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LEPROSY: CULTIVATION OF THE ETIOLOGIC AGENT;
IMMUNOLOGY; ANIMAL MODELS

THE ARMADILLO: ANIMAL MODEL FOR RESEARCH

WORKSHOPS

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LEPROSY: CULTIVATION OF THE ETIOLOGIC AGENTS;
IMMUNOLOGY; ANIMAL MODELS*

This Workshop was convened by the Pan American Health Organization (PAHO) to discuss and evaluate the current knowledge of leprosy in three critical areas of research: 1) cultivation of Mycobacterium leprae, 2) development and use of antigens and vaccines in the detection of leprosy and vaccination against leprosy and, 3) animal models for leprosy.

1. Cultivation of M. leprae

An important barrier to advances in research in leprosy is the lack of in vitro techniques for the cultivation of M. leprae. Studies by Hanks and collaborators on Mycobacterium lepraemurium (Mlm) show that reduced oxygen tensions in culture media promote growth of Mlm. Based on these observations Hanks has defined some physiochemical conditions that should be controlled in attempts to cultivate M. leprae and concludes that oxygen tension, oxidation reduction potential and temperature are important. Control of these factors may be critical in converting obligate intracellular mycobacteria (noncultivable) to in vitro adapted (cultivable) mycobacteria.

Barksdale emphasized the need to restudy the etiology of leprosy and to identify more precisely the acid-fast organism in tissues from leprosy patients. The possibility that leprosy is caused by more than one infectious agent acting in concert should be considered since some investigators have frequently identified several morphologically different microorganisms in tissue from patients with lepromatous leprosy.

*Presented by Dr. Jack W. Millar, Director Gorgas Memorial Institute, Washington, D.C.

Skinsnes presented the results of studies in his laboratory on attempts to cultivate M. leprae in a media supplemented by hyaluronic acid. The organism isolated has some features of Mycobacterium scrofulaceum, but Skinsnes feels that the organism also has enough properties (e.g. specific reaction with absorbed sera from lepromatous leprosy patients) that are consistent with M. leprae to warrant considering the organism as a cultivable form of M. leprae.

Claims for the cultivation of M. leprae raised the question of the validity of all currently accepted procedures used for the identification of M. leprae. A critical evaluation of the criteria developed by the Ninth International Leprosy Congress in 1968 (London) for the identification of M. leprae is warranted.

The in vitro cultivation of M. leprae has for over a century been an unrewarding endeavor, nevertheless, the Workshop encouraged support of continuing efforts in this area.

2. Antigens and vaccine of M. leprae

Sansarricq discussed the IMMLEP Program of the WHO that has as objectives: a) development of simple tests for the detection of early or subclinical leprosy, b) development of a vaccine for leprosy and, c) development of methods for the immunotherapy of leprosy.

Lymphocyte transformation tests can detect subclinical infection on exposure to M. leprae, but there is a need to improve the specificity of this test. A serious limitation of this technique is the requirement for skilled laboratory personnel. The immunofluorescence test devised by Abe has been evaluated in India and thought to be reliable for the diagnosis of leprosy. Further studies on this method are urgently needed. Suitable

skin test antigens for the detection of leprosy are urgently needed since skin testing is more adaptable to field conditions.

A vaccine is not available but must be sought. Effective vaccines may be derived from one or more of the following sources: 1) killed M. leprae or fractions thereof in an appropriate adjuvant, 2) a killed or live mycobacterium that cross-reacts with M. leprae.

The patients defense may be boosted by: 1) transfer factor, 2) infusion of allogeneic leucocytes, 3) BCG vaccination or 4) diphtheria toxoid.

The Workshop expressed a note of caution in the use of vaccines and antigens in patients. There exists the potential for the enhancement of cell-mediated immunity in the patient and this could lead to damaging reactional states. The theoretical possibility of the stimulation of suppressor T lymphocytes was considered. The use of oils as adjuvants is discouraged since all currently available oils are potential carcinogens.

3. Animal Models for Leprosy

Important considerations in choosing any animal model for an infectious disease are: 1) the time required to produce clinical disease, and 2) the cost per animal over the study period. Ideally, useful information should be obtained within one year. Suitable animal models should aid in the understanding of leprosy in the following areas: 1) chemotherapy, 2) pathogenesis and, 3) vaccination. Animals with lepromatous leprosy also provide a source of organisms for microbiological studies.

The slow ^wgro_h and probable low temperature requirements of M. leprae are factors that restrict the choice of animals as possible models for leprosy. Genetic uniformity of the animal and freedom from other myco-

bacterial diseases are desirable features. Animals bred in captivity are preferred to feral animals.

