

Infectious agents and cancer: criteria for a causal relation

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Abstract

Infectious agents, mainly viruses, are among the few known causes of cancer and contribute to a variety of malignancies worldwide. The agents and cancers considered here are human papillomaviruses (cervical carcinoma); human polyomaviruses (mesotheliomas, brain tumors); Epstein-Barr virus (B-cell lymphoproliferative diseases and nasopharyngeal carcinoma); Kaposi's Sarcoma Herpesvirus (Kaposi's Sarcoma and primary effusion lymphomas); hepatitis B and hepatitis C viruses (hepatocellular carcinoma); Human T-cell Leukemia Virus-1 (T-cell leukemias); and *helicobacter pylori* (gastric carcinoma), which account for up to 20% of malignancies around the globe. The criteria most often used in determining causality are consistency of the association, either epidemiologic or on the molecular level, and oncogenicity of the agent in animal models or cell cultures. However use of these generally applied criteria in deciding on causality is selective, and the criteria may be weighted differently. Whereas for most of the tumor viruses the viral genome persists in an integrated or episomal form with a subset of viral genes expressed in the tumor cells, some agents (HBV, HCV, helicobacter) are not inherently oncogenic, but infection leads to transformation of cells by indirect means. For some malignancies the viral agent appears to serve as a cofactor (Burkitt's lymphoma-EBV; mesothelioma - SV₄₀). For others the association is inconsistent (Hodgkin's Disease, gastric carcinomas, breast cancer-EBV) and may either define subsets of these malignancies, or the virus may act to modify phenotype of an established tumor, contributing to tumor progression rather than causing the tumor. In these cases and for the human polyomaviruses the association with malignancy is less consistent or still emerging. In contrast despite the potent oncogenic properties of some strains of human adenovirus in tissue culture and animals the virus has not been linked with any human cancers. Finally it is likely that more agents, most likely viruses, both known and unidentified, have yet to be implicated in human cancer. In the meantime study of tumorigenic infectious agents will continue to illuminate molecular oncogenic processes.

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Keywords: Tumor viruses; Human papillomaviruses; Human polyomaviruses; Epstein-Barr virus; Kaposi's Sarcoma Herpesvirus; Hepatitis B virus

1. Introduction

In the past 25 years revelations on the genesis of human cancer have come at an increasing pace. The contributions of knowledge about oncogenic infectious agents, especially viruses, have been instrumental in that understanding because in transforming cells they mirror, often brilliantly, basic cellular processes that culminate in cancer. Infectious agents, chiefly viruses, are accepted causes or candidates as causes of diverse malignancies of people world-wide. From

a universal perspective infectious agents especially viruses account for several of the most common malignancies – up to 20% of all cancers. Some of these cancers are endemic with high incidence in certain geographic locations, but have sporadic low incidence in other parts of the world. The consistency of association of a given virus and a specific malignancy ranges from essentially 100% to as low as 15% depending on the virus, the cancer and the geographic location. The significance of association of a virus and a cancer when less than 100% is generally uncertain and problematic in terms of etiology, but these lesser associations may define subsets of tumors – those infected with a specific virus or those not – as with EBV and gastric carcinoma. In

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contrast helicobacter is consistently associated with gastric carcinoma. If a given agent is not consistently detected in a malignancy with which it is associated, the question arises whether the agent nevertheless contributes to some phase of oncogenesis.

The agents considered here are human papillomavirus (HPV), human polyomaviruses (JCV, BKV, SV40), Epstein-Barr virus (EBV), Kaposi's Sarcoma Herpesvirus (KSHV), hepatitis B virus (HBV), hepatitis C virus (HCV), human T-cell leukemia virus, (HTLV1) and *Helicobacter pylori*, most of which are believed to cause or contribute in a significant way to the genesis of a variety of malignancies, in particular cervical carcinoma (HPV), mesotheliomas and brain tumors (polyomaviruses), B-cell lymphoproliferative diseases and nasopharyngeal carcinoma (EBV), Kaposi's Sarcoma and primary effusion lymphomas (KSHV), hepatocellular carcinoma (HBV and HCV), T-cell leukemia (HTLV1) and gastric carcinoma (helicobacter). The human polyomaviruses have not attained the same status as the other agents as probable causative agents, but merit attention because of their compelling oncogenic properties and the increasing strength of their association with specific malignancies. In contrast although strains of human adenoviruses have clearly oncogenic properties including the ability to dysregulate the same tumor suppressor genes targeted by HPV and the human polyomaviruses, there is no known association with human malignancy. In addition to these major associations several of the viruses have lesser associations with other malignancies. Cofactors have been implicated for some of these agents especially in instances where the malignancy has high incidence in certain geographic areas (Table 1).

