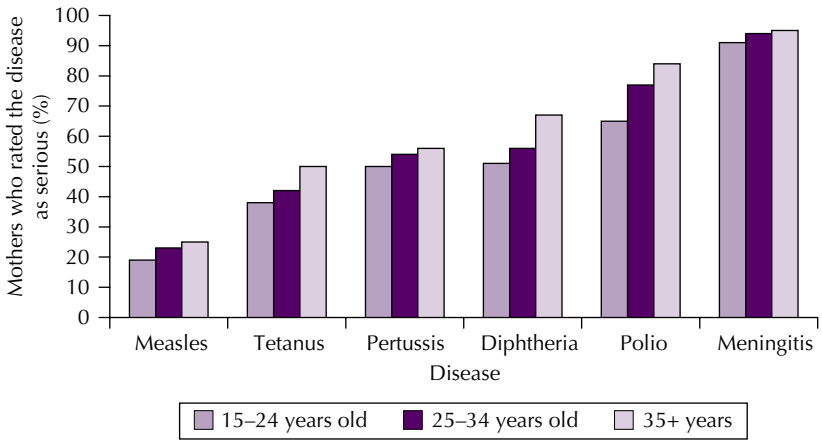
Results from the Tracking Studies

Parents, especially mothers, have been consistent in their responses to many of the core questions in the tracking research, particularly with regard to what they want the government to provide them in relation to the promotion of immunization. They want the government to openly provide information that is clear, consistent, and factual. Parents want to be able to access the information using resources that are easily available; they also want an evidence-based approach and want to be able to find the evidence. As a consequence, in its promotional science materials, the Department of Health now provides the references to the scientific information that forms the basis of its recommendations on vaccines. Though it is infrequent that parents will actually read them, they feel enormously reassured if they are given the references on which the claims and reassurances are based.

One of the starting points of the tracking studies is to establish the parents' perceptions of the seriousness of vaccine-preventable diseases. Figure 2 shows the perceived seriousness of vaccine-preventable diseases according to the mother's age. The youngest group—mothers aged 15–24 years—does not appear to view measles or tetanus as serious diseases, in both cases reflecting lack of experience with the diseases (measles) and lack of knowledge (tetanus). Consistently, in all studies, parents rate meningitis as the most serious disease. Interestingly, the youngest mothers have no experience whatsoever with polio, yet they rate it as one of the most serious diseases. The perceived seriousness of each disease correlates with age, with the youngest group perceiving the disease to be less serious and the oldest group perceiving it to be more serious.

The level of the mother's education also affects how serious she perceives a particular vaccine-preventable disease to be. The less se-

FIGURE 2. Perceived disease seriousness according to mother's age.



Source: British Market Research Bureau data, 1998.

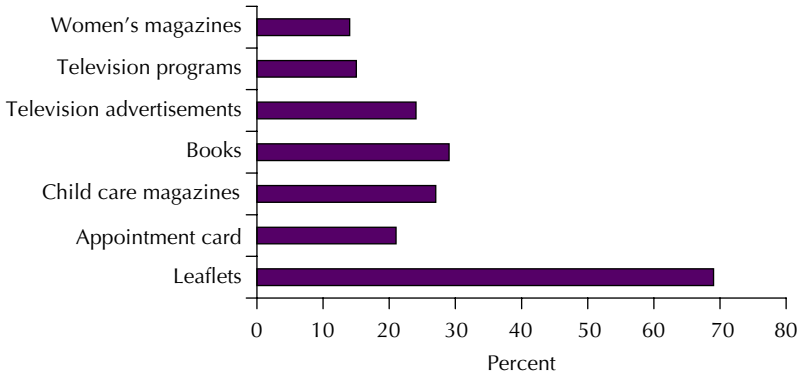
rious diseases are appropriately rated as less serious by the mothers with more education, and with increasing true disease seriousness, so the more educated mothers appropriately rate them as more serious. However, the perceived seriousness of meningitis overrides issues of education. Also, when asked about their knowledge of vaccines, mothers with more education are able to spontaneously identify more vaccines than those less educated. Thus, the background knowledge and perception of diseases and vaccines are not homogeneous across all of the target population. This means that communication materials need to be heterogeneous, according to the specific needs of each group.

Sources of Information on Immunization

If the national immunization program aims to target information on immunization to parents, then it is essential to know what sources parents use to obtain their information. The tracking studies consistently show that leaflets were by far the commonest source used by parents, although the Internet is rapidly becoming a very widely used alternative. Parents actively seek leaflets and they find it helpful when information on forthcoming immunizations is provided along with the appointment card. Figure 3 shows the percentages of different materials used by mothers to inform themselves about immunization.

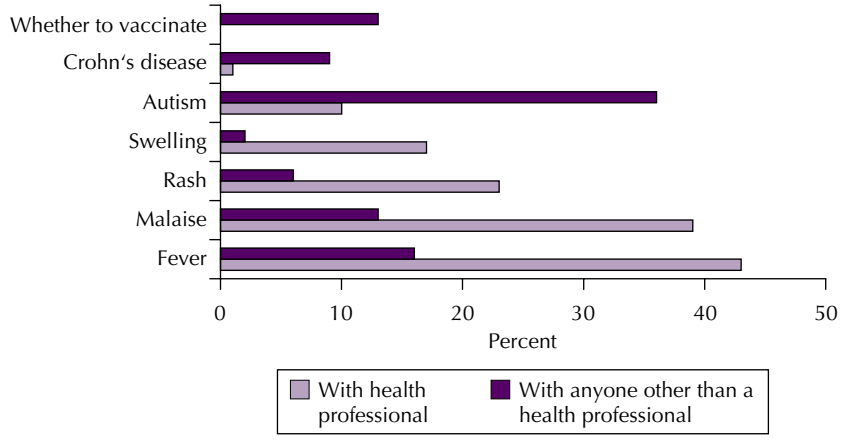
Ideally, parents discuss immunization with well-informed health professionals who have the time to deal with their concerns and have accurate information to impart. However, parents also discuss their children's health matters with their informal networks. These networks include family members—especially mothers' mothers—and friends. When they rely on informal networks, parents may be getting information from individuals whose knowledge is very limited. Figure 4 shows the side effects of immunization that mothers discussed and with whom they discussed them. While minor side effects, such as fever and injection site reactions, are frequently discussed with health professionals, the mothers interviewed revealed that they did not discuss the issues that they really feared, such as autism, with health professionals but with friends and family. This selection of uninformed sources increases the potential for misinformation and decreases opportunities for reassurance about the actual

FIGURE 3. Sources of information consulted by mothers before immunizations due.



Source: British Market Research Bureau data, 2003.

FIGURE 4. Side effects of immunization discussed by mothers, and with whom.

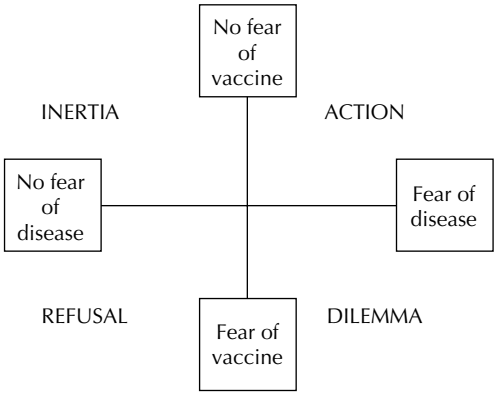


Source: British Market Research Bureau data, 2003.

safety of vaccines and affirmation of the absence of evidence for the feared adverse events. This bias in the source of advice is important. Parents' anxieties may not be addressed if they choose not to discuss their specific concerns with well-informed health professionals.

In the light of these potentially contrary influences, parents may find decision-making very difficult. Figure 5 shows the balance between no fear of disease and fear of disease, and a counterplay between no fear of vaccine and fear of vaccine. When parents fear the dis-

FIGURE 5. Influence of the fear of a vaccine versus the fear of disease on the decision to immunize.



ease in question and do not fear the appropriate vaccine, then the decision to vaccinate is the most likely outcome. When there is no fear of the disease and no fear of the vaccine, the likely outcome is inertia until there is some other stimulus to action. When parents fear the vaccine and do not fear the disease, they are likely to refuse immunization. When they fear the disease and the vaccine equally, they are faced with a dilemma, with delaying the decision being the most likely outcome.

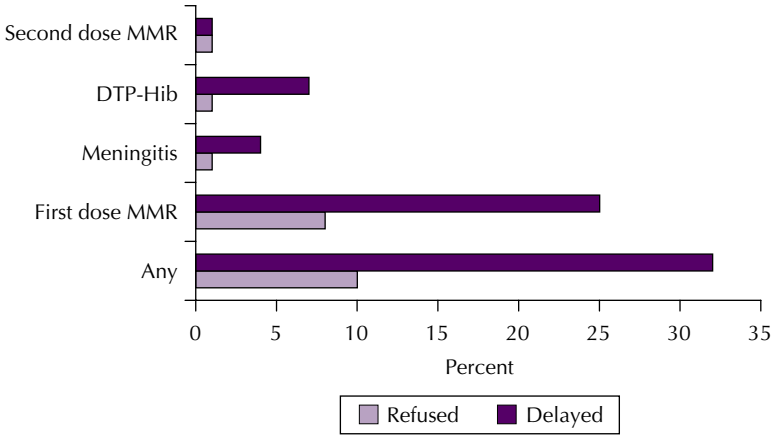
Figure 6 shows the spontaneous responses of mothers of young children when they were asked about their actions regarding immunizations, at a time when there was much adverse publicity about vaccine safety. The publicity focused on MMR vaccine, and the most common response reported by parents during this time of concern about vaccine safety was to elect to delay immunization.

Figure 7 shows the responses from the parents according to their newspaper readership. Parents' confidence in vaccine safety can be fragile. Research has found that the parents most likely to delay or refuse immunizations were those who read the two mid-market UK tabloid newspapers that have been consistently and vocally against MMR vaccination. It also showed that increases in the proportion of parents saying they would withhold vaccination coincided with increases in (mostly negative) media reporting on vaccine safety.

Responding to the Public's Needs for Immunization Information

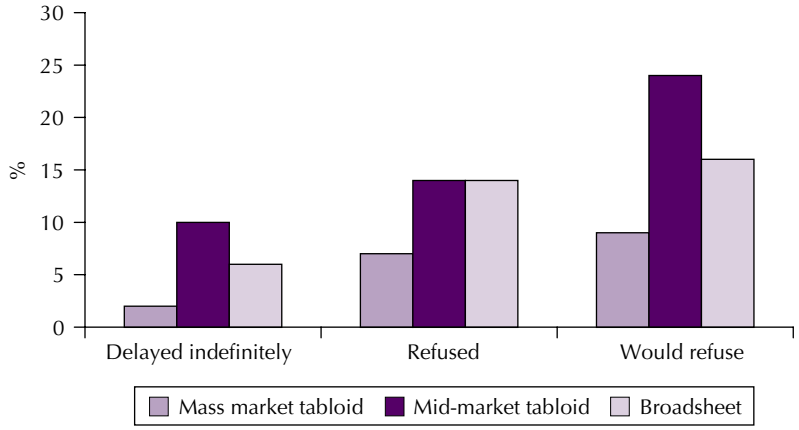
As pressures on the public's perceptions about immunization have increased, and as new vaccines have been introduced, it has become increasingly important for work on immunization communications to be integrated into

FIGURE 6. Vaccinations delayed or refused.



Source: British Market Research Bureau data, 2003.

FIGURE 7. Decision to delay and refuse MMR vaccine, by newspaper readership.



Source: British Market Research Bureau data, 2003.

policy development and program management in the UK. The team responsible for the National Immunization Program at the Department of Health now has dedicated immunization communications staff, consisting of two health professionals trained in communication, one editor, two advertising/media staff, and one administrator; they also have support from the Department of Health's Media Center. The routine program budget for immunization communication work is approximately US$ 4 million per year.

The responsibilities of the immunization communications staff include translation of policy and program management goals into communications for health professionals and the public; production of printed materials, such as leaflets, fact sheets, and posters; radio and television advertising; and website development and management. Recently, a dedicated MMR immunization information service was set up on the National Health Service's website (*10*). The MMR website has a facility through which parents can ask their MMR-related questions if the information is not already available on the site. The staff also undertake training for health professionals, research, and advocacy.

From April 2001 to April 2003, the immunization communications team undertook 67 MMR training sessions with health professionals and 15 meetings with parents; produced 4,000 information packets on MMR for health professionals; distributed 160,000 MMR information packets for parents; distributed 624,000 leaflets to primary care facilities; issued 31,000 posters; sent out 50,000 MMR videos for parents and 35,000 MMR videos for health professionals; and developed a specific MMR website—and answered all of the questions submitted by parents.

Conclusion

Public acceptance of immunization will be of increasing importance for the maintenance of successful immunization programs, irrespective of the scientific virtues of vaccines and the benefits they bring. The media provide the interface between immunization programs and the public. Health and immunization program managers must recognize the independence of the media and cannot assume that the media share their views. Increasingly, the public is actively seeking information, and this need for clear, truthful, and accessible information

must be provided for. The Internet, where information is unregulated, contains much that is potentially wrong and harmful. Those providing immunization services must compete effectively with those providing misinformation and must dedicate as much effort to communicating about vaccines as they do to providing them.

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Part VIII

Vaccines, Prevention, and Public Health

THE ROLE OF PREVENTION IN HEALTH AND PUBLIC HEALTH: CHALLENGES FOR THE FUTURE

Carlyle Guerra de Macedo[1]

INTRODUCTION

In this chapter I will cover four issues. First I will sketch a brief retrospective of the inextricable link between public health and prevention; more specifically, vaccines and prevention. Second, I will summarize what I consider to be a sea change in public health thinking and practice. On the basis of that, I will reexamine the relationship between prevention—including vaccines—and public health. Finally, I will outline some of the leading challenges that await us.

Public health and prevention, two intervention areas that benefit people's health, were practically born together and evolved as parts of a single process. In antiquity, even before public health became a concept in its own right, there was the idea of (or feelings about) prevention. Even if sparked only by fear of illness, or by a guilty feeling in the eyes of the gods, or by trying to escape punishment by the gods, people behaved in certain ways to avoid health risks, avoid becoming ill, or avoid worsening their health. Almost 3,000 years before Christ, in Ancient Egypt's Old Kingdom, the architect and physician Imhotep made recommendations for maintaining or improving health. In Ancient Greece, the mythical Aesculapius also issued many recommendations for preventing disease. Then, in Classical Greece, Hippocrates, particularly in his treatise on "Airs, Waters, and Places," clearly defined the concept of prevention as an aspect of medicine.

Once mankind transcended the doctrines, the humors, and the miasmas, the concept of specific prevention took hold. In this regard, one could cite Girolamo Fracastoro's experiments in the 16th century that demonstrated contagion, thus beginning to destroy the idea of the miasmas, or the quarantines against plague that, in fact, acknowledged disease transmission. Finally, in the 19th century, with the arrival of the era of microbiology with Robert Koch and Louis Pasteur, and the era of experimental medicine with Claude Bernard, public health and prevention were established as concrete and independent entities.

To some extent, this evolution peaked in the recently ended 20th century. And if the 21st century is to be the century of vaccines, it will still be a continuation of our past experience, especially in the second half of the 20th century.

Public health and prevention—and by extension the field of vaccines—have developed in tandem throughout the centuries, since antiquity up to modern times. In fact, they are aspects of a single process. Public health has been so identified with prevention that, in some forums and at some times, public health has actually been defined as the prevention of disease.

But despite the many successes, the concept of public health as the control of communica-

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ble diseases, or as identified with hygiene, or with taking care of the environment, or with responsible actions by public authorities or public institutions falls short in confronting the vast challenges that the people's health now poses.

