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HERPESVIRUSES AND HUMAN CANCER[1]

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There is now good evidence that herpesviruses play a role in some kinds of human cancer. This article reviews that evidence, placing particular emphasis on the development of Burkitt's lymphoma and cancer of the cervix uteri.

Introduction

Although it has been established that some viruses can cause cancer in animals, the evidence for a viral etiology of at least some human cancers has been slow to accumulate. At present, viruses acting alone or in association with such factors as hormones, chemicals, genetic determinants, or other unknown cofactors have been linked to five human cancers (see Table 1). Three of these malignancies (Burkitt's lymphoma, nasopharyngeal carcinoma, and carcinoma of the cervix) are associated with herpesviruses. The other two (mammary carcinoma and acute myelogenous leukemia) bear similarities to diseases of mice caused by retroviruses, a group of viruses containing RNA.

The herpesviruses, constituting a large family of DNA-containing viruses, cause a variety of human illnesses (see Table 2). In general, herpesvirus infections are characterized by long periods of latency and sometimes by recurrent episodes triggered by organic or emotional factors. A number of herpesviruses have been associated with animal cancers—including Marek's disease (*48*), a lymphoproliferative disease of chickens (Table 3). This latter association is especially noteworthy because Marek's disease is the first neoplastic disease to be prevented by the effective use of a live attenuated vaccine (*49*).

The purpose of the present article is to review the evidence linking herpesviruses with human neoplastic diseases, placing particular emphasis on Burkitt's lymphoma and carcinoma of the cervix, two diseases having a significant impact on human populations in the developing world.

Epstein-Barr Virus and Cancer

Burkitt's Lymphoma

Burkitt's lymphoma is probably the most prevalent type of cancer in African children (*7*), accounting for 30 to 60 per cent of all malignant tumors in children between 1 and 15 years of age (*12*). The tumor is frequently located in the jaw; however, the ovaries, thyroid, testes, and abdominal viscera can also be affected.

Burkitt called attention to the peculiar distribution of cases in equatorial Africa (*8*) and noted that more cases were found in areas where the climate is warm (never below 16°C), the annual rainfall is greater than 100 cm and the altitude is below 1,600 m (Table 4). Burkitt's lymphoma is also found in New Guinea, where similar climatic conditions exist, but is relatively rare in the rest of the world. Burkitt postulated that a virus, possibly a vectored virus, infecting a population where malaria is hyperendemic, was responsi-

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Table 1. Known associations between viruses and human cancer.

Cancer type	Virus type	Virus family
Burkitt's lymphoma	EBV[a]	Herpesvirus
Nasopharyngeal carcinoma	EBV	Herpesvirus
Carcinoma of the cervix	HSV-2[b]	Herpesvirus
Mammary carcinoma	Type B	Retrovirus
Acute myelogenous leukemia	Type C	Retrovirus

[a]Epstein-Barr virus.
[b]Herpes simplex virus type 2.

Table 2. Human illnesses caused by members of the herpesvirus family.

Virus	Illness
Herpes simplex virus type 1	Gingivostomatitis, encephalitis, keratoconjunctivitis
Herpes simplex virus type 2	Genital herpes, disseminated neonatal herpes
Cytomegalovirus	Congenital defects, transfusion mononucleosis, interstitial pneumonia
Epstein-Barr virus	Infectious mononucleosis
Varicella-zoster virus	Chicken pox, shingles

Table 3. Tumors in animals from which herpesviruses have been isolated.

Animal	Tumor and reference cited
Frog	Renal carcinoma (*40*)
Cottontail rabbit	Lymphoma (*31*)
Spider monkey	Lymphoma (*43*)
Chicken	Marek's disease (*48*)

Table 4. Epidemiologic features of Burkitt's lymphoma.

Geographic distribution	Africa, New Guinea
Minimum temperature	16°C
Maximum altitude	1600 m
Annual rainfall	> 100 cm
Endemic vector-borne disease in affected areas	Malaria
Age of peak incidence	5 years of age
Population groups affected	Africans, Indians, Arabs, Portuguese

ble for the tumor (*9*). The search for such a virus led Epstein and Barr (*16*) to discover the Epstein-Barr herpesvirus (EBV) in cell cultures derived from a Burkitt's lymphoma.