In the case of DNA-containing tumor viruses, infection is latent, the viral genome persists in some form in the tumor tissue, and a subset of viral genes is expressed in the tissue. If viral genome persists it may do so in either integrated or episomal form. HTLV1 and HBV also persist as integrated genomes. For the hepatitis viruses and helicobacter infection is not latent; rather there is persistent infection and replication of the agent at the site of tumor formation.

The goal of this article is to come to grips with the criteria that establish a given infectious agent as oncogenic. In the context of the many associations between a virus or microbial agent and a given malignancy, the distinction between associated versus causative agent frequently arises and may be difficult to decide. However, since some of the associations are considered to be causal or probably causal and others are not, it is instructive to consider by specific cases what evidence is generally accepted as sufficient to establish a causal relation, and which factors may be dispensable. The approach taken here will be to review briefly the salient associations for each of the commonly accepted agents in terms of consistency of disease association, oncogenic properties, nature of the association, mechanism of oncogenesis and essential or suspected cofactors. Alternatively it is possible that an infectious agent may establish a noncausal relation

with a tumor, that is, it may modify the phenotype of a tumor cell. Based on this information inferences can be made and provide an additional perspective on issues of causality.

2. EBV

The Epstein-Barr virus was originally isolated from biopsy tissue samples of the childhood malignancy, African Burkitt's lymphoma [1]. This unusual cancer occurs with high incidence in an endemic region that is also holoendemic for malaria. An association with EBV was subsequently discovered in nasopharyngeal carcinoma, a cancer that develops with extraordinarily high incidence among the Cantonese Chinese and with elevated incidence among Alaskan Inuits and in Mediterranean Africa. EBV is accepted as cause of post-transplant lymphomas and is linked to a subset of Hodgkin's lymphomas, gastric carcinoma, and rare examples of T-cell lymphoma, especially in Japan, Taiwan and Korea, and rare smooth muscle sarcomas in children with AIDS.

BL was the one of the first tumors shown to have characteristic chromosomal translocations. The reciprocal translocations involved chromosome 8 near the location of the *c-myc* oncogene and either the immunoglobulin heavy chain locus on chromosome 14 or the light chain loci on chromosomes 2 and 22 [2]. These rearrangements alter the regulation of expression of *c-myc* and place this cellular gene under the control of the immunoglobulin promoters. Rare childhood lymphomas in Western countries were subsequently shown to resemble Burkitt's lymphoma histologically. The incidence of these tumors is 100-fold less frequent than the endemic form, and only approximately 20% are associated with EBV. Interestingly, these tumors are also marked by the characteristic translocations between *c-myc* and the immunoglobulin loci although the breakpoints are different. In sporadic BL, the breakpoints are usually within the first exon or intron of *c-myc* whereas in endemic BL the breakpoint is far 5' to the *c-myc* gene [1].

Deregulated *c-myc* expression is probably critical to the development of BL. The characteristic chromosomal translocations may be triggered during variable gene rearrangement or class switching. The cell-surface markers, CD10 and CD77, which are characteristic of BL, suggest that BL may represent a germinal center B cell. Germinal centers are greatly expanded in chronic malarial infection.

Nasopharyngeal carcinoma is an unusual tumor with intriguing epidemiologic and biologic characteristics. The tumor occurs world-wide but with exceptionally high incidence in particular populations in specific geographic regions [3]. The incidence is highest among the Cantonese in Southern China and in Hong Kong, Singapore and Taiwan Chinese and appreciable in Southern Chinese living in Malaysia and California; in the USA incidence declines in second generation Chinese-Americans. NPC occurs with intermediate incidence in Mediterranean Arabs

and in Malays in Singapore. The incidence is quite low in American Caucasians and Europeans. The extraordinarily elevated incidence of NPC in distinct populations suggests that environmental and genetic elements and viral infection contribute to the development of this disease. However, unlike BL, all cases of NPC whether from areas of low or high incidence are associated with EBV infection [4].

