Viruses and Human Cancer*

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The contention that viruses may cause cancer in man rests mainly on analogy with observations in other species, particularly laboratory animals. However, even if tumor viruses were known not to cause human cancer they would be a suitable starting place for the study of genetic mechanisms in neoplasia. Oncogenic viruses have from seven to 50 genes, only a few of which are required to confer malignancy on an infected cell. These viruses obviously represent a more promising place to search for oncogenic genes than mammalian cells, which contain two million genes or more. The electron microscope has been most useful in the discovery of suspected oncogenic viruses, since some of these agents have a distinctive appearance, leaving little doubt of their presence and identity. Cell culture is also an early step in identifying a virus and in evaluating its oncogenic potential. In culture, the presence of an oncogenic virus may be inferred from the formation of miniature tumors or "foci" of multilayered cells. A major difficulty is caused by viruses that are frequently present in cells used to grow the agent to be isolated. Immunologic methods, including complement-fixation tests and immunofluorescent, cytolytic and neutralizing-antibody technics, are extremely sensitive and useful for detecting a virus, its proteins and antiviral antibodies. However, the necessary purified antigens and specific immune sera can be difficult to obtain. Thus, when a preparation of purified virions is not available as antigen and tumor tissue must be used instead, as with human tumors, immunologic findings can have several interpretations. For example, the carcinoembryonic antigens of some tumors may be confused with viral antigens. Technics of biochemistry and molecular biology are also now being used to identify viral changes in cells. The assay for an enzyme (an RNA-directed DNA-polymerase), which may be specific for RNA tumor viruses, and methods to identify the nucleic acids of adenoviruses in human tumors are examples of such technics.

Epidemiologic studies of the association of virus with cancer generally have one of three goals: to evaluate evidence for person-to-person or animal-to-person transmission of disease or the sharing by affected persons of some common exposure; to investigate evidence that a cancer and a virus exhibit concomitant variation in time or geographic location; and to assess evidence for higher rates of antecedent infection by a specific virus among persons with cancer as compared to controls.

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Transmission

Person-to-person transmission or common exposure to an etiologic agent would be suggested if a cancer exhibited time-space clustering. For example, an apparent excess frequency of acute leukemia was found among children in Niles, Illinois, from 1957 to 1960. However, few studies of such occurrences have designated a priori the population-at-risk among which clusters of cancer are to be sought or the personal inter-relations that join the members of a “cluster.” To evaluate even careful positive studies it is necessary to recall that, with many clusters available, some clustering must occur from chance alone. The question is not whether clustering has occurred but whether there are more clusters than would occur by chance alone.

Familial aggregation of a cancer is usually inferred to support a “genetic component,” a particular type of person-to-person transmission, in the etiology of the disease. However, familial aggregation is consistent both with vertical and horizontal transmission of an infectious agent, and with noninfectious causes of disease, since family members share many environmental exposures. A valuable but rarely used approach is the study of disease concurrence among biologically nonrelated family members such as spouses and adopted children.

Concomitant Variation

Many viral diseases show marked seasonal or other temporal variation. If a human cancer were to show such variation, a viral etiology would be supported. However, search for seasonal variation in onset of disease is unlikely to be rewarding in the study of cancer because of the probable long induction-incubation period. There may be value in the search for seasonal variation in births of children in whom cancer develops. This would presumably correlate with exposure better than time of onset. Such an association has been found for boys with Hodgkin’s disease.

For many neoplasms geographic variation in risk is among the most striking epidemiologic features of the disease. Such variation, particularly when coupled with studies of populations that migrate from high-risk to low-risk areas, or vice versa, should provide considerable insight into etiology of disease. Such studies have generally allowed only the inference that “environment” has a major role in cancer etiology. It is probably true that cultural characteristics of populations living in different locations are more direct determinants of disease frequency than geographic factors.