Clearly, public health's sphere of action must broaden, and many other actors must be brought into the effort to make people healthier and to prolong lives, lives that can be fully enjoyed. Thus, the population, as it always has been but more so now, is the focus of a new public health. And when the population becomes the focus of public health, not just as an object (health for the people) but also as a subject (health through the people that makes the population the fundamental actor of the new public health), the way opens for understanding prevention much more broadly. This approach continues to emphasize the importance of disease prevention, particularly of vaccines, and expands it to cover risk prevention, root causes, and the conditions that make it possible for people to become ill or to be healthy. This, then, is a public health with a social dimension that has not been seen before, even though pioneers such as Virschow, Laennec, Chadwick, and many others did acknowledge such a social dimension within public health and pointed to it for more than two centuries.

This public health with a social dimension culminates in actions and results, insofar as it is expressed in social practices; in other words, actions and results that are expressed in the daily lives of the persons, families, communities, and society as a whole.

These social practices reflect positive values in terms of life and health, as well as in regard to solidarity among peoples and among social groups, and in regard to the environment and the organization of society's resources for caring for health and well-being. This makes it possible for public health to truly function as a basic vehicle, a fundamental tool for the peoples' well-being. In this regard, public health as an expression of these values and these social practices transcends the realm of health, health services, or health care systems, although for practical purposes it is operationally defined within those systems.

Public health also transcends national borders. Today there is a growing awareness and acceptance of the existence of goods whose utility reaches beyond national borders. These are global or regional public goods and services that cannot be harnessed exclusively within an individual nation's borders, but that demand solidarity, cooperation by all peoples or by many of the world's peoples. This international dimension is another dominant trait of this new public health that we are trying to build. In this context, today's new public health uses not only the tools of the biological sciences, specifically the medical sciences. It also must avail itself of tools acting upon other disciplines and other fields, including, fundamentally, the political sciences. We have even seen that part of the decisions regarding the use of the products created through science require social decisions, decisions by the state, or decisions by important social groups, so that they can be put into practice for the population's benefit. This is politics: it is acknowledging the distribution of power in society and identifying society's essential actors, the relationships among them, and the mechanisms whereby decisions are made. These decisions include both individual group decisions (whose validity is limited to those groups) and decisions that must be imposed on all of society, if you will, and which, therefore, fall to the state through government.

Politics in this context means recognizing the relationships among the three great social entities that must be considered by any student of today's social actions: civil society, the state, and the market.

For quite some time now we have been seeing an overstatement of the market's importance, with the state being seen as little more than a tool for creating favorable conditions for the market's operation, and society being relegated to a substratum designed to justify the existence of the market and the relationship between the state and the market. Although this clearly is an overstatement, in many, many

areas this understanding has predominated. This is what is known as neoliberalism, which was given some voice about 12 years ago in the so-called "Washington consensus."

But day by day the conviction grows that those relationships must return to their traditional track. The population is society's most important entity. It is *for* the population that everything must be done. It is *through* the population that everything must be done. It is the population and society, in fact, that through history have created that greater institution—the state—to serve them, not to dominate them. The state must again act in terms of what is best for society, for the state is a product and an instrument of society. The state must view the market as the most efficient mechanism humankind has created to produce the goods and services to satisfy society's needs, but when all is said and done, the market is just that, a mechanism.

I stress this because I believe that for this new public health with social dimensions to resolve the root causes of ill health—which means living conditions—it is essential that we understand this overarching political dimension in which the great entities interrelate and function.

This may not be as difficult as it first appears. To facilitate putting public health into operation, we have created the concept of essential public health functions—functions that fall directly under responsibility of the so-called health authorities, or of the public authorities expressed as the state and the government. These functions carry with them specific responsibilities; when performed, they open the way for the full content of public health to be conducted and brought to fruition, especially the growing involvement of all private, public, and quasi-public actors. In fact, one of the essential functions of the state and the public authorities is the mobilization of those other players, so they can join in the effort to meet the population's needs.

Viewed from that perspective, prevention becomes, more than ever before, an essential tool and component of public health. This newly conceived public health is no longer purely reactive, or merely responding to a need in terms of a specific disease. This is a proactive public health that can foresee problems and anticipate action, before any risk or damage to health occurs. Disease prevention, coupled with health promotion, thus becomes the core of this new public health.

Happily, the contribution of science and technology has opened up new opportunities; it even has justified this conceptual change. For it is science, principally through vaccines, that has made it possible to extend the limits of prevention. This, in turn, has strengthened public health.

Today, vaccines and immunization—in other words, the specific prevention of disease—lie at the heart of the new public health. And, as we achieve success in this area considered inherent to public health, thus enhancing our credibility, we can create conditions so that we can address other aspects of this new public health. On the other hand, if we do our job poorly—failing at specific prevention, particularly vaccination—no one will take us seriously when we try to promote the need for intervening in development models or in macroeconomic, employment, or overall welfare policies. Starting from this core, modern public health will prevent risks, both environmental and in individual and group behavior, as a key complement to specific prevention. To that end, prevention will join hands with health promotion, and so open up extraordinary opportunities for defining the characteristics of the new health care systems that must be created.

As stated earlier, specific prevention and risk prevention must be expanded to include the factors that create living conditions, and consequently represent conditions for the expression of these risks and for the occurrence of disease. Moreover, technology and science daily give us more and more tools to be able to predict risks and the possibility of disease more precisely and with a wider reach. This, in turn, also expands the opportunities for prevention, and makes the corresponding inter-

ventions better able to be targeted. But perhaps the final phase, which may well be beginning to emerge, is the conditioning of human beings to prevent the damage to health.

Based on this, we must design new models of care that have prevention and promotion as their core, but without overlooking the necessity of recovering lost health.

Given this scenario, what are the major challenges ahead? They are many, varied, and daunting. I will deal with four of them.

The first great challenge is to continue accelerating scientific and technological progress to produce the goods, particularly vaccines, that are necessary to give people more effective health care. Progress should come under appropriate regulation, which not only should guarantee the products' quality and safety, but also be a stimulus for progress itself.

The second great challenge is ensuring universal access—at the very least, equitable access—to these goods for people wherever they are, whatever the goods are. This should apply in every country of the world, in every community of the world, and for every individual in the world—in other words, for all of humanity. This means that not only should health systems be equitable and effective, and generate and produce health and satisfaction, but there should also be sustainable human development policies in place that foster equity and guarantee a life lived in liberty and well being.

The third great challenge is an ethical challenge, and it permeates all the rest. I don't want to touch here on the ethical problems of research or product development. Instead, I want to focus on the ethical problems inherent in the allocation and delivery of resources in the countries by the governments, in society, and between countries. One of the negative—in fact, unhealthy—characteristics of today's world, is the vast inequality between men and women and between social groups seen within countries and between countries. Consider this. The 500 largest personal fortunes in the world, the 500 wealthiest people in our world, have a net worth greater than the aggregate gross domestic product of all the Latin American countries, or about that of the 100 least-developed countries in the world. Those enormous gaps, those enormous disparities in living conditions, imply a situation that hinders and even prevents the use of specific health care products such as vaccines. So, this ethical dimension of human progress is one of our concerns and the greatest challenge to an improved delivery of scientific progress and the products it generates, particularly vaccines for caring for people's health.

The fourth challenge is the corollary of what I have said up to now. In order to better deliver scientific progress and its products—particularly vaccines—for the people's health, we need to have the collective effort of scientists, health workers and administrators, men and women everywhere, civil society organizations, businesses, the media, churches; in short, all social actors. The mobilization of all, and their coordination in the collective effort, is the greatest and most essential challenge. It has to do with building a new world which, by taking advantage of the opportunities created by science, does not forget that human beings are the purpose and the reason for everything.

EXTERNAL FINANCE OF IMMUNIZATION PROGRAMS: TIME FOR A CHANGE IN PARADIGM?

Dean T. Jamison[1]

INTRODUCTION

This chapter will look at economic issues surrounding immunization programs, particularly cost and finance issues, as they bear on the introduction of the new antigens now available, as well as on the expansion of coverage of the traditional six antigens. What are the economic and financial issues? How substantial are they? What are the implications for external financial assistance to countries?

After covering some general background observations, the chapter encompasses three parts. The first lays out basic numbers, depicting the magnitude of today's financial problems, particularly for low-income, high-fertility countries as they expand their immunization programs in terms of coverage and the inclusion of new antigens. The second steps back from the immunization discussion, briefly recounting a number of important recent debates and analyses of what has worked and what has not worked in development assistance. A long overdue critical look is now under way to examine what the instruments of development assistance have been; where the money has gone; where it has worked; and where it hasn't. There are lessons to be drawn from that literature that are quite challenging to those who are concerned with the long-term expansion of financing for immunization programs. The messages are reasonable and need to be taken seriously. That said, properly approached, development assistance for immunization programs can avoid most of the problems raised in the recent literature. Finally, the chapter presents how those who are concerned with long term finance for immunization can specifically address these problems.

FINANCING REQUIREMENTS AND BENEFITS

The financing perspective presented here is that of lower income countries—some six low-income countries of the Western Hemisphere and a much larger number of very-low-income countries in South Asia and sub-Saharan Africa.

The discussion about problems of financing immunization comes at an odd time. As we look back over the accomplishments of mankind in the 20th century, it is notable that not only have material conditions improved dramatically for the planet's population, but life expectancy has increased far more in this century than in all of previous history (*1*, for a brief overview of the magnitude of these changes). Health conditions—mortality and morbidity conditions—of almost all of the human population have never been better, and immunization was an important element in that success. Immunization, in fact, exempli-

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fies much of the rest of what has created that success, bringing to bear new scientific knowledge to underpin technology and to guide behaviors in ways that improve health. Science and technology—not income growth—has driven this enormous improvement in human health. The very real worries concerning erosion of coverage of immunization programs need to be placed in the context of the enormous successes that the health community as a whole has achieved, and in the fact that an important part of those gains result from the successes of those who have been involved with immunization.

In a different way, the problems that we face in maintaining immunization coverage levels, or in reversing declines in coverage levels seen in many countries in the 1990s, also come at a peculiar time. There is an emerging consensus within the economics community about the role and significance of improved health in the development process. One line of thinking concerns the intrinsic value of health. When one tries, in a very crude and approximate way, to put some dollar value on the large improvements in health, those numbers become huge. So large, in fact, that they often overshadow improvements of growth in material GDP as a measure of improvement in human welfare. (See reference *2* for results concerning the United States; the relative contribution to economic welfare of health improvements relative to income growth would be larger in developing countries.)

In addition, there is the instrumental value of better health in increasing national incomes and in improving household incomes. Economists today have a much better picture than they did ten years ago of the significance of better health as an instrumental strategy for improving national economic growth rates or enhancing poverty reduction strategies (*3*, *4*). So, there is a disjuncture between the intellectual community dealing with development and the degree of support that we see manifest financially and intellectually for health systems and for immunization.

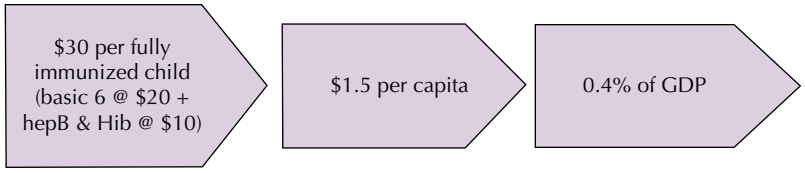
At the same time, new opportunities have opened up that are costly but very promising. The introduction of hepatitis B (hepB) immunization has been a success in some areas, but there is a long way to go with *Haemophilus influenzae* type b (Hib) and other vaccines that are just around the corner, such as conjugate pneumococcal vaccines and rotavirus vaccines. What is significant to an economist about all of those items is that the numbers are very big. Each of these new vaccines addresses a huge burden of disease, but they do get to be potentially costly, and attention needs to be paid to the cost-effectiveness of their use in different contexts (*5*–*7*).[2]

In the context of countries with relatively low incomes, most, although not all, tend to have high fertility rates with the concomitant additional demands on financial resources for the immunization of large birth cohorts. The global average cost for one fully immunized child is estimated at around US$ 20 for the basic six antigens, plus an incremental cost of US$ 10 for hepatitis B and Hib, much of which (in the case of Hib) would be for the vaccines themselves. Given fertility rates and the size of birth cohorts, this might come to US$ 1 to US$ 2 per capita for the population as a whole. On the surface this is not a great deal of money, but for low-income countries it could represent half a percent of GDP. Figure 1 schematizes how vaccine financing in a low-income country implies a significant fraction of that country's GDP.

The WHO-supported Commission on Macroeconomics and Health included a review of domestic expenditures on health (*8*). The Commission's work suggests that the amount of money indicated in Figure 1 is, on average, about 30% of all public sector expenditures on health in typical low-income countries. This refers to all public sector expenditures: hospi-

[2] Miller and Hinman (*5*) provide a thorough overview of the literature on cost-effectiveness of immunization programs. Earlier, the World Bank's *Disease Control Priorities in Developing Countries* (*6*) devoted four major chapters to assessing cost-effectiveness of immunization programs and these assessments fed into the Bank's *World Development Report, Investing in Health*. Jamison and Saxenian (*7*) summarize the *World Development Report*'s findings concerning immunization.

FIGURE 1. Estimated cost of high vaccine coverage in low income/high fertility countries.



= 30% of public expenditure on health

tals; primary health care programs; malaria, tuberculosis, AIDS control programs; and others. Therefore, when a country's minister of health asks the minister of finance to allocate money for completion of a high immunization coverage goal, with the addition of a couple of very important new antigens, the minister of health is asking for quite a lot. This is likely to be quite cost-effective on the whole, but it is still a lot to be financed.

Trends in the Thinking about Development Assistance

In recent years, somewhat less than US$ 50 billion is spent a year on official development assistance (ODA). Both in absolute terms and as a percentage of GDP of high-income countries, ODA has been declining over the last decade. Something less than 10% of the total is spent for health (*9*). This amount of money is not huge but for some countries it constitutes a significant fraction of their GDPs. Economists have recently returned to the question of what works in development assistance, and several trends that have emerged from recent thinking have important implications for immunization.

Aid Effectiveness

Much of the recent work has been done at the World Bank, and several questions are being increasingly asked. Is there any evidence that infusions of development assistance have affected economic growth rates? Is there any evidence that infusions of economic assistance have affected mortality rates, or levels of poverty? These are clearly not easily answered; it's not like a clinical trial with double blinded procedures and sharp endpoints. Nonetheless, some data do provide insights into these questions. Today we have much better data on economic growth of low-income countries, as well as other characteristics of those countries, than a decade or 15 years ago. Moreover, there has been an accumulation of data on how much and for what purposes development assistance has been used. The fundamental conclusion of this line of work (which is becoming sufficiently ingrained in the development banks to be considered a new addition to the "Washington consensus") is that, on the whole, aid doesn't work—countries getting more aid don't seem to grow any faster than countries getting less aid (*10*). This statement obscures a fundamental difference between countries that have underlying reasonable policies and those that lack reasonable policies and reasonable institutions. If the world is divided statistically into those two categories, development assistance does seem to work in countries where there is a good policy environment and a good institutional environment. Otherwise it does not. The effect is actually a substantial one, in that a 1% of GDP level of development assistance sustained over a period of time can result perhaps in a 0.5% per year increase in the rate of economic growth (*11*). With good policies and good institutions, ODA works.

But what does this mean for many of the countries that we are most concerned about?

The tendency is to withdraw aid from countries with poor institutions and poor policies and to put it where it will pay off. Increasingly, this is the position of the United States and United Kingdom governments, as well as of the World Bank. The dilemma is that the countries that most need the aid are often the very ones that have very weak policies and very weak institutions (see *12* for a discussion on this issue and its implications). From the perspective of those knowledgeable about immunization, however, it doesn't look as if this broad-brush conclusion about ODA effectiveness is quite right. Polio has certainly been eliminated in countries with good policies and good institutions. But it also has been eliminated from most countries with bad policies and bad institutions. And there is no smallpox today in countries with bad policies and bad institutions. Moreover, several of those countries have immunization coverage rates of 60% or 70%, as high as those in the United States.