Hodgkin's disease (HD) is an unusual malignancy in which the malignant cells, termed Hodgkin/Reed-Sternberg (HRS) cells, usually comprise less than 1% of the total cell population of the tumor. These cells appear as atypical immunoblasts or multinucleated giant cells with prominent nucleoli and marginated heterochromatin. The cells are believed to derive from germinal center B cells. The HRS cells are surrounded by reactive lymphoid stroma, plasma cells, eosinophils, and granulocytes. Classical HD is divided into three subtypes: nodular sclerosis, mixed cellularity, and lymphocyte depleted. In the United States, these variants differ in their association with EBV, histology; and prognosis. Nodular sclerosis HD has a good prognosis; approximately 20% are EBV-infected. Lymphocyte-depleted HD has the poorest prognosis; almost all cases are EBV-associated. Mixed cellularity HD has an intermediate prognosis; approximately 70% are EBV-infected [5]. In less developed countries almost 100% of HD are associated with EBV.

2.1. Nature of association

The association of EBV with some of these malignancies is quite compelling. The first link to EBV was demonstrated in BL and NPC as patients have significantly elevated antibody titers to viral antigens, including VCA and EA. Elevated EBV VCA antibodies precede the development of BL by months or years [6]. Similarly, early seroepidemiologic studies revealed that patients with NPC had elevated IgA antibody titers to VCA and EA [7]. Appearance of IgA antibodies to EBV precedes the development of NPC by several years and also correlates with tumor burden and recurrence. IgA antibodies may predict later occurrence of NPC. A link of EBV infection to Hodgkin's disease was initially suspected due to development of the lymphoma in populations with a profile similar to that of patients in whom IM developed earlier. Analyses of the serum repository at Yale revealed that elevated EBV titers preceded the development of HD by 2–3 years or more [8].

In all EBV-associated tumors, the viral antigen EBNA-1 is detected in BL and NPC in all the tumor cells. Moreover, the EBV genome is located in the malignant epithelial cells and not the infiltrating lymphocytes in the NPC tissue [9]. Similarly in HD, the viral genome and expression of the abundant nonpolyadenylated EBV-encoded RNAs (EBERs) were detected in HRS cells [10].

An additional compelling factor is that all the malignancies associated with EBV including BL and NPL contain homogeneous episomal genomes detected with the use of the EBV termini assay [11]. This assay is based on the fact

that the linear EBV genome found within the virion contains variable, multiple copies of a 500-base-pair direct tandemly repeated sequence at each terminus (TR). After entry into the cell, the virus circularizes via the TR, forming a fused restriction-enzyme fragment that will hybridize to probes containing unique DNA from either end of the genome. The detection of a homogeneous fragment, despite the heterogeneity possible in this fragment, indicates that every copy of the viral episome within a cell is identical with regard to the number of TR and that within a tumor, the EBV episomes in every cell are identical. The clonality of the EBV genome suggests that it can be used as a marker of cellular clonality. In addition, this assay suggests that the tumors develop from a single cell that was infected with EBV prior to outgrowth. If EBV had infected the tumor secondarily one might expect that multiple tumor cells would be infected and continue to proliferate with multiple, distinct forms of the EBV episome. The detection of a single form of the episome in EBV-associated tumors suggests that EBV infection has preceded clonal amplification or that a single EBV-infected clone has predominated. This observation also suggests that EBV infection is an early event in the development of cancer. Similarly to BL and NPC, EBV DNA and EBERs are detected in the malignant RS cells of HD, and analysis of the EBV termini reveals that HD has clonal EBV episomes indicating that HD develops from a single EBV-infected cell.

In addition, although the infection in the tumors is latent and does not produce virus, viral genes are expressed in all tumor cells [1]. In BL, viral expression is most limited with only expression of EBNA1 and the EBERs. In NPC and HD, EBNA1 and the EBERs and the viral transforming proteins, LMP1 and LMP2, are expressed. In post-transplant lymphoma, all of the viral proteins expressed in latently infected lymphocytes that have been transformed in vitro are expressed, including the major CTL targets, the EBNA2 and EBNA3 proteins. The expression of these CTL target proteins enables these tumors to be successfully treated by reducing immunosuppression or by infusing EBV-specific cytolytic T-cell preparations that have been expanded in vivo [12,13].