Antecedent Infection

The more successful epidemiologic studies of viruses and human cancer have been case-control studies of antecedent infection. Because the problems in selecting suitable controls are difficult, it cannot be excluded that many factors might independently be associated both with exposure to viruses and with risk of development of cancer. Another difficulty is that persons in whom cancer is more likely to develop and afflicted persons may differ from others in susceptibility to virus infection.

Oncogenic DNA Viruses

Papova Viruses

The papova viruses are small DNA viruses including the papilloma, polyoma and simian vacuolating viruses—hence the name. The human papilloma virus is typical of the group with a diameter of 55 nanometers (nm) and a symmetrical coat or capsid, which forms an

| Abbreviations Used |
|---------------------|
| EBV: Epstein-Barr Virus |
| gs: group specific |
| HVH-2: Herpesvirus hominis, Type 2 |
| mRNA: messenger RNA |
| SV40: simian virus 40 |
| ts-3: temperature-sensitive conditional mutant |
icosahedron with 72 capsomers. The virus is 12 percent DNA arranged in a circular double strand with a size of 5 x 10^6 daltons (d). The human papilloma virus produces in humans both the common wart and the laryngeal papilloma. The mechanism of oncogenesis is not well understood for the papilloma viruses, which do not grow well in cell culture.

Two other papova viruses, polyoma and the simian virus 40 (SV40), do grow well in cell culture and are excellent for study of oncogenesis. Polyoma virus occurs naturally in adult mice without apparent ill-effect. However, when injected into newborn rodents, it produces several different tumors. SV40, discovered in cultures of rhesus-monkey-kidney cells, produces sarcomas when injected into newborn hamsters. These two viruses are similar to papilloma virus in their physical properties but are smaller. Both have a diameter of 40–45 nm, and the size of polyoma DNA is 3 x 10^6 and that of SV40 is 2.5 x 10^6 d. This amount of DNA is sufficient to code for only about six to 10 proteins of 20,000 molecular weight. The small amount of genetic material in which oncogenicity is coded and the success of investigators in finding mutants in which oncogenicity is "conditional" make these viruses ideal for elucidating the genetic mechanism of oncogenesis.9

Both polyoma and SV40 may exert either of two cellular effects: productive infection with lysis of permissive cells or transformation. (Fig. 1.) Productive infection usually occurs in permissive cells of a natural host and resembles the lytic action of virulent viruses. Proposed major steps in the process are as follows: (1) adsorption of the virus to the cell membrane and penetration of the cell; (2) uncoating of viral DNA at the nuclear membrane; (3) transcription of part of parental viral DNA in the nucleus to form early viral messenger RNA (mRNA); (4) translation of early viral mRNA into "early" proteins involved in viral DNA synthesis and in altering cell metabolism; (5) replication of viral DNA; (6) transcription of progeny viral DNA to form late viral mRNA; (7) translation of late viral mRNA to form viral capsid and other proteins; (8) self-assembly of the virus in the cell nucleus; and (9) lysis of the cell.10

Cell transformation by polyoma and SV40 represents a permanent, heritable change most readily studied in nonpermissive cells of a foreign host. This occurs in up to 40 percent of cells exposed to the virus. In transformation only some of the first steps in viral infection occur. Viral DNA, capsid protein and infectious virus are not produced, possibly because of a lack of some host-cell function. The infecting virus does not lyse the cell, but its DNA is inserted in the host-cell genome, is replicated with it, and may alter certain properties of the host cell. (Fig. 1.) Radiobiologic evidence indicates that only a fraction of the polyoma genome is required for transformation, relative to that required for virus reproduction. However, with SV40, the entire viral genome is integrated in transformed cells even though no infectious virus can be demonstrated. The virus can be "rescued" by fusion of the transformed cells with permissive cells, causing cell lysis and release of mature infectious virions. It is not known how the genome of a transforming virus is integrated with the host-cell chromosome. Rapidly growing cells and those damaged by radiation or by incorporation of abnormal bases into their DNA are more susceptible to transformation, suggesting that integration of the DNA of the virus occurs more readily when host-cell DNA is synthesized or repaired.