So, there is an important disconnect in the story when you move from the very broad aggregates down to the specifics. A stronger case needs to be made over the next few years for more and better development assistance around immunization programs, pointing in a very explicit way to how and why it differs from the general case. And the fact that it differs from the general case is of particular significance precisely for those countries where the new consensus says we lack policy instruments—the countries with the poorest institutions and policies.

Project Support vs. Budget Support

A certain amount of aid is moving away from project support—for example, an immunization program, or an AIDS control program, or the extension of a road network—and towards more general budgetary support, often to be provided through pooling of donor assistance. There are many reasons for this, some of which are good (*13*). At the risk of oversimplifying, it is fair to say that there is a set of budget support modalities that are increasingly viewed as the broadly correct way to provide development assistance. The British government has taken a lead in this in many ways, and the World Bank also generally supports this. The approach involves making the transfer of funds to the ministry of finance or to a sectoral ministry conditional to policy. The Global Alliance for Vaccines and Immunization (GAVI) is pointing to ways in which support for immunization programs can be advanced within the context of this strong trend toward providing general budget support. GAVI's innovation involves supporting immunization programs based on performance. The country gets US$ 20 for immunizing each child, however it decides to do it; this, then, provides general budget support that is conditional on performance. GAVI's concern has been with transitional finance, but their approach points the way to designing long-term budget support that is conditional on measurable immunization performance.

Macroeconomic Consequences of Aid

Another concern in the aid community, particularly with the International Monetary Fund (IMF), is that some countries may be getting too much aid. For example, in 2002, newspapers reported that Uganda was getting too much aid. The IMF was then telling the World Bank and the government of Uganda that the latter could not accept money for immunization or for AIDS control programs from the development community—even though the development community was quite prepared to provide the money—because it would have adverse domestic macroeconomic consequences, essentially inflationary consequences. This is an argument that needs to be taken seriously (*8*, chapter 8). In essence, it is an argument that revolves around the generation of domestic inflationary pressures, of projects chasing after those few good engineers or doctors with an increasing amount of foreign money, thus creating an inflationary spiral. If, however, the money being proposed will be mostly used for drugs or vaccines (e.g., the

US$ 10 increment for adding Hib and hepB vaccines to an immunization program), almost all of this will represent foreign exchange, and the macroeconomic arguments about inflationary consequences simply would not apply.

As a community, we need to design external assistance requests for immunization programs much more carefully, in order to respond to what are serious concerns from the macroeconomic segment of the development assistance community and to make sure that general policy prescriptions do not seriously hinder external financing for immunization.

The Millennium Development Goals

An additional, and significant, direction in thinking about ODA concerns the achievement of the Millennium Development Goals (MDGs) (see *14* for a valuable overview and critical discussion). The goals are very specific targets for improving education, health, and income-related poverty. A central MDG for health is the decrease in child mortality by two-thirds between 1990 and 2015. Immunization can play a key role in this. Careful, country-specific analyses of the contribution of immunization to MDGs can be valuable both in program design and in advocacy (*15*).[3] Interestingly, the trend toward a focus on MDGs is, at least partially, opposed to the trend toward budget support.

Directions for External Finance of Immunization

The above considerations point to several directions for the design of development assistance for immunization programs. They have led to the following conclusions on the directions in which ODA should move:

1. long-term perspectives (10–30 years);
2. predictability;
3. an emphasis on "demand side" support (with a concomitant country control of resources);
4. incentives for countries to maintain (relatively) high coverage rates (in immunization or basic education);
5. avoiding creating perverse incentives; and
6. including a transparent exit strategy (e.g., reduced grant support with per capita GDP growth).

Point 4 is well-exemplified by the work of the Bill and Melinda Gates Foundation with GAVI and the World Bank in providing a financial incentive for enhanced immunization coverage. Payment for a fully immunized child, as GAVI is doing over a limited horizon, could be extended to predictable long horizons (*12*).[4] And this leads to points 5 and 6, on incentives and exit strategy, respectively. Countries shouldn't do extra well financially just because they started with low coverage rates for their income levels: the goal should be to maintain an environment where there is incremental reimbursement for incremental coverage increases above a certain expected level (*16*).[5] Other instruments might be used to help bring initially poor performers up to that level. In addition, another essential element for creating the right incentive environment is to avoid penalizing immunization successes: support can be withdrawn as incomes rise, while maintaining a high *marginal* payment for above norm coverage levels.

Finally, it is especially important to design external financing for immunization programs in ways that are consistent with donors' increasing reliance on budgetary support. Box 1 provides a simple categorization of budget support modal-

[3] Jones *et al*. (*15*) provide a valuable discussion of the potential for reducing child mortality at the global level that includes the role of immunization. This global discussion can provide a framework for country-specific analyses.

[4] Radelet (*12*) provides detailed quantitative examples to show that, even under very favorable circumstances, in a lower-middle income country development assistance is likely to be needed for decades.

[5] Radelet (*16*) provides a valuable exposition of the current United States administration's "Millennium Challenge Account" and its stated emphasis on meeting performance goals for continued aid. Actual creation of the account appears to be proceeding slowly, however.

BOX 1. Budget support modalities.

- Transfer (including debt relief) to treasury (unrestricted, with or without macroeconomic understanding.
- Transfer (including debt relief) to treasury (restricted to having additional anti-poverty expenditures).
- Unrestricted transfer to sector (with policy understandings).
- Unrestricted transfer to sector (performance-based).

ities. The fourth modality—performance-based but otherwise unrestricted support to the sectoral (or possibly national) budget—can be used to catalyze demand for immunization. It is very much a demand-side measure (point 3 above), while leading to budget support at a level dependent on performance.

Sustainable Incentives to Expand Immunization

Let's examine a concrete example to illustrate the above. Consider a GAVI-type policy that provides incentives for immunization (or primary education enrollment, to look at another example) that are proportional to the amount that *current levels exceed a baseline rate*. In addition, it would be useful to set ways in which to attain independence from donor finance while maintaining incentives. What follows is a hypothetical financial mechanism for meeting these objectives.

There are several things that might be desirable in such a financial mechanism for an immunization reimbursement schedule (IRS).

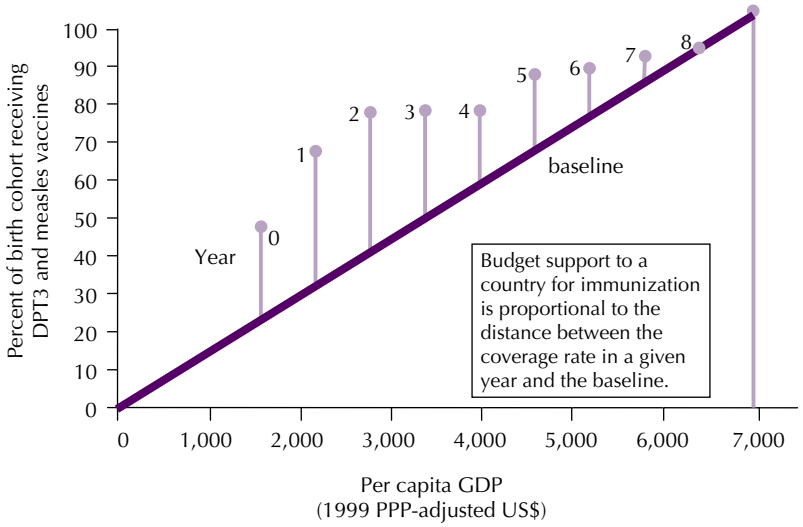
1. The IRS should motivate the mobilization of new effort.
2. The IRS should preserve incentives, so that greater program success always leads to reimbursement increases (as long as the country is eligible for the program in terms of income and technical qualification).
3. The IRS should be equitable, in the sense that eligible countries in similar circumstances get the same incentive payment.
4. The country's share of the total cost should rise as per capita income rises, until, ultimately, it reaches 100%. This is the exit strategy by which financial sustainability without external assistance would be reached.
5. The IRS should be easily modified to provide additional incentives for covering hard-to-reach populations and for introducing new antigens (where they are cost-effective).

Figure 2 shows an IRS in which the key concept is of a baseline that rises with per capita income up to some maximum level (US$ 7,000 in 1999 PPP-adjusted dollars in this case). At any given level of income, the baseline defines the level of immunization coverage above which the country will be reimbursed (say, at X% of the cost per fully immunized child (FIC) above the baseline, where X could even be greater than 100). In the example in Figure 2, the baseline is the straight line running from lower left to upper right.

Consider a hypothetical country followed over time. In year 0, its GDP is about US$ 1,500, its immunization coverage rate is 50%, and its baseline (or expected) coverage rate would be 30%. Reimbursement under the IRS would then equal 20% $\times$ size of birth cohort $\times$ the amount of reimbursement for each FIC. (Of course, this is not like GAVI, in that the baseline is not the country's own starting point, but, rather, the common baseline.)

Returning to Figure 2, we see the country responding to financial incentives by markedly increasing its immunization coverage. Its income also has gone up. Its reimbursement *goes up in proportion to coverage increases*; its reimbursement *goes down in proportion to income increases*. If the country experiences an income reversal, its reimbursement would go up, providing an automatic buffer to protect against coverage cuts. In years three and four, there is no coverage increase, but increases in income

FIGURE 2. Hypothetical performance-based reimbursement schedule for an immunization program.



Note: This plot shows the time line of a hypothetical country's per capita GDP, immunization coverage rate, and immunization reimbursement. Reimbursement equals the size of the birth cohort times the deviation from the baseline expressed as a fraction times the incentive amount per FIC.

reduce reimbursements. By year eight, the country has reached the baseline level and reimbursement stops. It is worth noting that up to that point, the *full incentive* to expand coverage was still there. Only if the country's coverage were to fall below the baseline would there be no financial incentive for (small) increases in the coverage rate. The country in this example displays unrealistically large income growth rates to better illustrate the point about the mechanisms for reducing reliance on external finance that nonetheless leave, at the margin, continued strong financial incentives for increased coverage.

This hypothetical IRS would need to be modified in two important ways to become practical. First, there would need to be a means for incorporating new antigens within the incentive structure. Probably the best way to accomplish this would be to have a separate reimbursement scheme (with a different baseline, reflecting much lower initial levels of coverage). Likewise, incentives might need to be boosted for expanding coverage to hard-to-reach areas. To this end, the reimbursement per FIC might be increased at some coverage level—say, 80%. The second would be to divide each country's population in two: the standard population where each fully immunized child would be reimbursed at US$ 15 to US$ 20, and a "remote" population where reimbursement might be twice or three times as high. Each population group would have a separate baseline and a separate reimbursement calculation. (Probably the way to approach this would be to let the country apply to have a defined subpopulation be designated as remote for these purposes.)

The conclusion, and hope, is that the immunization community will be able to design external finance mechanisms that create strong incentives for coverage, that give countries maximum budgetary flexibility, and that will allow for the widespread introduction of powerful new antigens. The example just described provides one such mechanism. Per-

haps the place to begin is in the Americas, with PAHO leadership. PAHO's past leadership has set a fast pace for global immunization. Forging new and more effective aid instruments for immunization in the Americas would point the way for immunization elsewhere (and for other sectors as well).

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A VISION FOR THE FUTURE SUSTAINABILITY OF NATIONAL FINANCING OF IMMUNIZATION PROGRAMS

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Roberto Tapia Conyer,[2] *and José Ignacio Santos*[3]

INTRODUCTION

The efforts of the individuals and ministries of health of the Pan American Health Organization's Member States in the area of vaccines and immunization have led to the design and implementation of policies that have enabled the Americas to be the first region to be free of polio, to control the circulation of autochthonous measles virus, and to have the most complete immunization schedules and the highest vaccine coverage of all of the regions of the World Health Organization (WHO). While there is little debate that vaccines and vaccination programs continue to be among the most cost-effective methods for disease prevention and health cost containment, several issues will certainly raise questions regarding the sustainability of national financing of new and future vaccination programs, as well as the value of alternative disease prevention methods. These issues include the growing list and costs of new vaccines; vaccines once thought to be shelved for life or unnecessary for human use, such as smallpox and anthrax vaccines; and the change in the immunization paradigm from a strictly preventive approach to one that seeks to use vaccines to ameliorate disease or impact disease progression.

Despite major advances in the prevention of vaccine-preventable diseases in the Americas, significant challenges have to be overcome. The most important one facing countries in the Region and the rest of the world is the sustainability of public financing of immunization programs. Operational self-sufficiency, which is achieved if a country purchases or produces all the routine Expanded Program on Immunization (EPI) vaccines it requires, is a key element in immunization program sustainability. The perception that vaccine affordability requires that vaccines be inexpensive is another.

Recent experiences in Mexico with the introduction of new vaccines, including *Haemophilus influenzae* type b (Hib), hepatitis B, mumps, and rubella—which increased the country's EPI from the basic six vaccines to 10 immunogens—implied an increase in the investment for vaccines from 0.4% of the 1996 health budget expenditure to 0.9% of the overall health budget expenditure by the year 2001, and an increase

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in the per capita investment from US$ 0.10 to US$ 0.60. The experiences of other countries in the Region have been similar.

What Has Helped the Countries to Meet These Challenges?

Two main factors have significantly contributed to make it possible to meet these challenges: the leadership of the PAHO Technical Advisory Group (TAG) on Vaccine-preventable Diseases and the PAHO Revolving Fund for Vaccine Procurement.

The PAHO Technical Advisory Group on Vaccine-preventable Diseases

The strong immunization programs in the Region of the Americas are due, in large part, to the positive impact of the PAHO Technical Advisory Group on Vaccine-preventable Diseases on the political will of decision-makers to invest in local vaccination programs. The TAG was established in 1985 during the successful initiative to eradicate poliomyelitis in the Americas. The group is composed of distinguished professionals in the area of immunization from Latin America and the Caribbean, the United States, and Canada. Every year, it brings together health authorities and experts from the Western Hemisphere, as well as Europe and other regions of the world. The TAG provides a forum for the debate and promotion of Regional initiatives aimed at controlling and eradicating vaccine-preventable diseases. One of its main objectives is that of strengthening policy dialogue on immunization in the Region of the Americas and in the international community (*1*).

The PAHO Revolving Fund for Vaccine Procurement

The second fundamental factor in meeting these challenges has been the participation of more countries in the Region in the PAHO Revolving Fund for Vaccine Procurement, with consequent decreases in the prices of high-quality vaccines and expansion of the participants' immunization programs. The Revolving Fund was established following a resolution of PAHO's Governing Bodies in 1977, with a capitalization of US$ 1 million. It began to operate in 1979 with the purchase of vaccines, syringes, needles, and cold chain equipment (*2*).

The Revolving Fund was designed to provide participating PAHO Member States with a means of assuring the smooth and constant flow of vaccines and related supplies for the implementation of immunization programs. In this process, PAHO does not sell vaccines to its Member States, but rather establishes annual vaccine contracts on their behalf (*2*).

Operation of the Revolving Fund

The Fund operates on an annual cycle. The ministry of health of each participating country establishes annual vaccine requirements for the following year using a quarterly system. PAHO consolidates these annual requirements and issues an international request for bids. Criteria for selecting suppliers are based on WHO/PAHO vaccine quality specifications, price, and the suppliers' track record for timely delivery. Once suppliers and prices are established, PAHO averages the prices across sellers for each product and distributes lists to the participating countries. PAHO then places a quarterly order to a supplier, specifying the quantity, as well as destination and date of shipment. A second phase of PAHO's role involves monitoring orders, expediting delivery, and arranging freight-forwarding services.

Following satisfactory delivery, PAHO sends an invoice to collect reimbursement, adding a 3% service charge to the cost of the vaccines. The service charge is held in a special reserve account to which PAHO charges losses incurred by the Fund due to shipment problems and/or currency transactions. If the reserve account exceeds US$ 100,000, the surplus reverts to the Fund's capitalization. At the end of 2002, the capitalization of the Fund was US$ 17,000,000. Countries have 60 days to repay the Fund. If a country is in arrears, no

further orders will be placed until debits are cleared. It should be noted that the Fund has had an excellent track record of members paying their invoices (3).