In a serologic study of Burkitt's lymphoma patients and control children in Africa, Henle et al. (*26*) found high antibody titers against EBV in Burkitt's lymphoma patients and low titers in controls matched by age, sex, and tribal origin. Also, it has been found that when children have Burkitt's lymphoma, antibodies are elicited against several viral antigens: the virus capsid antigen (VCA), the EBV-associated nuclear antigen (EBNA), the membrane antigen (MA) (*35*), and the "early" virus antigen (EA). Relatively high antibody titers against EA are found in Burkitt's lymphoma and nasopharyngeal carcinoma patients, but not in patients afflicted with other types of cancers or in control populations (*27*).

By means of nucleic acid hybridization techniques, zur Hausen et al. (*69*) have demonstrated that EBV DNA is present in Burkitt's lymphoma biopsy specimens, even though no infectious virus is present in the

cells. However, the virus can be induced to replicate in the tumor cells when they are cultured in vitro.

Even though normal lymphocytes do not usually grow in culture, it has been shown that cord blood lymphocytes can be established as continuous cell lines after infection with EBV (*11, 55*). The transformed cells have a characteristic chromosomal lesion on the long arm of chromosome number 10, a lesion that is also found in cultured Burkitt's lymphoma cells (*28*). The inoculation of EBV into owl monkeys or cottontop marmosets induces malignant lymphomas with many of the characteristics of Burkitt's lymphoma, including the presence of viral nucleic acids in the tumor cells and the expression of EBNA (*45*). EBV can also be recovered from the cultured tumor cells.

Nasopharyngeal Carcinoma

In addition to its association with Burkitt's lymphoma, EBV is associated with another tumor of the oral cavity, nasopharyngeal carcinoma. A relatively high incidence of this tumor (10 to 20 cases per 100,000 persons) is found among adults in the southern provinces of China, as well as in Viet Nam, Thailand, Malaysia, and the Philippines (*25*). A histocompatibility antigen, Singapore-2 LD antigen, is also associated with nasopharyngeal carcinoma in Chinese patients, suggesting a probable genetic susceptibility to this type of cancer (*63*). Patients with nasopharyngeal carcinoma have high antibody titers to EBV, VCA, and EA (*39*), and the presence of viral nucleic acid and EBNA can be demonstrated in the tumor cells (*3*). Production of infectious EBV can be induced in cultures of epithelial cells from nasopharyngeal carcinomas by treating the cells in vitro with bromodeoxyuridine (*67*).

Although there is no doubt that EBV is present in both Burkitt's lymphomas and nasopharyngeal carcinomas, this in itself is not proof of a causal relationship. The virus could simply be a "passenger" replicating more or less synchronously with the tumor. If this were true, however, the virus should be detectable in other tumors of the oral cavity or lymphoid system—such as in Hodgkin's disease. So far, scientists have been unable to detect the virus in such tumors (*30*), although a recent study has reported finding EBV antibodies in patients with tonsillar carcinoma (*68*).

Diverse EBV Associations

Seroepidemiologic studies in different parts of the world have shown that EBV is a ubiquitous virus, that most EBV infections are asymptomatic, and that most of them occur in early childhood (*18, 24, 65*). However, EBV causes infectious mononucleosis in adolescents and young adults not exposed to the virus during childhood (*29*).

It is not unusual for a virus infection to have a variety of clinical manifestations. What is unusual is the geographic distribution. One would like to know why EBV causes Burkitt's lymphoma in Africa, nasopharyngeal carcinoma in the Far East, infectious mononucleosis in North America and Europe, and primarily asymptomatic infections in other parts of the world. Clearly, other factors besides those already noted are involved.

In the case of nasopharyngeal carcinoma, a genetic predisposition to inhaled carcinogens appears to create an added risk (*25*); but in the case of Burkitt's lymphoma there is no analogous explanation. Climatic conditions similar to those of equatorial Africa are found in Brazil, and endemic malaria is also present in some areas, yet the incidence of lymphomas resembling Burkitt's is low in that country (*13*). It has been postulated that endemic malaria could provide the conditions necessary for EBV to cause cancer, either by creating an immunosuppressive effect or through the production of mitogens (*12*). This is not an entirely satisfactory explanation, since patients given organ transplants are placed on immunosuppressive

therapy and are undoubtedly exposed to EBV; yet there are no reports of Burkitt's lymphoma occurring in such patients, even though the risk of other types of cancer is increased.

Although no strain differences among EBV isolates from different parts of the world have been found, it is possible that certain virus strains cause cancer while others do not.