Transformation in vitro implies only that there has been a permanent and heritable change in cell-growth properties. There is no necessary association of transformation in vitro and malignancy in vivo. Transformed cells usually require passage in vitro before they can be
Fig. 1. Schematic and tentative outline of interaction of Papova virus with cells. The end products are cellular lysis with production of new virus (E), or cellular cells transformed by virus-specific proteins (G).
transplanted successfully into appropriate animals and metastasize. Transformation is accompanied by loss of certain cellular responses to environmental controls, such as the inhibition of DNA synthesis by topographic factors ("topo-inhibition"). Normal cells respond to contact with neighboring cells by becoming immobile (contact inhibition) and by ceasing to divide (mitotic inhibition). Transformed cells will move over one another and may form layers. The "foci" that are formed can be used in a cell-culture assay technic to enumerate oncogenic virus particles. Loss of contact inhibition is frequently accompanied by the ability of transformed cells to grow in soft agar, and this feature can also be used in a quantitative assay.

Several changes in the surface membrane of transformed cells are highly correlated with the loss of contact inhibition. The degree of loss of contact inhibition can be measured by determination of the cells per square centimeter of the culture dish, the saturation density. Some cytoagglutinins will agglutinate related murine fibroblast cell lines in order of their saturation densities. These changes in agglutinability are not an invariable accompaniment of transformation, and do not correlate at all with binding of cytoagglutinins by the cell. They reflect, however, some change in the configuration of the cell membrane that is also accompanied by chemical alterations; analyses of the cell membrane of mouse fibroblast cells, before and after transformation by SV40, show alteration of the glycoprotein composition.

Loss of topoinhibition may also include loss of mitotic inhibition. When this occurs eight or nine enzymes required for premitotic DNA synthesis are induced. Since there are too few viral genes to code for all these enzymes, it is thought that a viral gene product derepresses host-cell enzyme synthesis. This hypothesis is supported by the finding that a cell line lacking one such enzyme, thymidine kinase, remains unable to produce this enzyme after transformation by polyoma virus.

A virus-specific tumor antigen, the "T antigen," is an early viral gene product and may be concerned with the stimulation of cellular DNA synthesis (inductive function). T antigen is present in the nucleus of a transformed cell but not in the virus itself. The function of the T antigen is unknown, but it does serve as a useful indicator of viral transformation. Another antigen present in the transformed cell, specific for the transforming virus but lacking in the virus itself, is the tumor-specific transplantation antigen. This antigen, on the cell surface, is believed responsible for the immunospecific rejection of a transplanted tumor by a host animal.

Transformation by SV40 and polyoma virus is accompanied by metabolic and chemical as well as physical changes in a cell. For example, such transformed cells produce more lactic acid and acidify the culture medium more rapidly than control cells growing at the same rate and density.

A recent discovery of value in the study of oncogenic mechanisms concerns the "conditional" mutants of polyoma virus. The conditions under which these mutants do or do not express oncogenicity can be manipulated experimentally. Mutants have been obtained that are temperature sensitive in their ability both to replicate themselves and to transform cells. A temperature-sensitive conditional mutant (ts-3) of polyoma virus has been found that can transform cells, with resultant loss of contact inhibition. However, when the cells are grown at the nontransforming temperature, they revert to normal morphology. This fact implies that the continued activity of the viral genome is required to maintain at least some aspects of cell transformation. The inductive function is also temperature dependent, as are alterations of the cell-surface membrane. Thus, a common gene con-
trolling cell-surface characteristics and DNA synthesis is suggested. This gene may represent the specific locus of derangement in cancer. It is readily appreciated that increased DNA synthesis would enhance viral genome reproduction, but it is not clear why this is related to cell-membrane changes. The product of the ts-3 gene may operate indirectly through a cellular structure involved in both functions. The study of temperature-sensitive mutants suggests, then, that viral genes produce and maintain transformation by the continued formation of a viral protein (or proteins), not by a single "hit" on the host cell. 16