Introduction of New Vaccines

The Fund has also contributed to the introduction of new vaccines. Given that the price of vaccines remains a determining factor for immunization programs, the Revolving Fund for Vaccine Procurement has played a major role in accelerating the incorporation of additional vaccines by allowing countries to acquire high-quality vaccines at affordable prices. Priority has been given to including those vaccines that have already been available in the market for the past 15 years, including yellow fever, measles, mumps, and rubella (MMR), and hepatitis B, as well as newer vaccines, such as Hib, and/or other combination vaccines.

In 1996, only two Latin American countries—Chile and Uruguay—were using Hib vaccine in their immunization schedules; however, by 2002, over 90% of children born in the Americas had had this vaccine as part of their routine immunization schedule. Similarly, in 1997, the hepatitis B vaccine was limited to risk groups and risk areas, but is now included as a combination vaccine in most regular immunization programs in the Region. It is important to highlight that the dramatic drops in the price of these two vaccines have been the direct result of economies of scale derived from bulk purchasing through the Revolving Fund.

The Fund was also instrumental in accelerating the introduction of MMR vaccine. Following the 1989–1990 measles pandemic and the introduction of the measles booster, few countries in the Region were vaccinating against mumps or rubella. With measles virus circulation on the wane, the TAG recommended that countries in the Region adopt the more ambitious goal of eliminating congenital rubella syndrome. By 1998, most countries in the Region had switched from the monovalent measles vaccine to MMR. The availability of a combination vaccine that could be easily incorporated into the existing schedule, coupled with affordable prices due to bulk regional purchases, was a significant factor in this accomplishment.

Cost Benefits of the Revolving Fund

A major benefit of the PAHO Revolving Fund for Vaccine Procurement has been its impact on vaccine costs. Studies carried out by PAHO in the early 1980s showed the wide price differences charged by manufacturers for the same vaccine. Competitive procurement through the Fund has kept price increases for vaccines under contract at a minimum (2, 3).

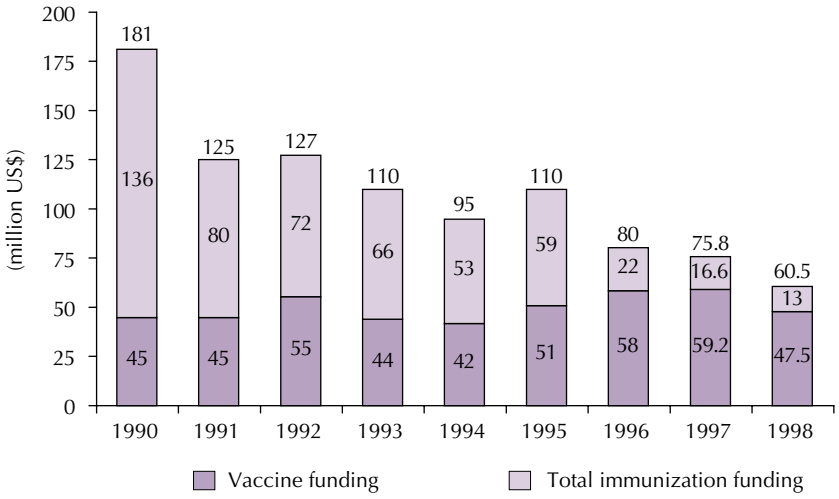
Recent Trends and Issues in Vaccine Development and Immunization Program Financing in Developing Countries

The past 25 years have seen dramatic changes in the world, among them health care reform for the financing of health services, including immunization programs in developing and developed countries. The financing of immunization programs in developing countries has experienced the most dramatic changes, due in large measure to the rapid expansion of the international vaccine market (4).

Multiple factors should be taken into account in strengthening the sustainability of national immunization programs. For many years, the regular immunization schedule in the non-industrialized countries of the Americas was limited to the original six vaccines included in the EPI program, due to the belief that in order to introduce other vaccines regularly used in industrialized countries, such as MMR, hepatitis B, and Hib, these biologicals had to be at rock bottom prices.

Predicated on a 1977 World Health Assembly declaration that "by 1990, all children in the world would be immunized," in 1982, the United Nations Children's Fund (UNICEF) established the Universal Child Immunization (UCI) target of 80% coverage by 1990 for the

FIGURE 1. Estimated contributions for immunization and vaccines through UNICEF, 1990–1998.



Source: United Nations Children's Fund.

six childhood vaccines included in the EPI program (*4*).

During this same period, donor funding increased substantially and international procurement mechanisms were put in place to help ensure that quality vaccines were provided on time and at affordable prices. Advances in molecular biology and biotechnology facilitated the development of recombinant and protein conjugate vaccines and significantly improved production processes. Also during this period, there was an important increase in the number of vaccine manufacturers. However, not surprisingly, the new vaccines—with patented technology—proved to be significantly more expensive than existing vaccines.

Once the UCI goal was achieved, donor funding had declined significantly, from US$ 181 million in 1990 to US$ 60 million in 1998 (Figure 1). These developments began to jeopardize the gains made through UCI and slowed progress toward introducing new vaccines into countries that needed them. In 1999, the Global Alliance for Vaccines and Immunization (GAVI) was established with a grant of US$ 750 million from the Bill and Melinda Gates Foundation and has helped curb the danger of reversing gains made in immunization coverage, especially in the poorer countries. Total commitments for 2001–2005 for GAVI's financing arm—the Vaccine Fund—exceed US$ 1 billion. The high profile alliance of public and private sector partners has rapidly mobilized large amounts of economic resources, both to procure new vaccines for poor countries and to improve their immunization programs overall (*4*, *5*).

The Mexican Experience—Where Are We Heading?

Mexico has a three-tiered health system: people with health insurance (both public and private), people without health insurance, and private health care. All public institutions, according to a Presidential decree promulgated in 1991, are required to guarantee the same immunization schedule. However, vaccine purchasing was done independently among the different Mexican health institutions, with

varying procurement mechanisms and, more importantly, varying costs, sometimes as much as three times the price for the same vaccine (*6*).

In Mexico, as in most other countries in the Region, the vaccines included in the EPI program remained unchanged for almost 25 years. However, in the last few years, there has been a rapid increase in the introduction of new vaccines, with a concomitant increase in investment. For many countries in the Region, these changes were possible with the assistance of external donor financing that has gradually changed to national financing. For Mexico, participation in the PAHO Revolving Fund made the initial difference, allowing for increasing the immunization schedule from six to 10 immunogens and for expanding immunization to adolescents. This purchasing option has permitted most countries in the Americas to expand their immunization programs; for many countries, their entire vaccine supply is purchased through the Fund (*4*, *5*).

In most countries in the Americas, the sustainability of immunization programs has always been a challenge due to the lack of entitlement funds for vaccine purchases, which are generally manufactured in developed, industrialized countries, and must be purchased on the international market. A major risk in this situation is that for many countries in the Region, the allotment for vaccine expenditures is determined on a yearly basis according to the annual budget negotiated by the ministries of health with ministries of finance and the legislative assemblies. This approach has several limitations and inherent dangers: the planning exercise is fraught with uncertainty and severely limits long-term forecasting, which in turn affects manufacturers' planning and production, causing shortages or delays in delivery. Moreover, when there is a supply problem due to limited forecasting, vaccine demand is accompanied by increased costs, delayed deliveries, altered immunization schedules, and increased risks in a susceptible population.

In addition, it became clear through international experience that there was little evidence that commercial health insurance provides significant levels of financing for immunization services. Furthermore, evidence from middle-income countries such as Romania, Bulgaria, Turkey, and the Philippines suggested that social health insurance—run by government mechanisms to pool contributions from the whole population to meet the costs of defined health services—can be used to finance immunization services (*7*, *8*).

With all this in mind, and in order to guarantee an appropriate supply of vaccines and the sustainability of high-quality health services, including vaccines and immunization, Mexico has implemented a two-pronged approach. The first and most expedient measure was for the health sector (the health institutions and the social security system for health) to join forces by forming a national Vaccine Consolidated Purchase Committee, whose main objective was to consolidate the procurement and purchase of vaccines for the entire population locally, from international manufacturers with registered and licensed biologics in Mexico. This is accomplished through a public call for bids, with common guidelines and specifications for the vaccines, delivery calendars, and institutional administrative specifications. The call for bids is published in a timely fashion in the *Diario Oficial de la Federación* (Federal Register).

In a parallel fashion, the Ministry of Health submitted a financial health reform package to the legislature in November 2002, which made explicit that the right to health guaranteed in Mexico's Constitution can be best accomplished through social health insurance that aims to protect the currently uninsured population from the financial burdens of catastrophic illness and, at the same time, guarantees essential health services. This is a tripartite investment by the federal government, the state governments, and the individual families according to their economic possibilities. The financing structure of the Ministry of Health is concentrated on achieving the availability of resources and promoting the success of public health programs, currently considered a matter of national security. Moreover, as part of the

national health financing reform, a special fund is earmarked to guarantee universal national coverage for all public health programs and services. In order to achieve this tremendous goal, 25% of the national health budget has been assigned for public health prevention and promotion services, including immunization programs (*9*). This health reform package was unanimously approved by both the Senate and the Chamber of Deputies on a most appropriate date, April 30, the Day of the Child in Mexico.

During this period of transition, in order to sustain the financing of immunization programs in our country and others in the Region, we must continue to negotiate the purchase of high quality vaccines at the lowest reasonable cost, guarantee that international manufacturers of vaccines comply with national licensure registry requirements, and establish plans that allow countries in the Region to promote competitiveness among manufacturers.

Development of a Strategic Plan for a Partnership for Immunization Financing for Countries in the Americas

Different countries in the Region have different goals for their immunization programs. For some, achieving higher levels of coverage with traditional vaccines is the highest priority. For others, which have already achieved high coverage for the basic vaccines, the primary aim may be to improve quality, enhance program efficiency, and expand the immunization schedule. However, a successful immunization financing strategy is a fundamental feature of the immunization programs for the countries in the Region (*5*).

The United States Institute of Medicine (IOM) report *Calling the Shots: Immunization Finance Policies and Practices* (*10*) underscores the importance of a strategic plan to guide a federal-state partnership in supporting immunization efforts in the U.S. that could also be applied to other countries in the Americas. In addition, the report disclosed that the absence of a national consensus about the roles and responsibilities of federal and state agencies in fostering immunization complicates efforts to extend benefits obtained by immunization to relatively small populations of high-risk children and the larger pool of adults who remain unprotected in the U.S.

In many countries, including the U.S., federal, state, and private sector investments in vaccine purchases are lagging behind opportunities to reduce the risk of vaccine-preventable diseases. A combination of new challenges and reduced resources has led to instability in the public health infrastructure in the U.S. and many other countries. In the U.S., three factors have contributed significantly to this trend. First, there has been a rapid acceleration in the science of vaccine research and production. Second, the health care services environment in the U.S. has grown increasingly complex, as seen in such trends as the emergence of private managed care organizations. Lastly, federal immunization grants to the states have recently been reduced, reflecting congressional responses to the shifting health care roles and responsibilities within the federal government, the states, and private health care providers, which followed the dramatic increases in vaccine costs in the early 1990s.

The IOM report concluded that a renewal and strengthening of the federal and state immunization partnership is necessary to overcome these challenges. Extrapolating this information to other countries in the Region, it can be said that the main role of this partnership is not only to financially sustain the program. The main objectives include the overall goal of preventing vaccine-preventable diseases; monitoring, sustaining, and improving vaccine coverage rates for children, adolescents, and adult populations; and responding to vaccine safety concerns. Achieving this renewal requires a consistent strategy, additional funds, and a multiyear finance plan that can help expedite the delivery of new vaccines and strengthen the immunization and long-term public health assessment, assurance, and policy development functions (*10*).

Immunization Financing Assessment in Vaccine Sustainability

The traditional assessment of an immunization program includes the evaluation of the delivery of immunization services; immunization safety information; disease surveillance; logistics; vaccine supply and quality; advocacy; and communications strategies. A more comprehensive assessment of an immunization program includes the overall financing environment, including the current financial picture and future financial needs. The overall financing environment assessment also takes into account the macroeconomic context, resources available to the government, the share of resources allocated to health, and the share of health resources allocated to immunization.

In addition, an immunization-financing database and financial assessments of immunization services may also add significant benefits for the countries in the Americas. These assessments may provide important information on the projected costs of existing programs, projected costs of proposed changes, financing strategies, and steps to enhance financial sustainability. Sound planning requires credible information about how much is spent, on what and from what source, and how much will be needed in the future. Countries need this kind of information to help strengthen the planning of immunization services and improvements to them. At a global level, donors need this information to assist them in prioritizing and planning for current and future support for immunization (*11*, *12*).

Conclusions

Whether an immunization program achieves its coverage goals, quality and access to both traditional and newer vaccines depends on many factors, ranging from quality control in national laboratories to the outreach strategy to the cold chain. Nevertheless, financing is one part of the overall challenge; it is the rate-limiting factor in how the rest of the system works, but is truly not sufficient to make it work well (*5*). We believe that the establishment of partnerships among federal, state, and private sectors in the countries in the Americas will help future investments in immunization and help develop strategies to make vaccines more accessible for the countries of the Region.

In addition to continued resource mobilization, it would be of strategic importance for the vaccine-producing countries in the Region to establish a consortium whereby, depending on the strengths and infrastructure of each country, all could participate in the production of combination vaccines of relevance to the Region and thus guarantee both production and a regional market. Strategic alliances with international vaccine manufacturers and other international agencies could also serve as mechanisms to facilitate access to new vaccines financing mechanisms.

Finally, the major roles of national immunization programs should be to assure vaccine purchase; ensure service delivery; carry out disease control and prevention activities; conduct surveillance of vaccine coverage and safety; and sustain and improve coverage levels. We recognize that while the financing of immunization options must be adapted to each country's needs, the main objectives are to promote equity; achieve efficiency; provide resources in an adequate, timely, and reliable manner; and encourage the highest level of self-sufficiency.

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THE ROLE OF MULTILATERAL FINANCING INSTITUTIONS IN SUPPORTING IMMUNIZATION PROGRAMS

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INTRODUCTION

Several types of organizations provide development financing. Private foundations give grants. Multilateral financing institutions, such as the Inter-American Development Bank (IDB) and the World Bank, provide loans (although some of them also give small grants). In the last 10 years, new entities have appeared that have changed the way development is financed. These are private-public partnerships, mostly between private philanthropic foundations and international organizations. In most cases, these partnerships are created to further a specific agenda, such as combating HIV/AIDS, tuberculosis, or malaria; to promote a particular technology; or to foster the production of needed information, as is the case of the Global Forum for Health Research. One of the interesting features of these new partnerships, particularly global funds, is that they give sizeable grants that complement other development financing efforts, such as loans or locally generated revenue.

For all practical purposes, multilateral financial institutions are credit unions of groups of countries. Through these institutions, countries jointly issue bonds, obtain financial resources, lend those resources for development to member states, collect loan repayments, and pay bondholders. Often, development banks are thought of as grant providers, but for the most part, they provide loans to finance development projects. Their main purpose is to support national development efforts through subsidized loans. However, since they try to be more than just banks and try to add value to the funds they provide, development banks participate in various development-related activities: 1) national policy dialogues and development planning efforts; 2) the provision of technical assistance, both at the national and the regional levels; and 3) the promotion of the supply of public goods, especially goods that will be undersupplied if left to market forces.

WHAT DO DEVELOPMENT BANKS DO FOR IMMUNIZATION PROGRAMS?