A disturbing finding is that Burkitt's lymphoma is a multifocal B-cell lymphoma affecting a variety of organs in the same individual; yet the tumor cells are monoclonal (*17*), that is, derived from a single cell. This indicates that malignant transformation is a rare event involving a single cell, much like a somatic mutation. If EBV is responsible for the transformation, one would expect that a number of cells would be equally responsive to the virus action, resulting in multiclonal tumors. However, it is possible that one cell type is preferentially transformed by the virus.

In general, the evidence for an etiologic role of EBV in Burkitt's lymphoma and nasopharyngeal carcinoma is fairly convincing; and it can be argued that Koch's postulates are fulfilled, at least for Burkitt's lymphoma (Table 5). However, the peculiar geographic distribution of Burkitt's lymphoma and nasopharyngeal carcinoma cases suggests that environmental factors are involved as well—factors that could be biological (e.g., malaria) in the case of Burkitt's lymphoma or chemical (inhaled carcinogens) in the case of nasopharyngeal carcinoma (see Figure 1).

Herpes Simplex Virus Type 2 and Carcinoma of the Uterine Cervix

Cervical carcinoma (see Figure 2) is the most common type of cancer affecting women of the developing world (*53*). In Africa and Latin America the incidence is over 50 cases per 100,000 women. A number of epidemiologic studies (*41, 60, 66*) have shown that cervical carcinoma occurs most frequently (Table 6) in women who are sexually active at an early age and who have had multiple sexual partners. The disease is rare in nuns and also in Jewish women whose husbands are circumcised. These facts suggest that an agent transmitted from men to women may be involved in initiating the disease. A list of agents that can be transmitted by sexual intercourse is shown in Table 7. After reviewing what is known about the possible oncogenic role of these agents, Alexander (*2*) suggests that the one most likely to be

Table 5. Koch's postulates and the role of EBV in the etiology of Burkitt's lymphoma.

Postulate	Application to Burkitt's lymphoma
(1) The microorganism must be regularly found in the lesions of the disease.	(1) EBV DNA found in Burkitt's lymphoma tissue is absent in other tumors (except nasopharyngeal carcinoma). However, EBV is present in the normal population.
(2) The microorganism must be isolated and grown in pure culture.	(2) EBV can be isolated from cell cultures of the tumor. It can be grown in virus-free lymphocytes in culture. The virus can be purified and characterized. However, other viruses or their products—including Reo 3 (*6*) and retroviruses (*64*)—have been detected in some Burkitt tumors.
(3) The disease must be reproduced in a susceptible animal by inoculation with the microorganism in pure culture.	(3) Multifocal lymphomas can be induced in owl monkeys and cottontop marmosets by inoculation of purified EBV. Histologically, the tumors are very similar to those of Burkitt's lymphoma, except that jaw tumors have not been observed.
(4) The microorganism must be found in the lesion produced in the susceptible animal.	(4) EBV DNA and EBV-associated nuclear antigen can be found in the tumors induced by inoculation of the virus into marmosets.

