BURKITT'S LYMPHOMA AND *HERPESVIRUS SAIMIRI* LYMPHOMA: COMPARATIVE ASPECTS[3]

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Burkitt's lymphoma is a common malignant tumor of children closely linked to the Epstein-Barr herpesvirus; Herpesvirus saimiri *lymphoma is a malignancy caused by injecting* H. saimiri *into appropriate test animals. The comparison of the two presented below reveals important differences between them. Nevertheless, use of* H. saimiri *in animal models is likely to be of considerable future utility in studying malignant human disease of the lymphoreticular type.*

Introduction

Special difficulties arise in the field of tumor viruses when a particular viral agent is suspected of causing human malignancy. With animal systems, viruses suspected of carcinogenic activity can obviously be tested in a variety of animal experiments. If they seem to bring about malignant change in animal cells *in vitro* the exact nature of such change can be seen when the cells are inoculated into isologous hosts and cause tumors.

In human systems, suspicion falls on a particular virus when evidence accumulates that indicates a close and invariable association between that virus and a certain type of tumor. But at this stage an impasse is reached in human work, for there are great difficulties in devising experiments to show conclusively that the suspect virus in fact plays an etiologic role in a particular malignant disease. The main problem is to decide on the value of accumulating more and more evidence of association between the agent and the tumor, since information of this type cannot give a final definitive answer.

It is therefore of vital importance to have animal models available for use in experimental studies of virus-cell interactions in malignant change—in order to elucidate, by extrapolation, comparable situations in man. For this reason *Herpesvirus saimiri* and its lymphoma are of particular significance.

Relevance of *Herpesvirus saimiri* to Burkitt's Lymphoma

It is now widely known that Burkitt's lymphoma was first recognized as a specific syndrome during the middle years of the 1950's by Denis Burkitt in Uganda (*1*). Early epidemiologic studies indicated that geographic distribution of the tumor was dependent in some way on temperature and rainfall (*2, 3*). While in London in 1961, Denis Burkitt gave the first account outside Africa of the dramatic tumor which now bears his name, including details of his geographic distribution studies.

The tumor has a bizarre nature, with multiple foci affecting sites quite uncharacteristic of the usual malignant human lymphomas (*4*). This, together with the tumor's curious epidemiology, made it immediately clear that a lesion of very special interest had been discovered. For, if distribution of the tumor was

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indeed determined by temperature and rainfall, some biological factor must be playing an etiologic role. A causative viral agent with an anthropod vector seemed the most likely in the context of tropical Africa.

Because of this intriguing possibility, extensive investigations of Burkitt's lymphoma were immediately undertaken in my laboratory, a particular effort being made to find and identify any viruses associated with the tumor. After many months of negative preliminary experiments (*5*), Epstein-Barr (EB) virus was finally discovered in cultured Burkitt lymphoblasts in 1964. It was immediately recognized on morphological grounds as being a member of the Herpes family (*6, 7*).

Tests were naturally undertaken to determine which herpesvirus was involved. Preparations from virus-bearing cultures were inoculated into various tissue culture systems, into eggs, and intracerebrally into suckling mice. All of these tests proved negative, and it became clear that the EB virus was highly unusual in that it showed negative biological behavior unlike that of any known member of the herpesvirus group (*8*). Since that early work, numerous studies have shown that EB virus is also immunologically distinct from known herpesviruses (*9, 10, 11, 12*) and that its relationship with Burkitt's lymphoma is extremely close.

Evidence implicating EB virus as a possible cause of the tumor has grown steadily. The virus has been found to stimulate human lymphoproliferation, both *in vitro* (*13, 14*) and *in vivo* (*15, 16*); to be intimately linked with Burkitt's lymphoma on seroepidemiologic grounds (*17*); to cause virus-determined neoantigens on the surface of tumor cells (*18, 19, 20*); and (as would thus be expected) to have its genome carried by the tumor cells (*21*).

Besides causing lymphoproliferation *in vitro* (*13, 14*), when EB virus infects normal human peripheral lymphoid cells and causes lymphoproliferation it brings about the following additional changes:

1) Cell morphology is altered to a blastoid form.

2) Cell proliferation appears to be unlimited.

3) Contact inhibition appears to be lost and the cells tend to grow in clumps.

4) The cells acquire what has been claimed to be a "specific" chromosomal marker (*13, 14*).