Although leprosy infections have been observed in other species only the mouse and armadillo have been studied to a significant extent. Immunologically intact mice have been used extensively but develop infections that are limited to the footpad. The development of a more susceptible strain of mouse is desirable. Armadillos develop a lepromatous form of leprosy following inoculation of M. leprae. Three species are known to be susceptible - Dasypus novemcinctus, D. sabanicola and D. hybridus. D. sabanicola seems to show a wider spectrum of histopathologic response to M. leprae and borderline forms of leprosy have been observed. D. novemcinctus native to Louisiana may be more susceptible than those in South America. An indigenous leprosy-like disease has been identified in D. novemcinctus in some areas of Louisiana, and the bacillus causing this disease is thus far indistinguishable from M. leprae. Because of the importance of the armadillo in studies on the pathogenesis and chemotherapy of lepromatous leprosy, an additional workshop on this animal is urgently needed.

The following recommendations are derived from the Workshop (listing is not necessarily in order of priority):

- a. Support of studies on the metabolism and cultivation of M. leprae is encouraged.
- b. The currently available set of widely accepted criteria for the identification of M. leprae should be critically evaluated and updated.

c. Studies in the early diagnosis of leprosy by serologic and skin testing methods are encouraged and should be supported. This is considered an important element in developing effective programs for the control of leprosy.

d. Vaccination and immunotherapeutic programs for the prevention and treatment of leprosy patients are encouraged; however, there is a need for a better understanding of the immunologic response of leprosy patients to minimize the possibility of reactions that may damage the patient.

e. The mouse and the armadillo are recognized as the most suitable available animal models for experimentation in leprosy; however, new models should be sought.

f. Since the armadillo is the only experimental animal that develops a recognized clinical form of leprosy in man, experimentation with this animal model should be encouraged and supported.

g. A workshop on the armadillo should be convened at an early date.

h. Infant thymectomized Lewis rats and nude mice are considered as useful animal models for specific studies and their use is encouraged.

THE ARMADILLO: ANIMAL MODEL FOR RESEARCH

Recommendations

In view of the differences in local facilities, species, and degree of development of research capacities, we have made the following general recommendations:

1. We strongly recommend that reproduction of armadillos under controlled conditions be given high priority in the immediate future. The armadillo must be bred in captivity before it can be utilized in biomedical research to its full advantage. This program should be carried out with different species of armadillos that have been demonstrated to offer particular promise for biomedical research (cross-reference here to text where species cited).
2. We recommend that research on the immunology of the armadillo be pursued. In comparison with other animals, several species of armadillos (Dasypus novemcinctus, D. sabanicola, and D. hybridus) appear to have sluggish, cell-mediated immune reactions; the humoral response appears to be vigorous. It may be helpful to bring this observation to the attention of immunologists in general.
3. Use of the armadillo in experimental chemotherapy of leprosy should be encouraged because the armadillo has several advantages not possessed by other animal models. Such advantages lie in the lepromatous features of the experimental disease and the presence of very large numbers of viable M. leprae.
4. Studies should be encouraged on the pathogenesis of infection by M. leprae in various species of armadillos.
5. Studies should be continued on the indigenous infections that

have been reported in D. novemcinctus with M. leprae-like bacteria in Louisiana and neighboring states. The geographic extent of the indigenous infection should be determined, and the possibility of such infections in other areas of the Americas and in other species of armadillos should be investigated. Exploration should be continued in various geographic areas in the Americas on possible infections by other mycobacteria of wild armadillos.

6. Investigations should be made of the suitability of the armadillo as an experimental model for other infectious diseases, particularly those caused by infectious agents whose temperature optima may be less than 37°C and for which there are presently no suitable animal models (cross-reference).

7. We recognized the hazards involved in work with infected armadillos. Conditions for breeding colonies are different from those for laboratories in which the armadillos are infected. The shipment of armadillos from one area to another should be carefully considered in light of the possibility of introducing infectious agents. The degree of infectious hazard of infected armadillos is unknown, so measures for the protection of the personnel should be carefully considered. Strict measures for the containment of infectious material would be necessary.

8. We recommend that methods for determination of armadillo age be investigated, with consideration of the use of the eye lens and tooth laminae, or other methods. We suggest that collections

of eye lenses and teeth be started now from animals of known age and from important experimental animals.

9. Because of the confusion and overlap in common or local names of armadillos, we recommend that the scientific identifications of armadillos be used exclusively. In some instances it may be necessary to carry out further research on the taxonomy of armadillos.

10. In view of the differences among facilities, opportunities, and capabilities mentioned initially, all possible means of financial and other support should be sought, particularly for Latin America, where leprosy and armadillos are abundant but where facilities are sometimes deficient.

11. We strongly recommend that the Pan American Health Organization promote the publication and distribution of the highly useful atlas on the histology of D. hybridus (insert correct Spanish title), as presented at this meeting by personnel of the Pan American Zoonoses Center.