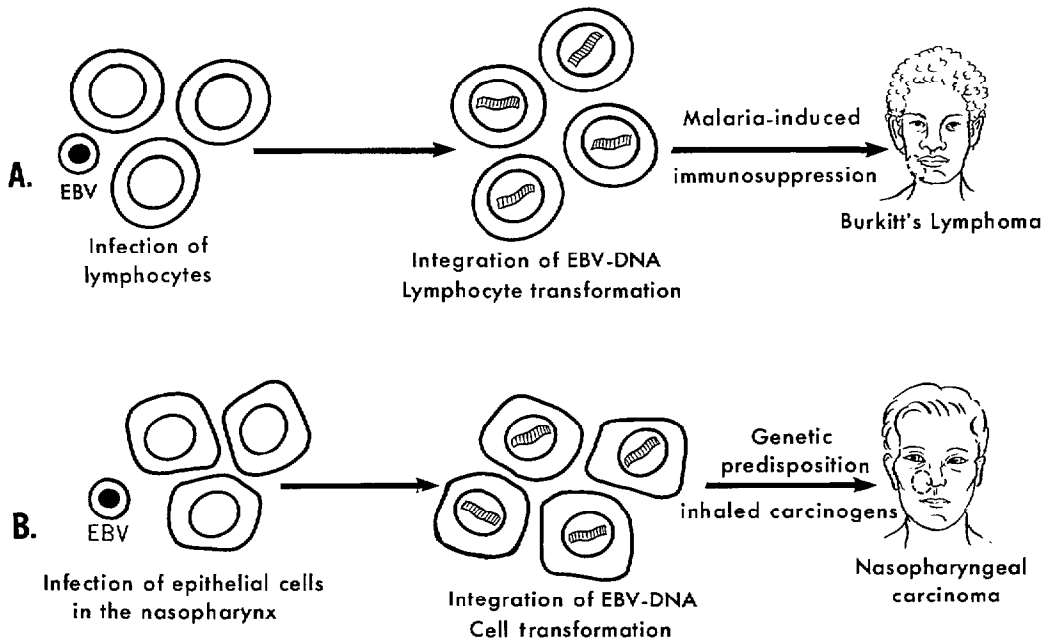

Figure 1. Possible participation of EBV in the etiology of Burkitt's lymphoma and nasopharyngeal carcinoma.

involved in the etiology of cervical carcinoma is herpes simplex virus type 2 (HSV-2), also known as genital herpesvirus—a close relative of the oral herpes simplex virus (HSV-1) responsible for cold sores.

Serologic studies (*46, 51, 59*) indicate that women with cervical carcinoma have an unusually high frequency and titer of antibody against HSV-2. It has also been shown that HSV-2 is transmitted venereally and that the rate of infection is greater in the sexually active population (*19, 47, 57*). It must be stressed, though, that the virus can be transmitted by nonvenereal means. This presumably explains why a recent study showed that nuns and their married siblings have the same frequency of neutralizing antibodies to HSV-2 (*4*).

Two recent studies have emphasized the role of the male in the development of cervical carcinoma. In Puerto Rico, Martínez (*42*) found 8 cases of cervical cancer among the wives of 889 men with cancer of the penis. Only one case was expected on the basis of incidence statistics. Also, Kessler (*34*) made a prospective study of 1,087 wives of men whose consorts had developed carcinoma of the cervix; this revealed a 2.7 per cent incidence among the wives—higher than the expected 1.1 per cent and the observed incidence of 7 out of 659 in the controls. Promiscuous men are known to harbor HSV-2 (*19, 58*). The frequency of HSV-2 isolations from the penis in different studies has ranged from 2 to 15 per cent, many of the infections being asymptomatic (*10*).

Although infectious HSV-2 is not present in cervical tumor tissue, several investigators have found evidence for the presence of the virus genome in tumor cells. Pacsa et al. (*50*) found HSV-2 antigens in exfoliated cervical cells from 94 per cent of a group of women with invasive carcinoma. Aurelian et al. (*5*) have described the presence of what they consider to be a virus-coded protein in cervical tumors from nearly all the patients they have tested. This antigen was found to disappear following effective therapy and to reap-

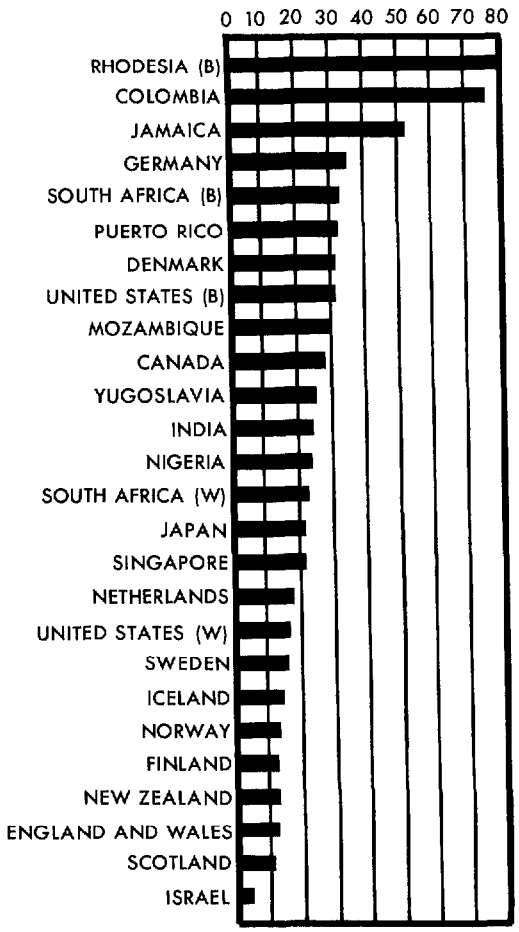

Figure 2. Age-adjusted cervical cancer incidences for 26 populations. B = black, W = white. From Persaud (*33*).

pear if the cancer recurred. Frenkel and coworkers (*20*) have reported finding a portion of HSV DNA in a cervical tumor specimen; and, by molecular cytohybridization, Jones et al. (*33*) have detected HSV messenger RNA in 5 of 8 cervical tumor biopsies.

