Bull Pan Am Health Organ 12(1), 1978.

HUMAN STUDIES FOLLOWING MODELS OF TUMORIGENESIS BY DNA TUMOR VIRUSES IN ANIMALS[1]

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A number of viruses that have produced cancer in laboratory animals are being investigated for their possible oncogenic effect in man. Among them are the adenoviruses, three papovaviruses, and herpesvirus type 2. Epstein-Barr virus is also suspected as an agent of certain human malignancies, and hepatitis B virus is being studied in this regard.

Papovaviruses and Adenoviruses

With few exceptions, solid tumors induced in experimental animals by papovaviruses and adenoviruses are free of both infectious virus and infectious nucleic acid. It is not surprising, therefore, that all attempts to isolate viruses from a variety of solid human tumors have met with complete failure.

Following the discovery of virus-specific messenger RNA in DNA virus-induced (but virus-free) tumors in laboratory animals, human tumor tissues were searched for messenger RNA that could be hybridized with the DNA of known oncogenic viruses. Special attention was given to the adenoviruses, since they are both oncogenic in experimental animals and widespread in man. However, the results to date have failed to indicate that any of the human tumors examined contain adenovirus-specific messenger RNA.

Earlier studies had demonstrated the presence of papovavirus-like particles in the brains of patients with progressive multifocal leukoencephalopathy (PML)—a rare demyelinating human disease that usually occurs as a complication of previous malignancy in the reticuloendothelial system or of immunosuppressive therapy. Two human papovaviruses (SV40-PML and JC) have been isolated from the brains of patients with PML, and another human papovavirus (BK) has been isolated from immunosuppressed renal allograft recipients free of PML. The three agents are antigenically distinct from one another but share common antigens with the simian papovavirus SV40. Evidence is accumulating that all three viruses are oncogenic for hamsters and can transform hamster cells in vitro; the same patterns of virus/tumor cell or virus/transformed cell interactions that are characteristic of SV40 are observed with the human agents—namely, production of tumor antigen and tumor-specific transplantation antigen, rescue of infectious virus through fusion with susceptible cells, etc.

The significance of these viruses in human malignancies remains to be determined. Seroepidemiologic surveys indicate that infections with JC and BK virus are very common in man, antibodies being present in about 70 per cent of adults tested. The human papovavirus infections do not appear connected with exposure to the simian SV40 virus, either through contact with rhesus monkeys (the natural host for SV40) or through vaccination with potentially SV40-containing poliovirus vaccines.

[1]Working paper, WHO Scientific Group on Virus Diseases (Geneva, 1-5 September 1975).

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Herpesvirus and Immunologic Control of Cervical Cancer

In recent years a great deal of attention has been focused on human herpesviruses as potential oncogenic agents. Herpes simplex virus type 2 (HSV-2) is venereally transmitted in man, and seroepidemiologic studies have revealed a high degree of association between infection with this virus and invasive carcinoma of the cervix.

HSV Antibodies

It has been observed that cervical cancer patients show a higher incidence of antibodies to nonstructural antigens induced by herpesvirus than do normal individuals or patients with breast cancer. In addition, the presence of herpesvirus DNA and messenger RNA in a cervical cancer biopsy has been reported. These findings, together with the known capacity of herpesvirus to transform hamster embryo fibroblasts, lend support to the idea that this agent has oncogenic potential in man.

Neutralizing antibodies against HSV-2 are more often found in women with cervical cancer than in matched control women. This appears to be true not only for cases of invasive carcinoma of the cervix but also for cases of carcinoma in situ and cervical dysplasia. Since both cervical cancer and HSV-2 infections are related to attributes associated with venereally transmitted agents, the association between the virus and the cancer could represent one of covariability. However, recent studies, including the comparison of cervical cancer patients with matched breast cancer patients of the same social group, support the hypothesis of a causal relation to cervical cancer. Also supporting the hypothesis are the recent discoveries of antibodies to herpesvirus-induced nonvirion antigens in cervical cancer patients. The data are compatible with a model in which infection by the virus early in life leads to oncogenic changes which are expressed a number of years later.

In addition to the previously reported data on complement-fixing antibodies, new information is being obtained. A radioimmune assay has been developed to detect the presence in cervical cancer patients of antibodies that react with specific nonstructurual polypeptides induced early in the HSV replicative cycle. Some cervical cancer patients were found to possess antibodies to early polypeptides but not to whole virus. Perhaps this test may be able to separate persons responding only to early antigen (that is, to incomplete or defective virus) from those responding to both early and late antigens (to both incomplete and complete viruses).