Development banks try to help strengthen immunization programs through activities at the national and international levels. At the national level, for example, they strive to include immunization programs in a country's policy dialogue and planning activities. The Poverty Reduction Strategy Paper (PRSP) is the methodology advocated by the World Bank for developing a strategy for reducing poverty

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and increasing growth. Immunization programs must be thought of within this context, for the way they get included in national budgets in developing countries is through inclusion in the PRSP. With the IDB, the country and the Bank develop a blueprint for cooperation: the Country Strategy Paper. Given the development framework contained in the PRSP, the Country Strategy Paper specifies how IDB will help the country finance some of its development initiatives, for example, how much lending will go for financing education, how much for infrastructure, and how much for public health services, particularly immunization programs. Immunization programs compete with other social development priorities in two instances: in the planning instrument (PRSP) and in the country assistance instrument (Country Strategy Paper). In both exercises, the guiding objectives of poverty reduction and economic growth should be kept in mind.

Development banks also help countries finance health needs with project loans. There are two basic types of loans: investment loans and policy-guided loans. A health investment loan, for example, may finance the development of a laboratory for ensuring the safety and efficacy of drugs (e.g., training personnel, purchasing equipment, etc.). The country finances part of the project and borrows the rest from a multilateral financing institution, such as IDB. Recently, however, the trend has been towards policy-based loans. Here, resources are transferred to the national treasury, which agrees to adopt some policy changes and to finance specific programs. Transfers are conditioned on the country's adoption of policy changes required to make development more effective. Therefore, policy-based loans not only finance development activities, but also ensure that those activities are carried out in the most effective way possible.

Another way in which development banks support national immunization programs is by financing the provision of technical assistance. Sometimes, the banks provide such assistance directly, and sometimes—as in the case of immunization programs—they do so in conjunction with a specialized agency, such as the Pan American Health Organization (PAHO).

Lastly, development banks support immunization programs through interagency coordination. Otherwise, given the norms and agendas of the various development agencies, the banks could contribute more to the confusion than to the solution.

In addition to these activities at the national level in support of immunization programs, development banks also support them at the international level. For example, IDB has contributed generously—through grants, not loans—at the regional level to the Plan of Action for the Eradication of Indigenous Transmission of Wild Poliovirus from the Americas. The World Bank Group is a member of the Global Alliance for Vaccines and Immunization.

PAHO, IDB, and the World Bank have created, under the leadership of former PAHO Director Dr. George Alleyne, a coordination system called the Shared Agenda for Health in the Americas. One of the specified areas for coordination is immunization. Within the framework of the Shared Agenda, the participating organizations work to strengthen PAHO's Revolving Fund for Vaccine Procurement, particularly in times of financial crisis, and to pursue equity and coverage goals. Overall, more than 85% of children under 1 year of age in Latin America are immunized. However, only 53% of the municipalities have reached a coverage level of 95% or higher. Thus, the three organizations are trying, together with the countries, to eliminate that inequity. Other goals of the Shared Agenda are to develop and use standardized procurement procedures regardless of the source of funds and to achieve financial sustainability. Although the ultimate goal for poor countries is self-financing, the immediate goal is to have a financial scheme that enables them to cover all of their short- and mid-term immunization expenditures. Sustainable financing of immunization programs in developing countries is a responsibility shared by the country itself and

the donor community, and the financial commitment of all partners must be reliable. If the external partners are here today, but gone tomorrow, then they do a disservice to the immunization program and to the country.

Accessing Financial Resources

Countries seeking to obtain financial resources to support their immunization programs should keep several points in mind when working on their PRSPs, country strategy papers, studies, and other related plans. Resources are mainly allocated according to cost-effectiveness criteria. In general, development banks do what yields the greatest benefit for the least expenditure. That is not easy to do in an area such as health, which has many political and emotional considerations. Anyone who makes decisions about resource allocation would recognize that some of the options selected are not cost-effective, but politically motivated. However, to the extent possible, cost effectiveness must be an important consideration. This criterion favors immunization programs because they are a very cost-effective health intervention.

"Development effectiveness" is another important consideration. Countries must do things that produce results and must measure those results. They must ensure that they do what they promise to do and achieve the impact they promise to achieve. Evaluating and measuring results and impact are essential to measuring program effectiveness.

Countries must recognize that efficiency and equity are not conflicting objectives, but complementary ones. More efficient use of resources permits higher levels of coverage, thus contributing to increased equity and, eventually, the incorporation of newer vaccines into the immunization program.

Finally, countries must ensure that domestic and external sources of financing are in complete agreement and strategically harmonious. If they are not, they may hinder program progress and effectiveness and lower cost-effectiveness, thus hampering achievement of development goals.

International Sources of Development Financing

The Heavily Indebted Poor Countries (HIPC) Initiative helps such countries reduce some of their external debt in return for a commitment to use monies that would have been targeted for debt servicing to finance development projects that are beneficial for poverty reduction and economic growth. This debt relief program is being used in large measure to finance immunization programs.

The International Development Association (IDA) is an organization that channels financial support to the world's 79 least developed and poorest countries. In July 2002, IDA put aside a good portion of its replenished capital for grants to support social programs—including immunization—in member countries.

The U.S. government's Millennium Challenge Account will provide increased funds for development assistance. Millennium Challenge Account aid will be conditioned on good governance and will be allocated to health, education, and other social programs to reduce poverty and foster economic growth.

Another potential source of funding for immunization programs is national trust funds. This entails establishing endowments whose interest earnings will be used to finance current expenditures of immunization programs.

Conclusion

Multilateral financial institutions can provide advice, but should not decide what a country needs to do to further its development. As with any other development program, immunization programs should seek to have country ownership, measurable results, and demonstrable impact. Development banks try to support their member states' public health efforts and to act as partners in their immunization programs.

THE POTENTIAL IMPACT OF HEALTH REFORM ON IMMUNIZATION PROGRAMS

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INTRODUCTION

It is essential to analyze the settings in which immunization programs are carried out in order to ensure the sustainability of these initiatives. The health systems of the Region of the Americas have undergone a series of changes collectively known as health reform. Since health reform is intended to improve access to and the quality of health services to which the population is entitled, it is important that it be understood, both because it has introduced changes in the traditional concept of health services delivery and because of the way it has changed existing processes. In some countries, health reform has resulted in a decline in the coverage of priority public health programs.

Health reform has been ongoing in Latin America for the past 20 years, and has primarily focused on funding and financial management, separation of the regulatory functions from the operating functions in health systems, and the operation of individual health services. The most important changes relating to the latter have been decentralization, or transfer of authority to the local level, and the development of basic packages of health services to be guaranteed to the entire population. In general, these processes have focused on individual health services and not on public health programs.

Since they have not been a focus of the health reform process, public health interventions have continued to be carried out following a central command and control logic that is contrary to the aforementioned decentralization trends, but to which many of their successes are due.

The health reform processes are not abstract changes—they have been implemented by persons carrying out the daily business of the health services. Thus, changes have affected the organizational culture and the conduct of the actors in the health sector. The smooth operation of the sector's activities and the practical translation of policy decisions into results that are congruous with the principles underlying the reforms and the reforms' objectives depends on the actors within it. Consequently, it is important to understand the opinions of

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those who work in the health field in order to better analyze the effects of the changes on such important programs as immunization.

Using information gathered through interviews of key informants working at various levels in the Region's health systems, this chapter compares the opinions of health professionals who work in immunization programs with the opinions of those who work in the realm of health reform policymaking or management regarding the potential effects of health reform on the Expanded Program on Immunization (EPI). Finally, it reviews certain factors related to the state's steering role in health from the standpoint of the so-called "essential public health functions" to emphasize the importance of improving the public health infrastructure in order to ensure the optimal performance of the health systems, particularly with respect to immunization programs.

Health Reform and the Expanded Program on Immunization

To explore the views of professionals who work in immunization programs and those who work in the health reform policymaking arena regarding the potential impact of health reform on the Expanded Program on Immunization, we built a matrix with the principal components of EPI and of health reform. Based on that, we designed a questionnaire for EPI professionals at the central level and managers at the subnational levels, and for professionals involved in the design and implementation of health reform policies at equivalent levels. The questionnaire covered six areas: separation of functions, funding, decentralization, drug policies, basic health services package, and cost containment. After it was validated, the questionnaire was administered to 40 professionals from seven countries of the Region: seven from Bolivia, one from Brazil, 13 from Chile, six from Colombia, three from Costa Rica, six from Guatemala, and four from Mexico. The group involved in health reform included 24 individuals, and the group working in EPI, 16. The participants represented 71% of those invited to answer the questionnaire by e-mail. We present the average scores for the responses of both groups to the questions in the questionnaire, which were constructed as statements with which the respondents indicated their level of agreement on a scale of 1 (lowest) to 5 (highest). By analyzing the principal components of EPI and of health reform, we demonstrate the similarities and differences between the two "worlds" studied.

The average scores for level of agreement among EPI professionals and health reform policymakers with the questionnaire's statements regarding *separation of functions* are very similar, as can be seen in Table 1. The opinions of these groups are farther apart where *funding* is concerned. There are differences in the groups' level of agreement with the statement about the autonomy of the different levels of the health services networks and their role in adjusting the funding allocated to the various program activities (Table 2). Although neither group agrees strongly with the statement, the health reform group agrees to a lesser extent than the EPI group does. Something similar occurs with the question on the strategies for fighting or opposing pressure groups and the impact of these strategies on EPI funding, although in this case, EPI professionals agree less with this statement than do the policymakers in health reform.

The issue of *decentralization* does not seem to divide the groups as much as one might expect. One of the most important differences is seen in the responses regarding the transfer of authority to the managers of the decentralized EPI, implying that EPI professionals see themselves in some conflict with those managing the changes (Table 3). Professionals working on health reform are more in agreement with the statement that decentralization forces a negotiation of objectives between the different levels of EPI, increasing its effectiveness, though both groups show a sufficient level of agreement with this statement. The opinions

TABLE 1. Average level of agreement (scale of 1 [least] to 5 [most]) among immunization program professionals and policymakers in health reform regarding statements about the separation of functions.

Statement	Immunization program professionals	Policymakers in health reform	Rank
Separation of functions forces the establishment of normative frameworks for the regulation of the roles of different entities intervening in EPI activities	4.9	4.8	1
Separation of functions must allow for a clear assignment of EPI's responsibilities to well-defined entities and persons	4.7	4.9	2
Separation of functions facilitates the definition of concrete and measurable goals for those entities responsible for EPI actions	4.2	4.5	3
Separation of functions calls for a redefinition of EPI's organizational structure	4.0	4.5	4
Separation of functions facilitates the identification of information needs and the development of information systems	4.2	3.9	5
Separation of functions facilitates EPI's relationship with its product suppliers	3.8	4.1	6
Separation of functions facilitates the implementation of EPI in seeking greater equity in access to health services	3.8	3.9	7
Separation of functions facilitates adaptation of EPI to population needs	3.7	3.9	(8)
Separation of functions strengthens the health authority	3.5	4.1	(8)
Separation of functions allows for better evaluation of EPI technologies	4.0	3.4	(10)
Separation of functions has meant low clarity of EPI roles	3.8	3.6	(10)
Separation of functions can increase the power of anti-vaccine pressure groups	3.5	3.3	12
Different levels of the health system should be autonomous in order to adapt EPI to their reality	2.5	2.9	13
Separation of functions degrades the unity of EPI objectives and negatively affects teamwork	3.0	2.1	14

of the two groups are most disparate regarding the statement that decentralization dilutes responsibility, with which the health reform group disagrees (average score of 2.6) as compared to the more moderate reaction of the EPI group (average score of 3.7).

With regard to *drug policies*, the overall conclusion drawn from Table 4 is that the EPI group does not support the inclusion of EPI in national drug policies and believes that the program should remain separate; the health reform group's opinions echo this sentiment, but less strongly. However, both groups feel strongly that EPI should be a priority element of any *basic health services package* (Table 5).

As seen in the responses to the statements regarding *cost containment*, there is also a high level of agreement about the cost of rationalization in terms of program operations (Table 6). The difference in the groups' level of agreement with the statement regarding the increase in costs brought about by developments in technology could be related to the background of the health reform group, which usu-

TABLE 2. Average level of agreement (scale of 1 [least] to 5 [most]) among immunization program professionals and policymakers in health reform regarding statements about funding.

Statement	Immunization program professionals	Policymakers in health reform	Rank
A basic responsibility of the health authority is to assure the program's funding and sustainability	5.0	4.8	1
Increases in EPI funding across time, due to the inclusion of new products, are compensated by economic and quality of life benefits from disease prevention	4.9	4.6	(2)
An adequate EPI organizational structure ensures the program's financial stability	4.7	4.8	(2)
The legal framework should establish that EPI be funded through general taxes and not funds coming from individuals (payroll taxes or out-of-pocket expenditures)	4.8	4.7	(2)
EPI is among those goods funded with general taxes that strengthen equity approaches	4.8	4.6	5
EPI funding through general taxes strengthens the Ministry of Health's normative role	4.6	4.3	6
EPI's defined goals must be closely linked to program funding	4.3	4.3	7
Amount of funding is the most important element determining EPI's coherence with population needs	3.9	3.9	8
Funding EPI with public resources facilitates enforcement of the normative framework established by the health authority for the program's development	3.8	3.9	9
Autonomy of the different levels of the health networks should allow them to modulate funding assigned to different program activities	3.8	3.2	10
Strategies for fighting pressure groups can have important impacts on EPI funding	3.2	3.7	(11)
Diversifying EPI suppliers contributes to the program's financial sustainability	3.2	3.7	(11)
EPI performance demands greater responsibilities than those required in other programs and should result in better salaries for EPI health workers	3.1	3.0	13

ally has more knowledge of health economics, as compared with the health science training and focus of EPI professionals.

Despite the fact that the results of this study are presented as examples only, since they may be influenced by the selection of the particular group of interviewees, it is possible to conclude that the areas in which they agree with each other seem to outweigh the areas of disagreement, despite their different organizational, operational, and funding rationales. The fact that these groups have similar opinions on several issues supports the strategy of considering EPI as an indicator for health reform monitoring.

Health reform is an opportunity for EPI and should be understood as a vehicle for the program's success. The building of teams comprised of people who come from both worlds can be a significant contribution to the evaluation of program changes in the context of health reform.

TABLE 3. Average level of agreement (scale of 1 [least] to 5 [most]) among immunization program professionals and policymakers in health reform regarding statements about decentralization.

Statement	Immunization program professionals	Policymakers in health reform	Rank
Decentralization must define the responsibilities of each level of EPI management	5.0	4.9	1
Information systems must stimulate the use of information where it is generated	4.8	4.8	2
Program norms allow for a positive use of the potential advantages of decentralization	4.8	4.7	3
Decentralized management can only be strengthened through a collaborative relationship among the different levels of EPI	4.5	4.6	4
Decentralization requires a stronger use of central health authority (to ensure adherence to national policies)	4.5	4.5	5
Decentralization must allow local managers to define their own strategies for achieving EPI objectives	4.3	4.3	6
Decentralization forces a negotiation of goals between the different levels of EPI, increasing efficacy	3.8	4.3	7
Transfer of authority to decentralized EPI managers is not harmonious	4.3	3.6	8
Decentralization demands technological changes to assure that EPI goals are reached	3.4	3.8	9
Decentralization exposes EPI to pressure from anti-vaccine groups	3.2	3.8	10
Decentralization increases equity gaps due to different managerial capacities of local authorities	3.3	3.5	11
Decentralization dilutes responsibilities	3.7	2.6	12
EPI's relationship with suppliers is more bureaucratic	3.0	2.8	13
Decentralization jeopardizes the coherence of national EPI objectives with health needs	3.3	2.4	14

It seems reasonable to consider EPI a protected area, and as such it would also be advisable to consider, in accordance with the actual conditions in the countries, a legal framework to ensure the program's protection. EPI should always be financed with public funds, not just because it is a public good, but because doing so fosters the central establishment of regulations and strategies and strengthens the health authority so that it can promote equity. It seems important to carry out larger studies of the opinions of decision-makers and health workers in order to accumulate more evidence like this that helps decision-making and helps legitimize the role of the health authority and the steering role of the government in health matters. This role should be strengthened to ensure a protected environment for priority interventions such as EPI.