Several groups of laboratory workers using a variety of methods have confirmed that HSV-2 can transform rodent cells in vitro (*14, 36, 56*). Such transformed cells will cause invasive tumors when injected into animals. Although infectious virus cannot be recovered from the tumors, some viral antigens are expressed in the transformed cells,

Table 6. Epidemiology of cervical cancer.

High-incidence factors	Low-incidence factors
Age: 35-60	Age: < 30
Early intercourse	Celibacy
Multiple sexual partners	Nulliparity
Low socioeconomic status	Jewish population
Poor genital hygiene	

Table 7. Infectious and noninfectious agents transmitted by sexual intercourse.

Genital wart virus	*Neisseria gonorrhea*
Cytomegalovirus	*Treponema pallidum*
Herpes simplex virus type 2	*Trichomonas*
Mycoplasma	Sperm
Chlamydia	Smegma

and the animals develop neutralizing antibodies against HSV-2. This suggests that the virus DNA or at least a portion of the genome is retained and expressed by the host cell (*38*).

No animal model for carcinoma of the cervix has been developed. However, preliminary studies (*52*) have shown that 13 of 129 cebus monkeys inoculated repeatedly in the cervix with HSV-2 developed cellular atypia approximately 14 to 50 months after infection. Animals inoculated with control material did not develop lesions.

A summary of the experimental evidence supporting an etiologic role for HSV-2 in carcinoma of the cervix is presented in Table 8. This evidence, although highly suggestive, is not compelling. There is, for instance, no satisfactory explanation of the fact that the cancer is relatively rare (about 1 case occurring per 2,000 women), while infections with HSV-2 are many times more frequent—involving at least 3 per cent of the female population (*19*). Hence, during the long interval between initial virus infection and the development of cancer, several factors must intervene to either facilitate or inhibit tumor growth. Such factors could be hormonal, immunological, or environmental. There are

Table 8. Evidence supporting a role for HSV-2 in carcinoma of the cervix.

(1) The epidemiology of carcinoma of the cervix suggests a transmissible agent.
(2) HSV-2 is usually transmitted sexually.
(3) Women with carcinoma of the cervix have higher frequencies and titers of antibodies against HSV-2 than matched controls.
(4) HSV-2-specific antigens can be demonstrated in cervical exfoliated cells.
(5) HSV-2 DNA and mRNA can be detected in cervical tumor tissue.
(6) HSV-2 is able to transform cells in vitro. Injected into animals, the transformed cells induce malignant tumors.
(7) Cervical inoculation of HSV-2 into cebus monkeys induces dysplasia.

experimental animal systems which support a multistep hypothesis for the development of cancer (*54*). A model that takes these factors into account is presented in Figure 3.

The Role of Cytomegalovirus

Another herpesvirus with oncogenic potential is cytomegalovirus (CMV), a virus most commonly associated with congenital anomalies and transfusion mononucleosis. This virus stimulates synthesis of DNA, RNA, and protein in host cells (*61*); and it transforms hamster (*1*) and human (*22*) cells in vitro to malignancy. These transformed cells will produce tumors when inoculated into hamsters or nude mice.

CMV is frequently cultivated from semen (*37*) and has been isolated from the cervix. Some investigators have found a weak serologic association between CMV and cervical carcinoma (*50*), but others have found no such association (*21*). In a recent study (*44*), CMV was isolated from 2 out of 10 cervical cancer biopsy cell cultures.

CMV may play a role in development of carcinoma of the prostate. An isolate from the prostate of a boy 3 years of age has been found to transform human embryo lung cells in vitro (*22*). Lymphocytes from patients with prostatic carcinoma have demonstrated cytotoxicity against cells transformed by this CMV isolate (*62*). CMV has also been isolated from patients with Kaposi's sarcoma (*23*), and CMV DNA has been detected in 4 out of 7 tumors of the colon (*32*). More research will be required to establish a firm association between CMV and these cancers.