5) Virus-determined neoantigens appear on the cells (*22, 23*).

It has been said that if such changes were to occur after a known animal tumor virus infected normal cells, they would indicate that the cells had undergone a malignant transformation. In animal systems it is possible to obtain ultimate proof that malignant transformation has occurred by inoculating the "transformed" cells into isologous hosts and observing that they grow progressively to form malignant tumors.

As already pointed out, this cannot be done with human material for obvious reasons. Therefore, animal malignancies analogous to Burkitt's lymphoma that are caused by herpesviruses are of supreme importance in studying the suspected viral etiology of human tumors.

It is also worth stressing that the EB virus is known to be the etiologic agent of infectious mononucleosis (*15, 16*). Although this disease is of course self-limiting, in other respects it is frequently very similar to the early stages of leukemia.

With its foregoing attributes and close association with Burkitt's lymphoma, EB virus seems very likely to be the etiologic agent of this peculiar tumor. But there are enormous difficulties in devising experiments to show that this is so. In this regard the importance of analogous animal tumors caused by similar types of virus becomes evident.

Comparison of Burkitt's Lymphoma and *H. saimiri* Lymphoma

Burkitt's Lymphoma

The features of this acute malignant disease of children have recently been reviewed by

Burkitt (*24*). The peak age incidence in areas of high endemicity is between six and seven years; the tumor is multifocal and involves bizarre sites uncharacteristic of classical human lymphomas. An association with leukemia is only very rarely seen as a terminal event (*25*). It is noteworthy that peripheral lymph glands are spared in all but about 2 per cent of the cases; when the glands are involved, it is almost always only those of the mesenteric region.

In addition to these clinical and pathological features, Burkitt's lymphoma has the epidemiologic peculiarity of being more common than the sum of all other tumors of children in tropical Africa and New Guinea, while being exceptionally rare in most other zones. Also, where it is common it is dependent on temperature and rainfall (*2, 3*). The idea is currently favored that this phenomenon is probably unrelated to a possible viral agent such as EB virus, which is ubiquitous, but may depend on a cofactor such as holo- or hyperendemic malaria (*26*).

At the cellular level the malignant cells of Burkitt's lymphoma show remarkable uniformity for any given tumor and have been characterized as lymphoblasts. They may either be well differentiated toward the lymphocytic stage or less mature, even resembling hemocytoblasts. Whatever form the tumor takes, it is quite clear that diagnosis cannot be made just on the basis of histology, or even histology and cytology alone. A full account of the definition of Burkitt's tumor has recently been drawn up by the World Health Organization, emphasizing that diagnosis must depend on history, the clinical picture, and gross pathology, taken in conjunction with light microscope features of the cells (*27*).

H. saimiri *Lymphoma*

The outstanding difference between *H. saimiri* lymphoma and Burkitt's tumor is that, so far as is known, the former does not occur under natural conditions. *H. saimiri* is apparently nonpathogenic for its natural squirrel monkey host (*28*) and causes malignant tumors only when inoculated experimentally into such test animals as owl monkeys or marmosets (*29*). Also in contrast to Burkitt's lymphoma, *H. saimiri* lymphoma consists of a marked and widespread reticulum cell invasion of many organs, with replacement of the normal cellular structure; the liver, kidney, spleen, lymph nodes, and adrenals are almost invariably involved. In some individuals peripheral blood changes have also been recorded (*30*). Thus, even the experimentally induced disease is markedly different from its nearest human analogue.

Comparative Virology

H. saimiri grows rapidly in owl monkey kidney cell monolayer cultures, where it induces foci of cytopathologic change (*28*). There is a rounding of the cells in which the virus is replicating, followed by viral release, cell death, and development of areas of cytolysis within the cell sheet. In the experimental animal, where malignant tumors are rapidly induced following viral inoculation, the agent must clearly bring about malignant change in the target cells–but so far such change has not been observed *in vitro*.

In contrast, EB virus will not infect a wide range of monolayer test tissue cultures (*8*), and until quite recently the only normal human cells which it could be made to infect were of the lymphoid series (*13, 14*). Both in such infected cells and in cultures of naturally infected cells from either Burkitt's tumors or cases of infectious mononucleosis, the virus shows an unusual relationship to the host cells in culture. Only a relatively small proportion of the cell population will be replicating the virus at any one time (it has long been known that this process ultimately leads to death of the virus-producing cells) (*8*). At the same time, however, the viral genome is present in all the cells in the population. This can be demonstrated by cloning experiments (*31*), by detection of virus-determined complement-fixing antigens (*32*), and by nucleic acid homology experiments (*33*).