Steering Role, Essential Public Health Functions, and Immunizations

According to the Pan American Health Organization (PAHO), the steering role of the national health authority encompasses many areas of health system performance, such as conducting, regulating, fulfilling essential

TABLE 4. Average level of agreement (scale of 1 [least] to 5 [most]) among immunization program professionals and policymakers in health reform regarding statements about drug policy.

Statement	Immunization program professionals	Policymakers in health reform	Rank
EPI must have specific and explicit goals, despite being included in general drug policies	5.0	4.8	1
EPI must have a special normative framework, regardless of its inclusion in general drug policies	4.8	4.9	2
To treat EPI differently from other programs included in general drug policies is a social recognition of the program as well as of the health teams in charge of it	4.3	4.0	3
Inclusion of EPI in drug policies without consideration of its peculiarities hinders the design of information systems to support its management	4.2	3.8	4
The level of attention required by pressure groups justifies that EPI vaccines be treated differently than drugs included in national drug policies	4.0	3.7	(5)
Management of EPI by health authorities can be simpler if the program is not included in general drug policies	4.3	3.4	(5)
The inclusion of vaccines in national drug policies can affect EPI's potential to promote equity	4.0	3.6	7
The legal framework should recognize EPI as a different program and should not incorporate vaccines in general drug policies	3.3	4.2	8
EPI organizational structure and management are different, despite being part of general drug policies	3.9	3.4	9
Including vaccines in drug policies facilitates coherence of EPI objectives with population needs	2.8	3.1	10
The legal framework should treat vaccines as it treats other products included in national drug policies	2.5	3.3	11
EPI should be included in general drug policies because providers of vaccines are the same as those providing supplies for other health programs	2.5	2.9	12
Each level of care must be free to make decisions about EPI in the context of its own drug policies	2.5	2.4	13
EPI's technical complexities favor its inclusion in general drug policies	2.3	2.4	14

public health functions, modulating health care financing, ensuring compliance with insurance schemes, and harmonizing health services delivery (*1*).

Since 1999, PAHO, in collaboration with the U.S. Centers for Disease Control and Prevention, has developed a project to measure 11 essential public health functions (*1*):

Function 1: Monitoring, evaluation, and analysis of health status.

Function 2: Public health surveillance, research, control of risks and threats to public health.

Function 3: Health promotion.

Function 4: Social participation in health.

Function 5: Development of policies and institutional capacity for planning and management in public health.

Function 6: Strengthening of institutional capacity for regulation and enforcement in public health.

TABLE 5. Average level of agreement (scale of 1 [least] to 5 [most]) among immunization program professionals and policymakers in health reform regarding statements about the basic health package.

Statement	Immunization program professionals	Policymakers in health reform	Rank
EPI must be part of the essential core of any basic package of health services in order to strengthen the package's coherence with population needs	5.0	4.8	(1)
The legal framework should assure EPI's inclusion in the basic health package, regardless of the design of the set of interventions	5.0	4.8	(1)
The political framework of health reform defines EPI as an explicit part of the basic health package mandatory for the entire population	4.5	4.7	(7)
The inclusion of EPI in basic health packages greatly contributes to the reduction of inequities in health	4.5	4.7	(7)
EPI must not be modified despite the levels of autonomy defined for the modification of basic packages at the local levels	4.5	4.4	10
Even though EPI's technology has a certain level of complexity, the nature of the program contributes to its success and justifies its inclusion in the basic health package	4.2	4.3	11
Political considerations related to pressure groups should not influence the decision to include EPI in the basic health package	4.9	4.7	4
The inclusion of EPI in the basic health package can increase the market for providers of supplies needed to develop the program	4.0	4.0	(12)
The inclusion of EPI in the basic health package requires the existence of norms and controls that assure all users of the same level of quality	3.7	4.3	(12)
The inclusion of EPI in the basic health package implies that the authority charged with assuring service delivery defines its organizational structure	4.9	4.6	5
Information systems are a requisite for the evaluation of different components of EPI when it is included in the basic health package	4.0	3.8	14
The inclusion of EPI in the basic health package forces the health authority to define minimal coverage goals to assure program efficacy	4.5	4.5	9
Health authorities, within the normative framework defined, must enforce EPI's implementation when immunizations are included in the basic health package	5.0	4.8	(1)
The inclusion of EPI in the basic health package can be an important motivator for health workers	4.7	4.6	6

Function 7: Evaluation and promotion of equitable access to necessary health services.

Function 8: Human resources development and training in public health.

Function 9: Quality assurance in personal and population-based health services.

Function 10: Research in public health.

Function 11: Reduction of the impact of emergencies and disasters on health.

These functions were defined in accordance with the findings of WHO studies and with the cooperation of various experts in the Region in order to construct a measurement tool, a massive questionnaire whose approximately

TABLE 6. Average level of agreement (scale of 1 [least] to 5 [most]) among immunization program professionals and policymakers in health reform regarding statements about cost containment and rationalization.

Statement	Immunization program professionals	Policymakers in health reform	Rank
The legal framework must protect EPI from cost containment measures	4.9	4.6	1
Limits to rationalization should be included in EPI's legal framework	4.7	4.7	2
EPI must be a protected program in order to maintain its coherence with population needs	4.9	4.4	(3)
It is easier to contain costs with financial information systems linked to program performance	4.6	4.7	(3)
Rationalization can be compatible with EPI's periodic goals	4.3	4.6	5
Rationalization can be more coherent when central health authorities intervene	4.4	4.3	6
Rationalization makes the relationship with EPI suppliers more demanding	4.3	4.3	7
Pro-vaccine groups are important for modulating cost containment measures	3.6	3.9	8
Rationalization must be a responsibility of each autonomous level	3.6	3.7	(9)
Technological development favors cost increases and pressures	4.0	3.3	(9)
EPI efforts at rationalization should center on its organizational structure	3.5	3.7	11
As EPI is a protected program, it is hard to introduce cost containment measures because of political constraints	3.5	3.5	12
Rationalization can help reduce inequities	2.9	3.8	13
Rationalization deteriorates working conditions of health workers	3.1	2.4	14

800 questions define these 11 functions and which contains indicators and standards for each function.

Figure 1 reveals some of the results of this study, published in *Public Health in the Americas: Conceptual Renewal, Performance Assessment, and Bases for Action* (*1*), reflecting the answers of groups of respondents selected from different areas of public health work in each of the countries of the Region. As the figure shows, only one essential public health function—function 11—exceeds 70% performance. Other functions, such as 8, 9, and 10, though performed to a high degree by some countries, are on average underperformed. The remaining functions, whose performances range from low to quite high, fall somewhere in between.

The success of immunization programs depends not only on the program's being given high priority within the context of health reform and on having adequate funding resources, but on the foundation provided by an adequate public health infrastructure. Table 7 shows how the essential public health func-

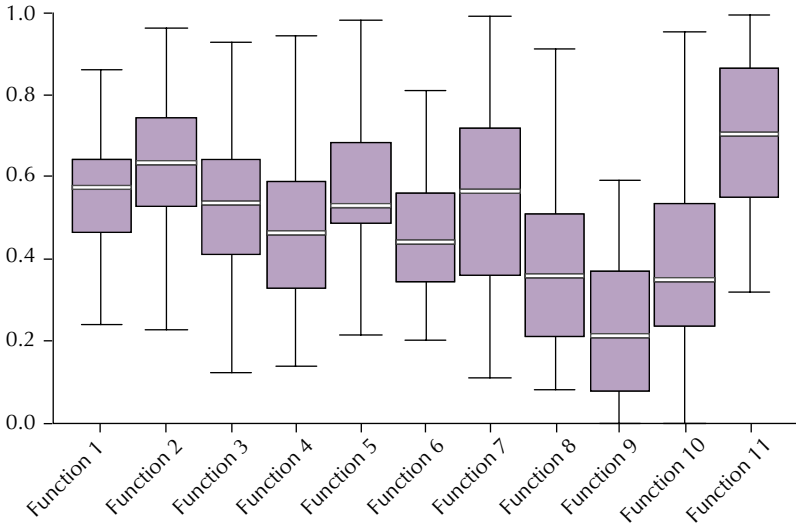
TABLE 7. Importance of essential public health functions to the Expanded Program on Immunization.

Essential public health function	Importance for Expanded Program on Immunization (EPI)
Monitoring, evaluation, and analysis of health status	• Permits detection of inequities in vaccine-preventable diseases
Public health surveillance, research, control of risks and threats to public health	• Permits monitoring of: Vaccine coverage Adverse effects Acceptance of and opposition to immunizations Laboratory failures
Health promotion	• Fosters the creation of healthy public policies supporting immunization programs
Social participation in health	• Empowers the population to support immunization programs
Development of policies and institutional capacity for planning and management in public health	• Promotes national plans that address medium- and long-term health goals • Promotes public funding for EPI
Strengthening of institutional capacity for regulation and enforcement in public health	• Facilitates central control and decentralized implementation of EPI • Ensures enforcement and fulfillment of performance contracts • Facilitates passage of laws making immunization programs mandatory
Evaluation and promotion of equitable access to necessary health services	• Strengthens primary care • Promotes the development of strategies to reach people in EPI activities
Human resources development and training in public health	• Ensures training and continuous education of health teams working in EPI
Quality assurance in personal and population-based health services	• Promotes better register, quality control, and eventually, production of vaccines
Research in public health	• Improves knowledge of vaccine-preventable diseases in the country • Facilitates evidence-based decision-making for introducing new vaccines and immunization strategies
Reduction of the impact of emergencies and disasters on health	• Permits the prevention and mitigation of damage due to infectious diseases spread during emergencies

tions apply to a priority public health program such as immunization. For example, monitoring, evaluation, and analysis of health status should permit the detection of inequities in the distribution of vaccine-preventable diseases and vaccine coverage.

Finally, given the vision and values of the various professional disciplines that exist within the Region's health systems, it is important to adapt tools such as those intended to measure the performance of the health infrastructure for use in the evaluation of the immunization programs' performance in the development of priority public health programs so that the immunization program's logistical needs can be determined and its effectiveness improved. In fact, health reform represents an important opportunity for the success of this initiative. Immunization programs have every opportunity to convince decision-makers of

FIGURE 1. Distribution of the performance of the essential public health functions in the countries of the Americas.



Source: Pan American Health Organization. *Public Health in the Americas: Conceptual Renewal, Performance Assessment, and Bases for Action*. Washington, D.C.: PAHO; 2002. (Scientific and Technical Publication No. 589).

their relevance, positive impact on individual and public health, and cost-effectiveness, as well as the costs of not deciding today to extend the benefit of immunization to all the inhabitants of the Region of the Americas.

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PERSPECTIVES FOR THE ELIMINATION/ ERADICATION OF DISEASES WITH VACCINES

Walter R. Dowdle[1]

INTRODUCTION

The eradication of smallpox in 1977 is often described as the single greatest accomplishment in public health. When the program began in 1967, there were an estimated 10–15 million cases and 1.5–2 million deaths (*1*). In 1988, when the World Health Assembly resolved to eradicate polio (*2*), paralytic poliomyelitis occurred in 125 countries, with an estimated 350,000 cases annually. By 2002, the number of cases and countries had been reduced by more than 99% and 95%, respectively. The Guinea Worm Eradication Program, with modest funding, has reduced the number of reported cases of dracunculiasis outside Sudan by more than 98% from the estimated 3.5 million in 1986, and the number of infected villages by more than 90%, from 23,000 (*3*). Clearly, global disease eradication initiatives can be powerful public health strategies—they are also subjects for legitimate debate, however.

Previous attempts to eradicate yellow fever, yaws, and malaria were unsuccessful (*4*), but they greatly contributed to understanding eradication challenges. Eradication must be biologically feasible (*5*). The anticipated direct and consequent benefits of eradication must be balanced against costs in terms of competition with other health activities, national and global health priorities, and allocations of scarce health resources (*6*). Perceived humanitarian benefits must be sufficient to generate the required political will and financial support (*7*). And today, national security concerns regarding the intentional release of an eradicated agent in an increasingly non-immune population have been added to the debate (*8*). This chapter reviews the relevance of the current definitions of elimination and eradication, explores the issues surrounding eradication as a public health strategy, and considers potential candidates among other vaccine-preventable diseases.

CURRENT DEFINITIONS OF ELIMINATION AND ERADICATION

Public health practitioners have used the terms disease elimination and eradication for years to describe ideal outcomes of disease control or goals distinct from it, where disease control is defined as the reduction of disease morbidity/mortality to a locally acceptable level. Both terms have been used inconsistently, with different meanings attached at different times to different diseases. The 1997 "Dahlem Workshop on the Eradication of Infectious Diseases" attempted to define elimination and eradication more precisely by using current models and building on earlier definitions (*5*).

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Elimination was defined separately for disease and infection, as follows:

- The definition of elimination in terms of disease used neonatal tetanus as a model, and reads: "Reduction to zero of the incidence of a specified disease in a defined geographic area as a result of deliberate efforts; continued intervention measures are required."
- The definition of elimination for infection used the declaration in 1984 that the Americas was polio-free, and reads as follows: "Reduction to zero of the incidence of infection caused by a specific agent in a defined geographic area as a result of deliberate efforts; continued measures to prevent reestablishment of transmission are required."

Eradication, using smallpox as a model, was defined along the lines of common usage, as "Permanent reduction to zero of the worldwide incidence of infection caused by a specific agent as a result of deliberate efforts; intervention measures are no longer needed."

At the 1998 Conference on Global Disease Elimination and Eradication as Public Health Strategies in Atlanta (*9*), some participants voiced strong concern that the distinction between eradication and elimination was artificial, confusing, and difficult to convey to those who were outside of the international public health inner circle. Although the Oxford dictionary defines "eliminate" as "to get rid of" (*10*) and "eradicate" as "to root out" (*11*), other participants noted that the term "elimination" was not directly translatable in many languages and called for its use to be discontinued. A post-conference ad hoc group was convened as recommended, and it attempted to combine elements of both terms (*12*): "The absence of a disease agent in nature in a defined geographical area as a result of deliberate control efforts. Control measures may be discontinued when the risk of disease importation is no longer present."

This definition also failed to satisfy everyone.

A Revised Basic Definition of Eradication

The Dahlem definition separates elimination into two categories, depending on whether the indigenous agent remains (as with *Clostridium tetani*) or does not remain (as with wild poliovirus) in the specific geographical region where the disease has been eliminated. The former was seen as the highest possible achievement for neonatal tetanus, the latter as a geographic step towards global polio eradication. The phrases that refer to the need for intervention measures further distinguish the two terms and convey the programmatic and economic advantages of eradication. The Dahlem definition of eradication is consistent with earlier interpretations, implying a state of permanence (*13*). It did not anticipate the current level of concern that the eradicated infectious agent could become a tool for bioterrorism in growing non-immune populations. For the first time in over 30 years, some countries have begun to vaccinate select groups against smallpox. Allaying national security concerns over the phrase "intervention measures are no longer needed" requires proof of agent extinction both in nature and in the laboratory. Documenting extinction in the laboratory is impossible, except for those parasitic diseases where eradication and agent extinction are synonymous. The definition of eradication has become an issue in achieving the ultimate goal in public health.

Modifying the 1998 Atlanta conference definition might accommodate differing national policies, for example, "Control measures may be discontinued when the risk of disease is no longer judged to be present." This change would allow for policy flexibility and would also permit the inclusion of the Guinea worm disease situation, but the added value of such a phrase is questionable. Elimination and eradication might be replaced with new terms, such as interruption of indigenous transmission or interruption of global transmission, which are frequently being heard in reference

to measles and polio, respectively. De Serres et al. (*14*) have already pointed out that the Dahlem definition of elimination as "reduction to zero" is unrealistic and functionally unnecessary. They proposed elimination to be more appropriately defined as a situation in which sustained transmission cannot occur and secondary spread from importation will end naturally. These terms are epidemiologically precise and may apply as well to a revised definition of eradication, but they are unlikely household words. Finally, the intervention qualifiers might be removed altogether from the Dahlem and Atlanta definitions of eradication, and the geographical qualifiers might be removed from the Dahlem eradication and elimination definitions. Doing so provides a revised basic definition of eradication as "The absence of disease in a defined geographical area as a result of deliberate control efforts."