An important next step in advancing present knowledge on the role of herpesvirus in human cancer would be carefully executed prospective studies to determine: (1) the relative risk of developing the disease among women with and without a post-herpesvirus type 2 infection; and (2) the occurrence of cervical neoplasia in relation to the occurrence of antibodies to herpesvirus nonvirion antigens, as well as the diagnostic and/or prognostic value of patterns of antibody to the virion and nonvirion antigens. The results should make it possible to characterize high-risk women who should be followed more carefully and to whom existing preventive measures could be more intensively applied.

Detection of HSV DNA in Tumor Cells

Studies of nucleic acid hybridization between HSV and cervical cancer have been made. With a single exception (yet to be confirmed), all attempts to find HSV-2 DNA in human tumors have failed. Moreover, there has not been any success in finding HSV DNA in HSV-transformed rodent cells, even when assayed under

conditions permitting the detection of 0.1 genome equivalent per cell. The HSV-transformed cell system differs in this regard from cells transformed by other herpesviruses (Epstein-Barr virus, Marek's disease virus, and herpesvirus saimiri). It should be kept in mind, however, that 0.1 of an HSV genome has a molecular weight of about 10 million daltons, and this amount would be much larger than the amount of DNA (1 million daltons) found in papovavirus-transformed cells. A more sensitive approach might be to look for other evidence of genetic information in tumor cells. An example is the work employing temperature-sensitive mutants of HSV to detect a residual helper viral genome in the transformed cells.

Tissue-Culture Research

One urgent problem that requires more attention is the usual inability to grow malignant cells from cultured human tumors. Tests for such malignant cells have utilized mice treated with antithymocyte serum or, more recently, nude mice. Another approach using an in vitro marker of malignancy is now being used in which the fucose compounds in membranes of cultured malignant cells are being studied. A novel series of four major radiolabeled fucose compounds have been identified in a variety of normal cultured cells from rat, hamster, mouse, baboon, and human tissue. Transformation of the animal cells by oncornavirus, herpes simplex virus, or SV40 has resulted in a characteristic and marked decrease in the incorporation of radiolabeled fucose into the least chromatographically mobile (and presumably most complex) fucose compound, with a concomitant increase in the precursor fucose compound. The observations in animal cells have recently been extended to a variety of cultured human tumor cells—in which the decreased incorporation of radiolabeled fucose into the most complex fucose compound was also demonstrated. The results thus suggest that fucose metabolism could possibly be used as a diagnostic marker of in vitro malignancy in cultured human tumor material.

Animal Models

Research on model systems has been encouraging, particularly the genital infection of female cebus monkeys with HSV-2 and the preliminary finding of animals with persisting anaplastic cervical cytology. Several experimentally infected monkeys have developed atypia and others dysplasia in contrast to none of the controls. The oncogenic potential of HSV for hamsters is well known.

Successful herpesvirus vaccines of the live attenuated variety, including one for controlling oncogenic Marek's disease, have been developed for use in veterinary medicine. However, because of the undesirable properties inherent in an attenuated live virus vaccine, particularly that of potential reversion to wild-type, latent infection, and even oncogenicity (Table 1), there is little to expect from such attenuated vaccines in the forseeable future. Despite this gloomy outlook, attenuated HSV strains

Table 1. Some expected features of attenuated vs. inactivated HSV vaccines.

	Attenuated live virus vaccine	Inactivated vaccine
Vaccine dose	Low (replicates)	High
Antibody persistence (immunity)	Long	Short
Boosters needed	Infrequently	Frequently
Reversion	Possible	None
Latency	Possible	None
Oncogenicity	?	None
Subunit vaccine (free of nucleic acid)	Not possible	Possible

Source: Melnick (*3*).

have been obtained, particularly from among the temperature-sensitive mutants that have recently been described. Studies (*1*) on the nature of the immunity (e. g. to challenge virus or to challenge tumor cells) produced by attenuated HSV in rodents are now possible. These studies can even include primates if attenuated herpesvirus saimiri is used, for such an experimental vaccine has now been produced and has been shown to provide protection against lymphomas in marmosets.

Development of HSV Vaccine

It appears that the most straightforward and safest approach to obtaining immunity at this time would be through the use of inactivated or subunit vaccines. To date the use in humans of killed whole herpes simplex virions (as they exist in infected cell extracts) has not met with success. However, as it has just been mentioned, in the case of another oncogenic herpesvirus, marmosets have been protected with heat-inactivated herpesvirus saimiri against lymphomas induced by that virus.

• *Selection of strains for vaccine production.* Recent studies (*2*) indicate that the strains of HSV-2 are not all antigenically identical. The results of Cr 51 release assays, using antisera adsorbed with virus-infected cells, have demonstrated two subsets of HSV-2 strains; these subsets are called α and β. HSV-2 isolates from different geographic areas were subsequently examined. Ten of the strains were from Colombia, where no significant differences were found between the type 2 antibodies of cancer cases and those of controls. All 10 strains were identified as belonging to subset β. In contrast, it was found that five of 15 strains from the United States of America and one of two strains from India belonged to subset α. The data suggested a correlation between the virus subset and the oncogenicity of HSV-2 in hamsters: four of five α -viruses and five of eight β-viruses transformed cells in culture, but only those four cell lines transformed by α-viruses were oncogenic when injected into hamsters. More work is required to assess the possible roles of α- and β-viruses in cervical cancer and in the possible development of vaccine stocks.