2.2. Oncogenic properties

A key biologic property of EBV that underlies its clear link to cancer is its ability to alter B-cell growth regulation and induce permanent growth transformation. Virus detected in throat washings or produced by cell lines maintained in vitro can infect naive lymphocytes to establish immortal transformed cell lines. Similar lymphoid cell lines can also be established by spontaneous outgrowth of EBV-infected B-lymphocytes from the peripheral blood of infected individuals [14].

The ability of EBV to cause neoplastic growth is most clearly demonstrated by the development of B-cell lymphoproliferation in patients who are immunocompromised [15,16]. EBV-associated lymphoproliferative diseases may

develop in patients with congenital immune impairment including the X-linked lymphoproliferative syndrome, severe combined immunodeficiency, adenosine deaminase deficiency, and Wiscott-Aldrich syndrome and especially in organ-transplant recipients and patients with AIDS.

In addition to these key biologic properties, EBV encodes multiple viral proteins that have profound effects on cellular expression. The EBV oncogene, LMP1, is essential for EBV transformation of lymphocytes and is the only EBV gene product that has transforming ability in rodent fibroblasts [1]. The carboxyterminal portion of LMP1 interacts with cellular adaptor proteins that transduce signals from the tumor necrosis factor family of receptors [17]. These molecules, called TRAFs for tumor necrosis factor receptor associated factors, are activated by the receptor clustering that occurs after ligand binding. LMP1 apparently acts as a constitutively activated member of this receptor family. Two domains have been identified in the carboxyterminus of LMP1 both of which can activate NF κ B through interactions with TRAFs.

Activation of NF κ B contributes to the activation of expression of most of the cellular genes that are induced by LMP1. LMP1 activates NF κ B in both lymphocytes and epithelial cells, and many of the same cellular genes are induced in both cell types [18]. LMP1 induces expression of many important cellular genes that have profound effects on cellular growth, including the epidermal growth factor receptor, anti-apoptotic genes such as bcl2 and A20, B-cell activation markers including MHC class I, adhesion molecules such as ICAM1, and proteins involved in invasion and metastasis such as matrix metalloproteinase 9, vascular epidermal growth factor and hypoxia-inducible Factor 1 α (HIF 1 α). LMP1 is also intimately involved with induction of activation of Interferon Regulatory Factor 7 (IRF-7) [19,19a,19b].

LMP2 is also an integral membrane protein that has been shown to interfere with signal transduction from the activated immunoglobulin receptor [20]. LMP2 is phosphorylated on tyrosines by the cellular tyrosine kinases, *fyn* and *lyn*. LMP2 is thought to sequester these kinases and inhibit translocation of the B-cell receptor into lipid-rich rafts in the plasma membrane [21]. This transposition blocks B-cell activation and thus prevents activation of the viral replicative cycle. In epithelial cells, LMP2 inhibits differentiation and induces cell proliferation through activation of PI3 kinase and the serine/threonine kinase, Akt [22]. The block in epithelial cell differentiation may inhibit activation of EBV replication, which is thought to occur in differentiating epithelial cells. In epithelial cells, LMP2 activates Akt which phosphorylates and inactivates glycogen synthase kinase 3 β . The inactivation of GSK3 β by LMP2 results in increased cytoplasmic and nuclear β -catenin [23]. β -catenin-mediated signaling is an important pathway in carcinomas, and activated nuclear β -catenin has been detected in NPC.

β -catenin is also stabilized and becomes transcriptionally active in immortalized B-lymphocytes, which may contribute to the oncogenic properties of EBV-infected B-cells

devoid of the chromosomal translocations of BL. Failure of the β -catenin to be proteasomally degraded seems to be due to a novel specific deubiquitination mechanism [10,24].

Importantly, both LMP1 and LMP2 are expressed in many of the cancers associated with EBV. This expression may make possible treatments based on immunotherapy targeted towards these proteins or inhibition of essential signaling pathways that are activated by these proteins. Inhibition of NF κ B in EBV-infected lymphocytes with a constitutively active form of the I κ B inhibitor of NF κ B results in rapid death of the cells through apoptosis, indicating that NF κ B signaling is essential to growth transformation.