With this definition, elimination becomes redundant. National security concerns become a non-issue, as post-eradication intervention practices can differ according to disease and national policy. Eradication becomes national, regional, or global. Although this revised basic definition of eradication loses epidemiologic precision, it retains important word recognition and symbolizes the highest aspirations of public health. It is consistent with common use of the term in the public media and traditionally in the Region of the Americas, not the least because elimination is not readily translated into Spanish and Portuguese. The basic definition permits national and regional programs to consider their own goals independent of global implications. It gives credit to Cuba as the first nation to eradicate polio and measles. It gives credit to the Americas as the first region to do so. It declares Guinea worm eradicated in India and Pakistan. Nations and regions no longer have to wait for credit until the remainder of the world catches up. It is also consistent with the definition of eradication in agriculture. Diseases such as bovine pleuropneumonia, foot-and-mouth disease, rinderpest of livestock, and Newcastle disease of chickens are eradicated within nations or regions, without reference to continuing methods of intervention.

National Eradication

Disease eradication at the national level can be the outcome of good public health practice, often without eradication being the stated goal. The constellation of conditions that make eradication feasible is well established:

- biologic feasibility (defined here as including the availability of effective intervention measures),
- political will,
- sufficient funding, and
- adequate public health infrastructure.

In countries where all four of these conditions exist, national eradication is highly probable over time. District health officers, state health commissioners, or ministers of health dedicated to their missions are unlikely to tolerate continued outbreaks of serious disease if prevention is possible and adequate funds are available. National eradication brings health equity, strengthens health infrastructure, and fulfills political and public health goals. High-income countries eradicated indigenous smallpox, poliomyelitis, and, more recently, measles, well before global initiatives were envisaged. It was desirable, doable, and the thing to do. The vaccine-preventable diseases considered at the 1998 Conference on Global Disease Elimination and Eradication as Public Health Strategies as potentially meeting the biological criteria for eradication (*15*) include: measles, rubella, hepatitis A, and hepatitis B. Whether these diseases meet the remaining three conditions of the eradication constellation is open to question.

Regional Eradication

Expanding from national to regional eradication extends to low-income countries the eradication benefits already being enjoyed by high- and some middle-income countries, but it

greatly increases complexity. A Region must collectively ensure that the essential conditions in the eradication constellation are in place in all member countries. Biologic feasibility requires overcoming the challenging environmental and social conditions that favor transmission of disease agents in low-income countries. Political will requires gaining eradication consensus among multiple countries with different health priorities and agendas. Sufficient funding requires generating outside support for countries where internal funding is highly competitive or lacking. Adequate public health infrastructure requires providing outside technical assistance, and, in many cases, compensating for weak or absent infrastructure through more centralized or vertical programs.

The list of potential candidates for eradication begins to shorten at the regional level, as differences in perceived eradication benefits begin to emerge among member nations. Low-income countries must address issues of scarce health resources, national health priorities, control of the health agendas, lost opportunities, and the influence of outside funding. Some middle-income countries may feel coerced through regional peers and national pride and the benefits of eradication may not be always apparent among the political realities of the day.

Key to regional eradication is the constitution of a formal representative body that has legitimacy and commands respect, so it can promote eradication if this is determined to be an appropriate strategy to address health inequalities within the Region. Such a formal body is crucial to generate political will, secure adequate financing, and provide the organizational and technical skills to achieve the eradication goal. The model, of course, has been seen in the Region of the Americas, with PAHO functioning as such a formal body with a legacy of more than 100 years of extraordinary leadership. Current progress toward the goal of measles eradication in the Western Hemisphere is continued evidence of that leadership (*16*).

Global Eradication

Global eradication provides global health equity. It promises that the necessary constellation of conditions for eradication will be in place for every country in every region. National and regional challenges to biologic feasibility become global. Political will requires developing consensus among 214 individual countries with differing aspirations and health priorities. Sufficient funding requires marshalling both public and private resources to support the Regions and countries most in need. Adequate public health infrastructure requires developing a centralized global mechanism to generate funds, coordinate global strategies, set global standards, provide regional assistance, and maintain a global database. The key to global eradication, as it is to regional eradication, is a formal body with legitimacy, respect, leadership, and infrastructure to ensure that the job is done. The formal body at the global level is, of course, the UN system, with the World Health Organization (WHO) as the lead agency.

Candidate Diseases for Global Eradication with Vaccines

Measles has been mentioned frequently as the leading candidate, after polio, for global eradication with vaccines (*15*). The biologic feasibility of national measles eradication has been confirmed by the interruption of indigenous measles virus transmission in Cuba since 1988 (*16*), England and Wales since 1995 (*17*), and the United States since 1997 (*18*). The feasibility of regional eradication is confirmed by progress in the Western Hemisphere (*16*). However, meeting the remaining three conditions in the constellation for global eradication is a formidable challenge.

Political Will

In the Americas, the 1994 resolution of the Ministers of Health to eradicate measles by

the year 2000 is clear evidence of political will (*19*). Political will also is high in sub-Saharan Africa and South Asia, where measles remains a major cause of death in children. Even so, a unified global position on eradication is problematic as long as key industrialized countries—such as Japan, Italy, France, and Germany—do not consider measles a national health priority (*20*).

Whether current national security concerns will influence the attitudes of key high-income countries toward future global eradication initiatives is unclear. Hopefully, bioterrorism will be viewed not as a public health issue, but as a foreign policy or criminal issue that could have major public health consequences. Nations that have national security concerns always have the post-eradication option of continuing immunization. Low-income populations who experience natural disease threats daily are likely to have fewer concerns about a remote threat of bioterrorism (*21*). National security is a contingency that requires having plans in place to deal with the consequences, but it is not an excuse to avoid achieving attainable global health goals. Reaching a consensus on global eradication of measles, or of any other disease, requires careful weighing of many factors, but national security concerns should not be one of them.

Sufficient Funding

A 1985 Resolution adopted by PAHO's Governing Bodies (*22*) and a 1988 Resolution adopted at the World Health Assembly (2) justified polio eradication on humanitarian grounds and the consequent benefits of boosting other childhood immunizations. In the early 1990s, however, polio eradication's economic benefits became a major selling point in generating financial support (*23*). A consensus on the economic analyses of disease eradication is not always clear (*24, 25*), but economic benefits have been implied in virtually all definitions (*5, 12, 13*). It had been estimated that US$ 1.5 billion in vaccine costs would be saved each year when polio was eradicated and all control measures stopped (*23, 26*). Discontinuing vaccination against smallpox was estimated to save hundreds of million dollars annually in direct costs (*23, 25*).

Estimates of direct economic benefits now risk disbelief, as some countries rethink the phrase that "intervention methods are no longer needed." Because of perceived post-eradication uncertainties, high-income countries are anticipated to continue routine use of inactivated polio vaccine long after global transmission of wild poliovirus has stopped. Low-income countries are expected to continue post-eradication use of oral polio vaccine, at least until a cessation strategy is devised that reduces the risk of circulating vaccine derived viruses (*8, 27*). National and regional measles eradication in the Americas has been shown to be cost-effective with continued immunization, based solely on reduced incidence, however (*28, 29*). Even so, as the fifth leading cause of death among children aged <5 years worldwide (*30*), generating sufficient funds for measles eradication is justifiable on humanitarian grounds alone.

Adequate Public Health Infrastructure

Eradication initiatives for vaccine-preventable diseases are strategies for national health systems to go beyond business as usual. Such initiatives are most effective when administered within functioning primary health care systems. Where infrastructure is weak, vertical initiatives are seen as offering opportunities for strengthening national health infrastructures and providing universal health benefit not otherwise available. A recent review of published evaluations on the polio eradication initiative reported overall positive effects on national health systems and other health services (*31*). Nevertheless, philosophical differences remain over the effectiveness of vertical versus horizontal health programs (*32, 33*). The current progress of national measles control programs in all WHO regions provides

direct evidence of the legacy of eradication programs. The existing polio eradication infrastructure has both facilitated (*31*) and reduced the costs of national measles initiatives in polio-free countries (*29*). Most developing countries today are more likely than at any time in the past to have public health infrastructures capable of sustaining national measles eradication goals.

Conclusions

A revised, basic definition of eradication has been proposed—"The absence of disease in a defined geographical area as a result of deliberate control efforts." Using this definition, disease eradication becomes the highest public health achievement at the national, regional, and global levels. National eradication, where biologically feasible, can be a natural outcome of good public health practices. Many countries eradicated smallpox, measles, and polio well before regional or global eradication goals had been set. Systematic application of country-appropriate strategies led to the global eradication of smallpox and the regional eradication of wild poliovirus.

National security concerns are not necessarily exclusive to global eradication initiatives. For example, it is conceivable that, in the absence of a formal global program, smallpox could have been eradicated over the last 30 years by step-wise immunization assistance to individual governments. Bioterrorism issues would be no different. High-income countries must place post-eradication security concerns in the context of global health needs and aspirations. National preparedness and a perceived need to continue some form of post-eradication intervention measures should not be obstacles to achieving global health equity. Eradication at the national level raises expectations, generating demands for similar goals in other countries, as has happened with measles eradication in the Americas. Reaching a consensus on global eradication requires careful weighing of many factors, but national security concerns should not be one of them.

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Epilogue

Conference on Vaccines, Prevention, and Public Health: A Vision for the Future

WELCOMING REMARKS

George A.O. Alleyne[1]

As usual at these conferences, we thank the sponsors last, but I am going to reverse that practice. I would very much like to thank those who have sponsored this conference and made it possible for such a distinguished group of persons to be here with us today. Of course, I want to thank all you participants for coming.

I am very pleased to see so many persons here today who have dedicated their lives to work in this field, especially those at the head table whom I would describe as the warriors in the fight to immunize against vaccine preventable diseases. We have here a wealth of talent and experience, and in a sense they represent the thousands, if not millions, of persons who share the vision of a world made better through the use of vaccines and through the strategy of prevention and acceptance of the values and virtues of attending to the public's health.

Ladies and gentlemen, I never tire of telling of the relationship between this and similar events to mark the Pan American Health Organization's centennial. I do not do this only because of the pride that I have in this Organization, but also because of my appreciation and sense of history—a sense that we who are privileged to be here today must recognize the debt we owe to those who founded this organization, and those who help it grow and prosper. My predecessor, Carlyle Guerra de Macedo, who we have here today, is one of those. Thank you very much for coming, Carlyle.

I also do this to emphasize what has been a defining theme for me during this particular year. The theme is that we do not celebrate primarily the work of the Pan American Health Organization, but we celebrate the work the countries have done together and what we have been able and privileged to do to help them to achieve that work.

Our founding fathers were aware of some aspects of the nature of disease and some sanitary measures that could be applied. As I go back and read their deliberations, I have doubted that ever in their wildest dreams they could have imagined that science and man's genius could take us beyond the technology for curing disease to the technologies, which when applied to the healthy, would prevent disease.

But we did not get here in one great leap. There were many refueling stops along the way. In 1970, a conference was held in this very room, the "International Conference on the Application of Vaccines Against Viral, Rickettsial and Bacterial Diseases of Man," eight years before the world, led by D.A. Henderson and his cohort of vaccination warriors, was freed from smallpox.

That was nine years before this country saw its last case of poliomyelitis. I like to believe that it was that conference that gave the impetus for the formation of the global Expanded Program on Immunization (EPI) and a similar version of EPI here in the Americas. It was that conference that detonated the interest in vaccination globally, and you are all witness to the events that have unfolded since then. I trust

[1] Director Emeritus, Pan American Health Organization.

that this conference will have a similar or greater impact, and that your deliberations will have a similar or greater visionary thrust than that which was engendered here thirty years ago. Judging from the abstracts, your debates will encompass facts and dreams of what vaccines can and will do, as I know that even the most pragmatic scientist among you dreams as well. I believe that your debates will show how in years to come, the number of vaccines licensed in this country will double or triple from the current twenty-six, and that we will see the same phenomenon in our world's developing countries, particularly the countries of the Americas.

The debate will continue on how to maintain and enhance the health of the public by embracing vaccines as one of the darlings of prevention. Your conclusions will represent a powerful and logical argument against the view that the usefulness of vaccines may be measured in terms only of economic benefits to be derived on the interruption of vaccination. And I hope that your vision of the future will be a rosy one, because I believe that a clear statement about the desired future is essential for and can influence the future we inherit.

I wish you a very successful conference. I can do no better than to repeat the words that the Secretary of State of the United States of America, the legendary Elihu Root, used when he welcomed the delegates to the second Sanitary Convention in 1905, which ratified the decision to create our Organization. He said in purple prose that was appropriate for that time, and I quote, "that you may promote the great work of elevating the standard from which you yourselves and your fellows and your successors may take new departures for the accomplishment of great things for humanity. That you may feel and may communicate this magnetic influence which tends to promote the successful activity of human intelligence. That is my sincere wish." I hope that a hundred years hence my successor will attest to the great things for humanity that were begun in this conference. That is my sincere wish.

SUMMATION

Donald A. Henderson[1]

This has been an extraordinary conference. It clearly has exceeded all expectations of its organizers. The program has been rich and varied and the speakers have offered a remarkable overview of accomplishment and a vision of what might be. It seems to me that there never has been a more auspicious time for the future of vaccine research, development, and application.

We salute Pan American Health Organization and the countries of the Americas for their efforts, especially over the past 25 years of the Expanded Program on Immunization (EPI), in accomplishing a revolution in disease prevention through vaccination. Special recognition is in order for the leadership provided by PAHO Directors Carlyle Guerra de Macedo and Sir George A. O. Alleyne, and especially for Dr. Ciro de Quadros and his staff. Over this brief time, we have witnessed the disappearance of polio from the Americas using an imaginative strategy which now serves as the template for the global eradication program. We have seen measles cases decrease to the point where, by the close of this conference, fully nine weeks have elapsed since the last known case of measles in the Western Hemisphere. Neonatal tetanus cases are now found in fewer than 1% of all Latin American districts. Diphtheria and pertussis have become rare, if not endangered, diseases.

New vaccines have been steadily introduced into immunization programs—vaccines for rubella, for hepatitis B, and for *Haemophilus influenzae* type b. An effective yellow fever vaccination program has been mounted. A revolving fund for the purchase of vaccines was inaugurated in the Americas in 1979, and this now serves as a model for other areas of the world. Most impressive are the annual national planning meetings that include national and international staff along with PAHO experts, and which set specific goals and identify present and future needs—another model which needs to be emulated elsewhere in the world. These are remarkable accomplishments that are not yet widely known or fully appreciated.

This meeting, as Dr. de Quadros has pointed out, occurs just 32 years after the first international conference on vaccines. That conference was held in this same room, in December 1970. It proved to be an historical turning point. Let me highlight a few points of special interest to give perspective to present events.

The intent of the 1970 conference was to review the status of available vaccines and their application and to develop recommendations for their use both in the industrialized and developing countries. The meeting had been made possible by a generous contribution from Merck & Co. The secretariat consisted of Charles Cockburn and myself, from WHO/Geneva, and Conrado Ristori and Mauricio Martins de Silva, from PAHO. During the course of the conference, two committees met to draw up recommendations for routine vacci-

[1] Special Advisor, Center for Biosecurity, University of Pittsburgh Medical Center, Baltimore, Maryland, U.S.A.

nation programs that seemed most generally appropriate for the industrialized countries and a design for immunization programs for the developing countries. The latter were eventually to materialize some four years later in the form of the EPI. Such was its birth.