This outline of the actions of polyoma and SV40 reveals the important contribution that their study has made to the understanding of viral oncogenesis. The study of these papova viruses may yield an unexpected additional benefit. Epidemiologic studies have identified groups of persons with inborn and acquired characteristics who are at increased risk of cancer. The skin fibroblasts of members of some of these groups are more sensitive to in vitro transformation by SV40 than fibroblasts from normal persons. It has been suggested that determining fibroblast transformation rates could identify persons at high risk of leukemia and other cancers. 17

Adenoviruses

The adenoviruses are human viruses that cause cancer in other mammals. This is a large group of medium-size DNA viruses, including 31 of human and 24 of other vertebrate origin. All have a diameter of 80 nm and an icosahedral capsid. Twelve to 13 percent of the virus is composed of linear, double-stranded DNA of 20-25 × 10^9 d. Therefore, the circularity of papova virion DNA is not necessary for oncogenicity. 18,19 Oncogenic adenoviruses penetrate nonpermissive cells of a foreign host and cause transformation and an abortive infection. T antigen is formed, host-cell DNA synthesis is stimulated, and the entire viral genome is integrated into the cell's DNA. Only a portion of the 23 to 46 genes of the virus function in the transformed cell, and late gene functions such as capsomer formation are blocked. The genes transcribed in the production of viral mRNA are the same as those active in early productive infection. Adenoviruses cause acute respiratory and ocular disease in man but have not been shown to cause cancer. Complement-fixation tests of serum specimens of cancer patients with adenovirus hamster T antigen and of human tumors with hamster T antibody have shown a low frequency of positive reactions both in patients and in controls. Moreover, 130 human tumors were assayed for adenovirus mRNA, and none was found, with the use of a method that would detect one tenth the amount present in adenovirus-transformed cells in culture. 10

Herpesviruses

Of all the viruses known to be oncogenic in animals, the herpesviruses have been most associated with cancer in humans. These are medium to large DNA viruses with a diameter of 180 to 200 nm. About three percent of the virus is DNA, doublestranded, with a size of 100 × 10^9 d. These viruses are sensitive to ether since they have a lipid-rich viral envelope, derived in part from the host-cell nuclear membrane. After assembly of the distinctive icosahedral nucleocapsid in the cell nucleus, the immature virus particles migrate to the nuclear membrane and are enclosed by a portion of this membrane as they pass into the cytoplasm. The mature virions are then held in cytoplasmic vacuoles and are released extracellularly when the vacuole opens through the plasma membrane. The mechanism of oncogenesis by herpesviruses is unknown, but the recent isolation of oncogenic herpesviruses from animals and their study in vitro may lead to such information. 20

A typical herpesvirus has been iso-
lated from the renal carcinoma of leopard frogs, and produces the disease when injected into tadpoles. Marek’s disease, a lymphomatous condition of chickens, has been shown to be caused by a herpesvirus. *Herpes saimiri* has recently been shown to produce lymphatic leukemia and a rapidly progressive disease resembling a lymphoma in marmosets and owl monkeys. This fatal disease is typical of lymphomas, with its displacement of normal tissues by immature lymphocytes, but is atypical in its brief incubation period and the associated tissue necrosis.

Latency is characteristic of herpesvirus infections. An equilibrium exists between host cell and virus, with infection apparent only when the equilibrium is upset as in the familiar cold sore or fever blister caused by *H. hominis* (*H. simplex*). The mechanism of latency is unknown. One possibility is a linkage of viral DNA with host-cell DNA, similar to the lysogenized state of certain bacteriophage-infected bacteria. Another possibility is a virus-carrier state in which the viral infection is confined to a few cells at a time owing to antibodies, interferon or the metabolic state of the cells.