2.3. Cofactors

The endemic patterns of incidence suggest that both genetic and environmental factors likely contribute to the development of tumors such as BL and NPC. An important contributing factor in the development of BL is co-infection with malaria. Chronic malarial infection results in expanded germinal centers (GC), and BL lymphocytes express markers of GC lymphocytes. It is likely that the increased viral reactivation in combination with expanded GC increases the likelihood that EBV will infect a GC lymphocyte. EBV may also affect immunoglobulin gene rearrangement and contribute to the development of the translocations. Similarly, viral reactivation occurs prior to the development of NPC. Tumor-promoting compounds have also been identified in Chinese salted fish and herbal medications and in food products in other populations with elevated incidence of NPC. Recently, several studies have identified regions of loss of heterozygosity on chromosomes 3 and 9 with loss of expression of p16 and a new member of the *ras* family [25]. The loss of these genes is likely induced by environmental insults.

These potential genetic or environmental factors may contribute to the increased risk of developing a latent transforming infection in epithelial cells. In combination with expression of LMP1 or LMP2 the loss of these potential suppressor genes likely induces a rapid progression to malignancy.

2.4. Causation

The consistent detection of EBV episomes and viral proteins in all cells in most of the tumors associated with EBV indicates that EBV and viral expression are essential for the development of these cancers. However, the association of a ubiquitous virus with unique cancers indicates that EBV alone is not sufficient for cancer development. As immunosuppression clearly leads to lymphoma (but not BL) development, it is possible that HLA-dependent immune recognition is also a factor in controlling EBV-infected cells in the non-immunocompromised. A recent study has shown that the EBV strains found in NPC have changes in predicted HLA epitopes in LMP1 [26]. Thus strain variation, HLA

type, and environmental and genetic factors likely all contribute to the development of this cancer.

3. KSHV/HHV-8

3.1. Malignancies

Kaposi's sarcoma-associated herpesvirus (KSHV) has been linked to several malignancies in the human population: Kaposi's sarcoma (KS), primary effusion lymphomas (PELs) and multicentric Castleman's disease (MCD).

KS is a multifocal vascular tumor of mixed cellular composition manifested most often as a cutaneous lesion. KSHV is always detected in the spindle cells of the KS lesion, which are thought to be endothelial in origin. There are four major forms: (i) Classic KS is seen in men of Mediterranean and Eastern European descent. (ii) AIDS-associated KS is a highly aggressive malignancy. In HIV-infected individuals, the KS lesion is not restricted to the skin and often disseminates to the liver, spleen, gastrointestinal tract and lung. KS is the most frequently detected tumor in AIDS patients. (iii) Endemic KS, a third type, is prevalent in certain parts of Africa like Uganda, and affects mostly HIV-negative adults as well as children. Endemic KS is generally more aggressive than Classic KS. (iv) The fourth type is an iatrogenic form of KS that occurs in some recipients of organ transplants receiving immunosuppressive therapy. More than 95% of all KS lesions, regardless of type, contain KSHV viral DNA thus strongly linking KS to KSHV infection [27].

Primary effusion lymphomas (PEL) are malignant B cell lymphomas representing a specific subset of lymphomas that present as body cavity effusions. The term PEL designates a distinct clinicopathologic group of lymphomatous effusions [28]. Since one criterion for characterization as PEL is KSHV-positivity, 100% of PELs are KSHV-positive. PELs often contain EBV as well. PELs are observed in both HIV-positive and HIV-negative individuals with both types of PELs invariably containing KSHV viral DNA. Interestingly, a fraction of PELs present as solid tumor masses rather than effusions, with all of the lymphoma cells positive for KSHV DNA. Thus KSHV-associated PELs display a spectrum of morphology [29,30].

Multicentric Castleman's disease (MCD) is a B-cell lymphoproliferative disorder. There are two types, a hyaline vascular form, which presents as a solid mass, and a plasmablastic variant, which is associated with lymphadenopathy. MCD is sometimes referred to as multicentric angiofollicular hyperplasia and is characterized by vascular proliferation of the germinal centers of the lymph node. Nearly 100% of AIDS-associated MCD is positive for the presence of KSHV, while approximately 50% of non-AIDS associated MCD contains KSHV viral DNA. AIDS-associated MCD is usually accompanied by the development of KS in the affected individuals. MCD is a polyclonal tumor and is highly dependent on cytokines such as interleukin-6 (IL-6) [31].