At the time of the conference, vaccination programs were not of high priority in most developing countries. As we were discovering in the course of the smallpox eradication campaign, only small numbers of children were being vaccinated against any diseases other than smallpox. Smallpox vaccination was offered in most countries because of the severity of the disease, but, as we discovered, less than 10% of the vaccine then in use met accepted international standards. Moreover, vaccination was primarily available only in urban centers; programs extending throughout a country were uncommon.

Other vaccines were seldom used in developing countries. UNICEF supported BCG programs in several countries. Measles vaccination had recently begun in countries of Western and Central Africa with assistance from the United States Agency for International Development (USAID) and the Centers for Disease Control and Prevention (CDC); DPT was being provided to less than 5% of children in the developing world. Yellow fever vaccine was periodically provided in some African countries when epidemics threatened.

The conference subcommittee for the developing countries recommended programs that provided the following antigens—smallpox, BCG, DPT, measles, and typhoid. There was no mention of polio vaccine. You may wonder why. Polio was not then considered to be a significant problem in the developing world. Not until 1972, when the first lameness surveys were conducted, did our understanding change. The lameness surveys, as you may recall, consisted of screening all children at school entry to ascertain what proportion had the characteristic flaccid paralysis of recovered polio cases. Such surveys were able to be performed rapidly and to cover a large population of children. First in Indonesia and later in Africa, it was demonstrated that paralytic polio was surprisingly more prevalent than any had appreciated. By 1974, when EPI started, polio vaccine was substituted for typhoid fever vaccine in EPI programs.

Two notable problems were highlighted at the 1970 conference. First was the comparative dearth of vaccine research and development; second was the lack of both interest and resources for vaccination programs in both industrialized and developing countries. The principal concerns were: how to obtain affordable vaccines in quantity; how to secure the interest and commitment of governments and potential donors; how to mount effective national programs. Three decades later, many of those concerns remain, but many solutions have been found. Dramatic changes have occurred, and in no region of the world more than in the Americas.

At this conference, there has been a veritable cornucopia of initiatives pertaining to vaccine research and development. The sheer number of potential new products and the opportunities now offered actually create a quite different problem, and that is one of prioritizing those to be pursued most actively and how best to do this.

Many additional resources have become available for program implementation. UNICEF has played a key role as have bilateral agencies, the Rotary Foundation, and development banks. Countries have begun to set aside funds from national budgets in order to mount and strengthen programs. A special impetus has been provided by the Bill and Melinda Gates Foundation, especially in the areas of research and development, as well as in program implementation. That Foundation's very generous support of public health has been an encouragement to other donors and foundations to examine the comparative benefits of prevention contrasted to therapy.

The capacity to deliver vaccines has vastly expanded throughout the Americas and, no less, in other areas of the world. "National Im-

munization Days" were a creation of Brazilian and Cuban health authorities, and this strategy spread rapidly across the Americas and to countries in Africa and Asia. It has proved to be a critical component of the polio eradication effort but effective with other vaccine programs as well.

During this meeting, two particular challenges have been posed which I believe deserve highlighting as problems now worthy of special attention. First is that of devising approaches that permit the targeted development of new vaccine preparations. The second is the problem of deciding regulatory measures that are appropriate to countries where disease risks warrant the development of vaccines and drugs to cope with diseases of the tropics, and which potentially may need to be produced under regulatory strictures that are best adapted to the country and the problem. There are no simple or obvious answers to address either of these problems. However, if they are not addressed, it is clear that future progress will be much more problematical.

As we have heard, laboratory scientists are now producing seemingly countless numbers of new discoveries and new ideas that offer enormous promise. What is not clear is how and by whom the most promising can be identified and supported in order to undertake the necessary developmental research to translate them into practical products for use in the field.

We have heard at this meeting how costly and time consuming it is to take a promising product from the research bench through the developmental stages, to production, to animal and human testing and, finally, to licensure. It requires a substantial involvement and commitment on the part of the private sector, wherein resides the skills and knowledge to undertake such steps in development. Companies, understandably, need reasonable assurance that if they make such an investment in a product, it will be able to be recouped through sales. Vaccines, however, have not proven to be especially remunerative, especially those that are particularly intended for use in developing countries. The fact that we have today so few manufacturers and that the number continues to diminish is alarming. This was highlighted for us specifically with regard to malaria. It pertains with equal force to many other products. Thus, it is critical to identify priority areas with care, to establish clear time lines for development, and to work out means by which commitments for final products can be made.

The second consideration is the question of what regulatory provisions are most appropriate for which products and in which countries. Ever safer and ever more carefully monitored products are desirable, certainly, but each increment in improvement in standards of manufacture, testing and documentation bears with it a cost. This was brought home to me this past year as the United States and other countries sought to purchase smallpox vaccine for the first time since 1975. It cost, at that time, between US$ 0.50 and US$ 1.50 per vial, each vial containing 100 doses. The cost of the vaccine is now more than 100 times greater. This far exceeds changes due to inflation. We are now told that to bring a new vaccine to market in Europe or the United States would require something between US$ 200,000,000 to more than US$ 500,000,000. If no mechanisms can be found to diminish these expenses, there will almost certainly be a number of products of immense potential value, especially to those in the endemic regions of the developing world, that will not make their way to market. It seems to me that it is appropriate and timely to reconsider regulatory and licensing mechanisms with this concern in mind.

There is a special need, as well, to consider regulatory measures in the context of programs and priorities. The implications of regulatory measures can be profound, as witness the turmoil and vaccine shortages that resulted from a recent decision to require that thiomersal be removed as a preservative from all vaccines. A problem, potentially as serious, occurred early in the course of the smallpox eradication pro-

gram. It was scarcely averted. For some 18 months after the program began, we had been successfully using jet injectors throughout Latin America and West and Central Africa. The injector deposited the vaccine in the superficial skin layers by injection instead of by scarification with a needle. Studies showed that the depth of vaccine deposition was about the same with each. However, quite unexpectedly, regulatory authorities in the United States decided that the vaccine used for jet injection had to be classified as an injectable product and so had to be sterile. We learned this only days before the proposal was to be brought before a WHO biological products control committee.

The smallpox vaccine was then being grown on the flank of calves, as it had been for a hundred years. However much the skin was cleansed, bacteria were carried forward in the vaccine. Tests monitored both numbers and the absence of pathogens. Making it a sterile product was impossible. Vaccine grown in eggs or tissue culture could have produced a sterile product, but laboratories that had tried to do so were unsuccessful in producing a sufficiently stable product for use in the field.

A decision to require that the vaccine be sterile would require a total restructuring of programs in at least 20 countries and possibly suspending most of them because vaccine for scarification was in short supply. Nevertheless, we were advised that the regulatory authorities were favorable to the idea. Fortunately, the WHO Director General understood the problem and insisted that the item be removed from the agenda.

The challenge for us in the years ahead is to appreciate that the discovery, development, application, and regulation of vaccines have to be viewed as integral processes with each element having important, sometimes critical, implications on the other components with decisions weighed accordingly.

What will another conference look like 32 years from now? Who could possibly have predicted in 1970 how far we would have come in just one generation? It seems to me that the next 32 years promise to be even more productive provided that we address some of the more difficult areas I have noted.

I would like to conclude by, again, wishing PAHO a very happy 100th birthday. This has been a wonderful event for all participants and, in particular, for all of us who have been privileged to work with PAHO in any capacity. Thank you all for all you have done and are doing in building bridges throughout the Americas and with the rest of the world.

AGENDA FOR THE "CONFERENCE ON VACCINES, PREVENTION, AND PUBLIC HEALTH: A VISION FOR THE FUTURE"[1]

DAY 1: 25 NOVEMBER 2002

8:00 a.m. – 8:30 a.m.	Registration	
8:30 a.m. – 9:30 a.m.	**Opening and Introduction**	
	Moderator: *Sir George A.O. Alleyne*	
8:30 a.m. – 8:40 a.m.	Welcoming Remarks	*Sir George A.O. Alleyne*
8:40 a.m. – 9:10 a.m.	Keynote Address: "The Role of Vaccinology in Emerging and Re-emerging Diseases: From HIV/AIDS to Bioterrorism"	*Anthony Fauci*
9:10 a.m. – 9:30 a.m.	100 Years of Vaccines and Immunization in the Americas	*Ciro A. de Quadros*
9:30 a.m. – 2:30 p.m.	**Session 1: The Present**	
	Moderators: *Walter Orenstein and Jesús Kumate*	
9:30 a.m. – 10:30 a.m.	**Polio**	
9:30 a.m. – 9:50 a.m.	Present Status and Post-eradication Vaccination Policies	*Daniel Tarantola*
9:50 a.m. – 10:10 a.m.	Potential for Circulation of VDPV	*Philip Minor*
10:10 a.m. – 10:30 a.m.	Discussion	
10:30 a.m. – 11:50 a.m.	**Measles**	
10:30 a.m. – 10:50 a.m.	Is Global Eradication Feasible?	*Ciro A. de Quadros*
10:50 a.m. – 11:10 a.m.	Coffee Break	

[1] Held as part of the Pan American Health Organization's centennial celebrations.

11:10 a.m. – 11:30 a.m.	Prospects for New Vaccines & Delivery Systems	*Teresa Aguado*
11:30 a.m. – 11:50 a.m.	Discussion	
11:50 a.m. – 12:45 p.m.	**Rubella and CRS**	
11:50 a.m. – 12:10 p.m.	The Burden of CRS	*Louis Z. Cooper*
12:10 p.m. – 12:30 p.m.	Elimination Experiences in the Americas	*Gina Tambini*
12:30 p.m. – 12:45 p.m.	Discussion	
12:45 p.m. – 2:00 p.m.	Lunch	
2:00 p.m. – 2:20 p.m.	**The Challenge of Yellow Fever**	*Thomas Monath*
2:20 p.m. – 2:30 p.m.	Discussion	
2:30 p.m. – 4:50 p.m.	**Session 2: The Newest**	
	Moderators: *Adel Mahmoud and José Ignacio Santos*	
2:30 p.m. – 2:50 p.m.	*Haemophilus influenzae* type b: The Burden in Asia	*John Clemens*
2:50 p.m. – 3:10 p.m.	Varicella: What is the Real Burden?	*Michiaki Takahashi*
3:10 p.m. – 3:30 p.m.	Hepatitis A	*Stanley Lemon*
3:30 p.m. – 3:50 p.m.	Meningococcal Conjugate Vaccines for Africa	*Marc LaForce*
3:50 p.m. – 4:10 p.m.	Coffee Break	
4:10 p.m. – 4:30 p.m.	Pneumococcal Conjugate Vaccines	*Keith Klugman*
4:30 p.m. – 4:50 p.m.	Discussion	
4:50 p.m. – 6:00 p.m.	**Session 3: The Future**	
	Moderators: *Myron Levine and Gustavo Kouri*	
4:50 p.m. – 5:10 p.m.	Rotavirus	*Roger Glass*
5:10 p.m. – 5:30 p.m.	Cholera and Typhoid	*Myron Levine*
5:30 p.m. – 5:50 p.m.	Shigella	*Karen Kotloff*
5:50 p.m. – 6:00 p.m.	Discussion	

DAY 2: 26 NOVEMBER 2002

8:30 a.m. – 10:30 a.m.	**Session 3: The Future (Cont'd.)**	
8:30 a.m. – 8:50 a.m.	Human Papilloma Virus	*Ian Frazer*
8:50 a.m. – 9:10 a.m.	Helicobacter Pylori	*Steven Czinn*
9:10 a.m. – 9:30 a.m.	Hepatitis C	*Michael Houghton*
9:30 a.m. – 9:50 a.m.	Influenza	*John Treanor*

9:50 a.m. – 10:10 a.m.	Respiratory Syncytial Virus	*Peter Wright*
10:10 a.m. – 10:30 a.m.	Discussion	
10:30 a.m. – 10:50 a.m.	Coffee Break	
10:50 a.m. – 3:00 p.m.	**Session 4: The Quest**	
	Moderators: *Stanley Plotkin and Samuel Katz*	
10:50 a.m. – 11:10 a.m.	Tuberculosis	*Michael Brennan*
11:10 a.m. – 11:30 a.m.	A New Polio vaccine?	*Eckard Wimmer*
11:30 a.m. – 11:50 a.m.	HIV/AIDS	*José Esparza*
11:50 a.m. – 12:10 p.m.	Dengue Vaccines	*David Vaughn*
12:10 p.m. – 12:30 p.m.	Discussion	
12:30 p.m. – 2:00 p.m.	Lunch	
2:00 p.m. – 2:20 p.m.	Malaria	*Regina Rabinovich*
2:20 p.m. – 2:40 p.m.	Other Parasitic Diseases	*Peter Hotez*
2:40 p.m. – 3:00 p.m.	Discussion	
3:00 p.m. – 3:20 p.m.	Coffee Break	
3:20 p.m. – 5:50 p.m.	**Session 5: New Concepts for Vaccine Development, Adjuvants and Delivery Systems**	
	Moderators**:** *John La Montagne and Luis Salleras Sanmartí*	
3:20 p.m. – 3:50 p.m.	Mucosal Immunity	*Jay Berzofsky*
3:50 p.m. – 4:10 p.m.	Maternal Immunization	*Paul Glezen*
4:10 p.m. – 4:30 p.m.	DNA Vaccines	*Margaret Liu*
4:30 p.m. – 4:50 p.m.	Edible Vaccines	*Charles Arntzen*
4:50 p.m. – 5:10 p.m.	New Adjuvants	*Moncef Slaoui*
5:10 p.m. – 5:30 p.m.	New Injection Technologies	*John Beadle*
5:30 p.m. – 6:00 p.m.	Discussion	

DAY 3: 27 NOVEMBER 2002

8:30 a.m. – 9:50 a.m.	**Session 6: Vaccines and Bioterrorism**	
	Moderators: *Donald A. Henderson and Akira Homma*	
8:30 a.m. – 8:50 a.m.	Smallpox	*D. A. Henderson*
8:50 a.m. – 9:10 a.m.	Anthrax	*Art Friedlander*
9:10 a.m. – 9:30 a.m.	Other Diseases	*C. J. Peters*
9:30 a.m. – 9:50 a.m.	Discussion	

9:50 a.m. – 11:20 a.m.	**Session 7: Regulatory and Safety Issues**	
	Moderators: *José Luis Di Fabio and Philip Russell*	
9:50 a.m. – 10:10 a.m.	The Public Sector Perspective	*Manfred Haase*
10:10 a.m. – 10:30 a.m.	The Industry Perspective	*Luis Barreto*
10:30 a.m. – 10:50 a.m.	Coffee Break	
10:50 a.m. – 11:10 a.m.	The Consumers' Perspective	*David Salisbury*
11:10 a.m. – 11:20 p.m.	Discussion	
11:20 a.m. – 4:15 p.m.	**Session 8: Vaccines, Prevention and Public Health: A Vision for the Future.**	
	Moderators: *Carlyle Guerra de Macedo and Mark Miller*	
11:20 a.m. – 11:50 a.m.	The Role of Prevention in Health and Public Health: Challenges for the Future	*Carlyle Guerra de Macedo*
11:50 a.m. – 12:10 p.m.	Sustainable Financing of Immunization in Low-Income Countries: A Role for Demand-Side Development Assistance?	*Dean Jamison*
12:10 p.m. – 12:30 p.m.	Sustainability of National Financing of Immunization Programs	*Roberto Tapia-Conyer*
12:30 p.m. – 2:00 p.m.	Lunch	
2:00 p.m. – 2:20 p.m.	The Role of Multilateral Financing Institutions	*Alfredo Solari*
2:20 p.m. – 2:40 p.m.	Immunization Programs and the Health Care Systems: Lessons Learned	*Fernando Muñoz*
2:40 p.m. – 3:00 p.m.	Coffee Break	
3:00 p.m. – 3:20 p.m.	Perspectives for Erradication/Elimination of Diseases with Vaccines	*Walter Dowdle*
3:20 p.m. – 4:00 p.m.	Summation	*D.A. Henderson*
4:00 p.m. – 4:15 p.m.	Closing Remarks: A Vision for the Future	*Mirta Roses*

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