A herpesvirus of major interest is the Epstein–Barr virus (EBV), discovered by electron microscopy in Burkitt’s lymphoma cells grown in culture. EBV has so little biologic activity that it is unlikely to have been discovered by any means other than electron microscopy. The EBV has since been found in many isolates from Burkitt’s lymphoma, and antibodies of the virus are present in high titers in 87 percent of affected persons as compared to 14 percent of controls. The virus also converts peripheral lymphocytes in culture into lymphoblastoid cells. The epidemiologic evidence supporting a virus etiology of Burkitt’s lymphoma consists, first, of limited geographic distribution of the disease, although sporadic cases do occur outside the few areas where the disease is endemic. Secondly, within the endemic belt, the decreased risk of disease within arid or cool microclimates suggests that an arthropod might have a role in disease transmission. Thirdly, time-space clustering and evidence of the systematic drifting of the clusters have been observed. Fourthly, the disease is restricted to the young, except among migrants from areas of low to high risk. Finally, higher prevalence rates and higher titers of anti-EBV antibodies among cases of Burkitt’s lymphoma than among controls. One interpretation of these laboratory and epidemiologic findings is that, at least in endemic areas, Burkitt’s lymphoma represents an uncommon manifestation of a common infection. It is proposed that malaria or some other insult to the reticuloendothelial system, in addition to that exerted by the virus, is usually necessary for this uncommon manifestation to occur.

It was found that, like lymphoid cells infected with EBV, the cells of infectious mononucleosis have the ability to proliferate indefinitely in culture. An accidental laboratory infection suggested that EBV was the etiologic agent of infectious mononucleosis, and that possibility is now supported by epidemiologic and immunologic findings.

Nasopharyngeal carcinoma has also been associated with the EBV. High prevalence rates of antibodies were found in persons with this disease. However, it appears that antibody levels may increase as the disease progresses, suggesting that the EBV is a passenger virus.

A herpesvirus has also been associated with cancer of the uterine cervix. Considerable epidemiologic evidence suggested that cervical cancer could be considered, essentially, a venereal disease. The idea that some substance or agent transferred to the cervix during sexual intercourse might cause the disease led to an intensive search for such a factor. Among viruses, *H. hominis*, for-
merly *H. simplex*, has been implicated as a likely candidate. Work with this agent revealed that there are at least two similar, but antigenically distinct, types of the virus.\(^2\) Type 1 causes most oral and cutaneous herpetic infections, whereas Type 2 causes most genital infections.

Several studies have shown a strong association between antibodies to Type 2 *H. hominis* (HVH-2) and cancer of the cervix.\(^3\) Among women with the disease, 80 percent or more have such antibodies, whereas among control women the prevalence rate has usually been about 30 percent. There is a major obstacle to interpreting this association as causal. Both the risk of cancer of the cervix and the prevalence rate of HVH-2 antibodies have a strong inverse association with social class. Most reports have provided little information on patient selection and even less on control selection. The study of Royston and Aurelian\(^4\) comes closest to being satisfactory in this regard, patients and controls having been matched according to residence. Antibodies were found among nearly 100 percent of patients in each of three residential strata, whereas among control groups the prevalence rates were 61 percent or less. Prevalence rates of HVH-2 antibodies were also uniformly high among women with carcinoma in situ. Lower prevalence rates had previously been found among women with carcinoma in situ, suggesting that virus infection of the cervix was not a cause of the disease but a secondary event. Most recently, it has been reported\(^5\) that New Zealand women with either carcinoma in situ or invasive disease have HVH-2 antibody prevalence rates of only about 30 percent. In this study, controls had a rate of 23 percent. Although the discrepancy might be attributable to methodologic differences, even if substantiated, these findings would not exclude HVH-2 as a cause of cervical carcinoma, but rather, they would suggest that the virus acts indirectly or, perhaps, is one of several causes of the disease.