The evidence linking KSHV to KS, PEL and MCD is very solid and has been confirmed by multiple laboratories. KSHV has also been linked to multiple myeloma, angiosarcomas, as well as malignant skin tumors in post-transplant patients such as Bowen's disease, squamous cell carcinomas, actinic keratosis and extramammary Paget's disease. However, these disease associations are controversial and not generally considered to be well established [29].

3.2. Oncogenic properties

KSHV is consistently associated with KS, PEL and MCD. It is believed that KSHV transforms cells through a paracrine mechanism since there is a plethora of evidence of high levels of cytokines and growth factors in lesions of KS and MCD. KSHV has been shown to infect but not immortalize CD19-positive B cells in vitro [32], perhaps due to technical difficulties with the cell culture system. In contrast, KSHV can immortalize primary bone marrow derived endothelial cells [33]; the virus induces cell proliferation, anchorage independence and survival of these cells. Interestingly, only a subset of the transformed endothelial cells contained viral DNA suggesting that the uninfected cells survived through a mechanism involving cytokines secreted by the infected cells [33]. These observations suggest that KSHV transformation is highly dependent on paracrine factors as well as the cellular microenvironment, a concept gaining favor in other tumor models as well.

Further, several of the KSHV viral gene products including viral G-protein coupled receptor (vGPCR) and K1 are transforming in vitro and in transgenic mice [31,34]. Since KSHV does not infect mice or rhesus macaques, animal model systems utilizing closely related simian rhadinoviruses are currently under development [35]. These simian rhadinoviruses have been shown to be associated with a B cell hyper-proliferative disorder resembling MCD, and retroperitoneal fibromatosis, which morphologically resembles KS [35].

3.3. Nature of the association

Methods for detecting KSHV viral infection run the gamut from assays identifying viral nucleic acids to techniques identifying viral proteins. Nucleic-acid-based methods include PCR technologies for different viral genes such as Orf73, Orf65 and viral DNA polymerase. In situ cytohybridization has also been used to detect viral DNA in KS specimens. Protein-based methods include immunohistochemistry of paraffin-embedded KS, MCD and PEL specimens with antibodies directed against KSHV latency associated nuclear antigen (LANA), viral IL-6 (vIL-6), and the Orf59 gene product. Techniques incorporating western blots, enzyme-linked immunosorbent assays (ELISA) and immunofluorescence assays (IFAs) have been utilized to measure the prevalence of KSHV in the human population. ELISAs containing recombinant LANA have been used

to measure antibody titers to the protein in patients and blood donors; this assay is more sensitive than LANA IFAs. Other ELISA-based assays include those against the lytic cycle KSHV K8.1 glycoprotein and the KSHV K12 antigen [29].

3.4. Mechanisms of oncogenesis

KSHV is a double-stranded DNA virus belonging to the γ sub-family of herpesviruses. Similar to other *gammaherpesvirinae*, KSHV establishes a life-long latent infection in the B-lymphocytes of its host. The viral genome is approximately 160 kb in size and codes for more than eighty ORFs. KSHV encodes a diverse array of genes involved in transformation, signaling, prevention of apoptosis, and immune evasion.

The KSHV K1 and vGPCR genes possess oncogenic potential. The K1 protein can transform rodent fibroblasts in vitro, and when injected into nude mice these cells induce multiple and disseminated tumors. Further, K1 can functionally substitute for the saimiri transformation protein (STP) of herpesvirus saimiri (HVS) in vitro and in vivo to induce lymphomas in common marmoset monkeys. Transgenic animals expressing K1 also develop sarcomas and lymphomas. K1 is capable of eliciting B cell signaling and proliferation through its immunoreceptor tyrosine-based activation motif (ITAM) and by blocking Fas-induced apoptosis of these cells [34]. Further, K1 can activate the NF κ B and phosphoinositol-3-kinase (PI3K) pathways. In endothelial cells, K1 has been shown to upregulate the expression and secretion of vascular growth factor (VEGF) and matrix metalloproteinase 9 (MMP-9) [36].