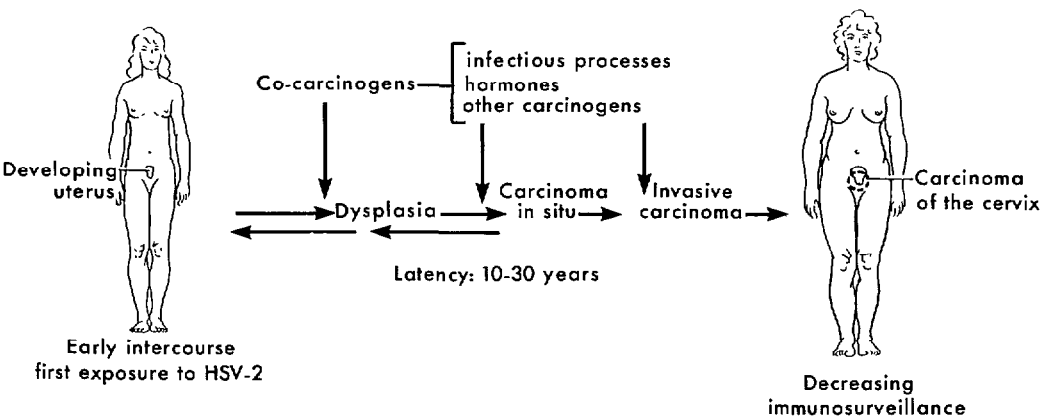

Figure 3. Possible role of herpes simplex virus type 2 in the development of carcinoma of the cervix.

Concluding Remarks

The evidence supporting a role for herpesviruses in some types of human cancer (Burkitt's lymphoma, nasopharyngeal carcinoma, and cervical carcinoma) is quite good. However, there is a general belief that these viruses do not act alone and that the tumors are a result of complex and ill-defined interactions in which genetic, immunologic, and environmental factors intervene (*15*).

Perhaps one way of proving the participation of a virus in cancer would be to demonstrate a reduction in incidence rates following an extensive immunization program. In the case of herpesviruses, however, it would be hard to carry out such a program for the following reasons: (1) the vaccine would have to be free of nucleic acid and therefore would be expensive; (2) infections are known to occur even in the presence of neutralizing antibodies; and (3) the incidence of cancer is relatively low, so a costly mass-immunization program would probably not be justified. On the other hand, the increasing incidence of venereal disease due to herpes simplex viruses should eventually result in an increased incidence of cervical neoplasia if the virus plays a role in this disease.

In the meantime, attempts to eliminate the other suspected co-factors involved in cancer development could be worthwhile. For instance, the reduction or eradication of malaria in Africa might have an effect on the incidence of Burkitt's lymphoma; a reduction or elimination of inhaled carcinogens might reduce the incidence of nasopharyngeal carcinoma; and a program of sex education might lower the frequency of carcinoma of the cervix. These measures would at the same time help to combat several other diseases that are far more prevalent than cancer, especially in the developing world.

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SUMMARY

At the present time, herpesviruses acting alone or in association with other factors have been linked to three human cancers: Burkitt's lymphoma, nasopharyngeal carcinoma, and carcinoma of the uterine cervix.

In general, evidence that the Epstein-Barr herpesvirus (EBV) plays an etiologic role in Burkitt's lymphoma and nasopharyngeal carcinoma is fairly convincing. However, the peculiar geographic distribution of these two diseases suggests that environmental factors are also involved.

Evidence that herpes simplex virus type 2 (HSV-2) plays an etiologic role in carcinoma of the cervix uteri, although highly suggestive, is less compelling. For instance, the fact that HSV-2 infection is fairly common while development of the cancer is relatively rare has not yet been satisfactorily explained. It would appear that sometime during the long interval between infection with HSV-2 and development of cancer, several factors must intervene to facilitate or inhibit tumor growth.

In addition, there is some evidence that cytomegalovirus (CMV) may contribute to development of Kaposi's sarcoma and to cancers of the cervix uteri, prostate, and colon. More research will be required to establish a firm association between CMV and these cancers.

Though a program of immunization against the herpesviruses involved would be hard to carry out, attempts to eliminate other suspected co-factors involved in cancer development could be worthwhile. Reduction of malaria in Africa might lower the incidence of Burkitt's lymphoma; reduction or

elimination of inhaled carcinogens might diminish the occurrence of nasopharyngeal carcinoma; and sex education programs might reduce the frequency of cervical cancer. Such measures would also help to combat other diseases that are far more prevalent than cancer, especially in the developing world.

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