If HVH-2 causes carcinoma of the cervix it may be possible to prevent the disease by a vaccine. This is a possibility even though latent herpes infection can persist in humans with circulating antibodies. Marek’s disease, a herpesvirus-induced neoplastic disease of chickens, can be prevented by vaccination.\(^3\) Other methods of prevention aimed at reducing the spread of the virus by modifying human behavior are unlikely to succeed. In addition to resolving whether the association with cervical carcinoma is causal, further work with HVH-2 should be directed to the role of men in its spread.

Herpesvirus may also be associated with Hodgkin’s disease, especially the form of the disease characteristic of young adults. Stewart et al.\(^6\) have identified “the herpes-type virus which has been described for the Burkitt’s tumor cell cultures” in a cell line derived from tissue from a young man with Hodgkin’s disease. Of interest is the report that “Reed–Sternberg-like” cells, the cells long considered pathognomonic of Hodgkin’s disease, are identifiable in tissue from patients with infectious mononucleosis.\(^7\) a disease almost certainly caused by the EBV. The same type of cell has been found in recurrent lesions of Burkitt’s lymphoma. Serologic studies have both supported\(^8\) and countered\(^9\) an association of EBV with Hodgkin’s disease.

There are also epidemiologic data that would link some cases of Hodgkin’s disease with a virus. It has been suggested that “Hodgkin’s disease,” as the term is now used, applies to at least two etiologically distinct entities.\(^10\) \(^11\) The entity that comprises most cases among young adults shows considerable geographic variation between countries and between different regions of the United States.\(^4\) The geographic variations are unexplained and have not been correlated with the prevalence of any virus. Support for an infectious agent in the
etiology of Hodgkin’s disease is provided by the finding that among young adults in New York, the risk of disease is three times as great among those who have been tonsillectomized as among those who have not. This observation, however, is not supported by data from a study in Finland. A small but definite amount of familial aggregation is reported in Hodgkin’s disease. Several reports have suggested person-to-person transmission of the disease. One of these, which come from Britain and Germany, show an excess of cases during winter.

**Pox Viruses**

Pox viruses are the largest animal viruses and can be seen with a light microscope. Brick-shaped or ovoid, measuring 250 by 300 nm, they have a complex structure without symmetry. The vaccinia virus, typical of the group, has DNA that is double stranded and about $160 \times 10^6$ in size.

Pox viruses have a predilection for epidermal cells and produce a spectrum of response from virulent to oncogenic. Whether a lytic, proliferative or oncogenic response occurs depends on the type of infecting pox virus. Vaccinia causes proliferation initially and cell lysis later. On the other hand, the virus of molluscum contagiosum produces chronic proliferation. The pox viruses multiply in the cytoplasm independent of cell nucleic acid synthesis. The location in the cytoplasm permits analysis of the replicative process by electron microscopy and autoradiography. With vaccinia, host-cell mRNA and DNA synthesis decreases rapidly three hours after infection and stops after four to six hours. Cytopathic effects are evident 18 to 24 hours after infection. Different events occur when the Shope-fibroma virus, an oncogenic pox virus, infects rabbit-kidney cells. Host-cell DNA synthesis is inhibited after three hours as in vaccinia infection, but is resumed after 36 to 48 hours. At that time virus production declines, and a steady state is reached, with constant production of small amounts of virus and continued division of the host cell. However, the cell displays altered morphology and loss of mitotic and contact inhibition. This steady state is dependent on a suppression of viral multiplication as demonstrated by the greatly reduced capacity of superinfecting pox virus to multiply.

The Shope virus causes a benign fibroma of cottontail rabbits, and other pox viruses produce similar fibromas in hares and squirrels and the rapidly fatal myxomatosis of European rabbits. Fibrosarcomas are induced when the virus is combined with tar, other carcinogens or cortisone. Yaba-monkey-tumor pox virus causes large, localized benign skin tumors, apparently histiocytomas, in monkeys and in humans.

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