Similar to K1, the KSHV vGPCR protein is capable of transforming NIH 3T3 cells in vitro. vGPCR immortalizes primary endothelial cells, and transgenic mice expressing vGPCR develop angioproliferative KS-like lesions. vGPCR can activate the phospholipase C (PLC) and phosphatidylinositol 3-kinase (PI3K) pathways. Additionally, vGPCR expression in many cell types results in the upregulation of a multitude of cytokines and paracrine factors. Thus, this viral protein may contribute to KSHV-associated neoplasia by inducing and sustaining cell proliferation [31,34,37,38]. Aside from K1 and vGPCR, KSHV also encodes a viral interferon regulatory factor 1 (vIRF-1) and the Kaposin/K12 gene, both of which possess transforming potential in vitro [31,34]. To date, these are the only four viral gene products that have been demonstrated to possess transforming properties in vitro or in vivo. Thus these gene products are likely to contribute to KSHV-associated pathogenesis.

KSHV also encodes several viral homologs of cellular cytokines and chemokines. The viral interleukin-6 (vIL-6) protein of KSHV has mitogenic properties and supports B-cell proliferation. Further, anti-apoptotic genes encoded by KSHV including viral Bcl-2 (vBcl-2), viral FADD-like interleukin-1 converting enzyme inhibitory protein (vFLIP/Orf71), and viral Inhibitor of Apoptosis

(vIAP/K7) ensure survival of the virus by preventing apoptosis of the infected cells. Finally, KSHV also contains several immune-evasion genes. The KSHV K3 and K5 proteins downregulate MHC I expression, thereby inhibiting the presentation of viral antigens. The KSHV vIRFs and Orf45 inhibit the host interferon response, while the KSHV complement control protein homolog (CCPH) prevents complement-mediated lysis of infected cells (reviewed in [31,34]). Thus, the sum of the functions of these KSHV proteins ensures life-long viral persistence in the host and likely contributes to KSHV-associated pathogenesis.

3.5. Cofactors

As with other tumor viruses, HIV is a co-factor for development of malignancy since the prevalence of KS in AIDS patients is unusually high. Immunosuppressive therapy is also a co-factor for the iatrogenic form of KS. However, KSHV-associated KS can occur in healthy, HIV-negative individuals, such as those seen in Africa and the Mediterranean. Finally, since the classic form of KS is predominantly observed in elderly men of Mediterranean and East European descent, genetic and environmental co-factors may also play a role in the development of the disease.

3.6. Tumor modifiers

KSHV may participate both in tumor induction and tumor progression. KSHV contains a number of transforming viral genes, and hence the virus may contribute directly to the induction of the tumor phenotype through the deregulation of cell growth pathways. Many of the viral genes, like viral IL-6, K1 and vGPCR, can function to promote cell proliferation. On the other hand, since oncogenesis is generally a multi-step process, KSHV infection may be only one of the events that eventually lead to neoplasia. KSHV may also contribute to the proliferation of uninfected cells through a paracrine mechanism. Finally, similar to EBV, KSHV viral proteins can induce MMP-9, an invasion factor, as well as the angiogenic factors, VEGF and bFGF [34,36].

3.7. Causation

The strong molecular epidemiological link associating KSHV with KS, PEL and MCD suggests that KSHV is necessary for the development of these malignancies. However, it is not entirely clear whether KSHV alone is sufficient for development of these neoplasms, since co-factors such as HIV co-infection and immunosuppression often play a contributory role in the induction of disease. Another issue relates to the clonality of the KSHV-associated tumors. Although all PELs and most KS lesions are generally thought to be monoclonal in nature, some KS lesions are polyclonal, as are most MCD tumors. The inference that most KS lesions and all PELs are monoclonal expansions of a single KSHV-infected cell supports the hypothesis that

KSHV infection precedes tumor progression and that the virus is required for tumor induction. The polyclonality of the MCD lesion suggests that KSHV may help to enhance or promote tumor progression, perhaps through a paracrine mechanism. In conclusion, the epidemiological studies linking KSHV to disease, the ability of KSHV to transform endothelial cells, and the identification of transforming and mitogenic genes encoded by KSHV all support the notion that KSHV is a tumor virus with oncogenic properties.