• *A subunit vaccine for HSV-2.* Work is going forward on the immunologic characterization of individually isolated HSV polypeptides. Pure polypeptides of HSV can now be prepared in large quantities and their functions can be determined (*1*). Furthermore, VP123, the major envelope glycoprotein, has been used to produce antiserum which exhibits type-specific neutralization when reacted with infectious virus. These methods suggest great potential for the production of pure polypeptides free of nucleic acid which could be used for raising levels of protective antibodies in humans. Such preparations could well become the vaccines of the future.

• *The role of HSV-2 in cervical cancer.* Despite the many retrospective studies that have been done, the etiologic role of HSV-2 in cervical cancer remains to be proven. The most direct and rewarding proof could come from vaccination of high-risk populations. If HSV-2 infections can be prevented by vaccination, or perhaps even limited (as in the case of vaccination against Marek's disease), then cervical cancer should disappear as a disease. The ultimate proof of causation would consist in controlling the disease by eliminating, or limiting the expression of, the etiologic agent.

Epstein-Barr Virus

A high degree of association exists between the Epstein-Barr (EB) herpesvirus and two human malignancies: Burkitt's lymphoma (a tumor with a predilection for the jaw that is peculiar to children of central Africa) and postnasal (nasopharyngeal) carcinoma, which is found with

higher frequency among Chinese male populations in Southeast Asia. The virus was detected initially by electron microscopy and subsequently by immunofluorescence in cultured Burkitt's lymphoma cells (but not in the original tumors)—which maintained their lymphoid character in the course of continuous in vitro propagation. High incidence and high titers of EB antibody (detectable by immunofluorescence, complement fixation, and gel difussion tests) in patients with Burkitt's lymphoma and postnasal carcinoma led to the assumption that the association could be etiologic.

Subsequent seroepidemiologic surveys, however, showed that infection with EB virus is widespread in normal populations, not only in Africa and Asia but throughout the rest of the world as well. Furthermore, EB virus has an extreme predilection for cells of lymphoid origin. The virus occurs regularly in cell lines derived not only from Burkitt's lymphoma and postnasal carcinoma but also from peripheral blood leukocytes of patients with infectious mononucleosis and various other diseases as well as from normal individuals.

The question of whether EB virus is etiologically related to lymphoma and postnasal carcinoma or whether it represents a passenger virus present in lymphoid cells trapped within the tumor remains unanswered.

DNA-DNA hybridization studies have detected small quantities of EB viral DNA in virus-free biopsy specimens of Burkitt's lymphoma and postnasal carcinoma. These findings, which are similar to those obtained with cell lines derived from the same tumors, still do not tell whether the partial viral genome detected was the cause of the malignancy or merely resulted from a secondary infection of the tumor cells by the virus.

In the laboratory EB virus is oncogenic for marmosets. Marmoset lymphocytes (akin to human lymphocytes) can be transformed in tissue culture by cell-free EB virus, resulting in continuous virus-positive lines. Cells of one such marmoset lymphoid line had been infected with EB virus that came from a lymphoid line from a patient with infectious mononucleosis. These marmoset cells, as well as cell-free virus derived from them, were found to induce malignant lymphomas in cottontop marmosets.

Hepatitis B Virus

In areas with a high prevalence of HB_S antigen there is also a high incidence of primary liver cell carcinoma. Since a vaccine is being developed to control hepatitis B infections, properly conducted field trials might determine whether control of hepatitis B virus infections has any suppressing effect on the incidence of hepatoma.

SUMMARY

• The discovery of virus-specific messenger RNA in virus-induced animal tumors has led to the search for messenger RNA in human tumors that can be hybridized with the DNA of known oncogenic viruses. Attention has focused on the adenoviruses, which have produced cancer in laboratory animals and are widespread in man, and on three papovaviruses that have been isolated in human disease and which are oncogenic in hamsters.

• In other research, the association between human infection with herpesvirus type 2, which is likewise oncogenic in hamsters, and invasive carcinoma of the cervix is being examined. An experimental vaccine is being developed, and nonhuman primate models are being studied as part of this work.

• Epstein-Barr virus is still another suspected agent of human malignancies, specifically Burkitt's lymphoma and postnasal carcinoma.

• High prevalence of antigen to hepatitis B virus has been seen to correlate with high incidence of primary liver cell carcinoma, and studies are attempting to elucidate the relationship.

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