Quite recently, certain unusual manipulations have enabled EB virus to infect normal human embryo fibroblasts. It is of interest that when this occurs there is neither a viral productive infection with cytocidal effects, as seen with *H. saimiri,* nor the peculiar low-grade viral productive cycle seen with EB virus in human lymphoid cells. In contrast, a striking focal morphological transformation can be detected (*34*), giving rise to a new, rapidly–growing cell type which does not produce the virus. As far as these preliminary results go, this morphological transformation looks as if it might correspond to true *in vitro* malignant transformation; if this is so, here again the behavior of EB virus follows a distinctly different pattern from that of *H. saimiri.*

Possible use of *H. saimiri* in Comparative Studies

Despite the important differences between *H. Saimiri* lymphoma and its causative agent, on the one hand, and Burkitt's lymphoma and its putative causative agent (EB virus) on the other, the *H. saimiri* system is likely to have considerable use as a model for the study of human malignant disease of the lymphoreticular type. Thus, if it could be shown that *H. saimiri* even occasionally caused malignant change in its natural host, an important area for epidemiologic studies of a natural animal lymphoma would be opened up. The fact that the etiology of Burkitt's lymphoma seems to require some cofactor (*26*) in addition to EB virus (if that agent is indeed involved) raises the possibility that in nature *H. saimiri* might also bring about malignant change in association with some as yet unknown second influence.

In another area, studies on the exact mechanism whereby *H. saimiri* virus causes malignant change *in vivo* are likely to be important in shedding light on the induction of malignancy in general; they might also help reveal the mechanisms underlying the causation of Burkitt's lymphoma.

In any event, *H. saimiri,* with its great oncogenic power in a variety of hosts, provides an important tool which is likely to prove of outstanding worth in the study of malignant transformation occasioned by herpesviruses in animals, and perhaps also in man.

SUMMARY

Burkitt's lymphoma, a malignant tumor in children, has been closely linked to Epstein-Barr (EB) virus, a member of the Herpes family. EB virus has been shown to stimulate human lymphoproliferation; to be intimately linked with Burkitt's lymphoma on seroepidemiologic grounds; to cause viral-determined neoantigens on the surface of tumor cells; to have its genome carried by the tumor cells; and to prompt the cells to undergo apparent malignant transformation. Nevertheless, ultimate proof that EB virus causes Burkitt's lymphoma is lacking, since for obvious reasons human subjects cannot be injected with "transformed" cells to see if malignant disease results.

This makes it highly desirable to find an animal model in which analogous tumors are caused by a similar virus. Attention has thus been drawn to *Herpesvirus saimiri,* which causes malignant lymphoma when inoculated experimentally into test animals such as owl monkeys and marmosets.

H. saimiri is apparently not pathogenic for its natural squirrel monkey host, and there are important differences between *H. saimiri* lymphoma and Burkitt's tumor. Despite these differences, however, *H. saimiri* is likely to be of considerable future use in studying malignant human disease of the lymphoreticular type.

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TWO NEW HERPESVIRUSES FROM SPIDER MONKEYS (*ATELES GEOFFROYI*)[5, 6]

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Experiments with two new viruses isolated from spider monkeys show them to be distinct from the previously known spider monkey herpesvirus (SMHV). One of the two, Herpesvirus ateles, *was found to cause a disease similar to malignant lymphoma with terminal leukemia in inoculated marmosets.*

Introduction

Two facts led us to find the new herpesviruses described in this presentation. One was the discovery by Meléndez and his associates in 1968 of the first lymphoma virus of monkeys, *Herpesvirus saimiri.* This virus, derived from squirrel monkeys, proved capable of inducing leukemic or aleukemic malignant lymphoma in several nonhuman primate species as well as in rabbits (*1, 2, 3, 4, 5*). The other fact was an association between human patients with lymphosarcoma and spider monkeys (*Ateles geoffroyi*) in Guatemala. A detailed description of this association has been presented elsewhere (*6*).

These two facts led us to search for the presence of *H. saimiri* in spider monkeys. However, *H. saimiri* was not isolated from this

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