4. HPV

Human papillomaviruses are consistently associated with cancer of the cervix. Virtually 100% of cervical cancers contain HPV (317-Fp 2264). However, only some types of HPV regularly have this relation. Of the approximately 130 HPV types so far distinguished, 30 are so-called anogenital types. A subset of these 30 types is considered “high risk” virus (types 16 and 18) based on the consistency of the association with cervical (and anal) cancer. HPV31 and 45 are also found in these cancers and together with 16 and 18 account for 80% of them. Some vulvar, vaginal and penile cancers are associated with HPV as well as several grades of cervical dysplasia. “Low risk” viruses are associated with benign lesions such as condyloma accuminata. More recently the virus has been associated with up to 20% of cancers of the oropharynx especially tonsillar. The latest emerging association is with basal cell and squamous-cell carcinomas of the skin. However, HPV DNA is also detected in normal skin so the last association is less firm. Finally HPV (especially HPV-5 and 8) causes skin cancer in the rare hereditary disease, epidermodysplasia verruciformis (EV).

HPV genes (E6 and E7) of high risk viruses have *oncogenic properties* including ability to immortalize the natural human target cells of the virus, squamous epithelial cells, and produce skin tumors in transgenic mice expressing these genes in basal epithelial cells. The nature of the association is both at the molecular level and epidemiologic. The viral genome persists as episomes in the basal cell layer of the uterine cervix, which is the source of episomes and virus replicated in the suprabasal cells. Replication of virus depends on stimulation of cellular DNA synthesis in these cells. In high grade cervical dysplasias which precede cancer, primarily E6 and E7 genes of high risk strains are expressed from integrated genome in the proliferating cells from the basal epithelium. Epidemiologically the most consistent association is between specific genotypes and cervical cancers. This selectivity is further evidence of a causal role of HPV in this cancer. Unlike EBV, HBV and HTLV1, which were highlighted by the endemicity of tumors from which the viruses were initially isolated, cervical cancer does not display endemicity although incidence varies considerably around the world.

The central *mechanism of oncogenesis* at the molecular level is disruption by HPV early genes of tumor suppressor

genes that result in dysregulation of cell growth and inhibition of apoptosis. E6 and E7 are overexpressed as a consequence of deletion of the E2 region in integrated HPV genomes; E2 ordinarily represses expression of E6 and E7 at the promoter level. E7 causes proteosomal degradation of pRb and related proteins so that cell growth is dysregulated, and E6 causes degradation of p53 with the consequence that the abnormally growing cells are spared from apoptosis. Degradation is brought about by an interesting mechanism: E6 binds to a ubiquitin ligase, E6-AP, forming a complex that binds p53 and leads to ubiquitination and proteosomal destruction of the protein [40]. In general E6 and E7 of high risk strains of HPV are more efficient at inactivating tumor suppressor proteins. In addition E6 and E7 may have inherent oncogenic properties. However, there is not a perfect correlation between oncogenic genotype and ability of E7 genes to transform cells and to disrupt pRb/E2F complexes. E6 of high risk strains also interacts with PDZ domains as do other oncogenic proteins of other tumor viruses. PDZ domains are located in tight junctions of epithelial cells and are believed to facilitate signal transduction. Finally, E6 operating through inactivation of p53 can immortalize some types of human epithelial cells as well as induce telomerase activity.

The E7 protein counteracts Rb function and allows cells to progress into S-phase. E7 binds the hypophosphorylated form of Rb and prevents it from binding to E2F and from repressing its ability to promote expression of genes required for cell DNA synthesis and thereby promotes cell-cycle progression by targeting Rb for proteosomal degradation [40]. E7 also inactivates INK4A, which is ordinarily upregulated when E2F is released, and in addition stimulates cyclins A and E and inactivates the cyclin-dependent kinase inhibitors, WAF1 and KIP1, as well as induces abnormal centriole synthesis and aneuploidy early in the oncogenic process. The ability of E6 and E7 to immortalize human cells is synergistic [41].

4.1. Cofactors

The genetic predisposition of patients with EV to skin cancer caused by HPV raises the possibility of another more subtle susceptibility to cervical cancer. There is evidence for a genetically determined risk for cervical cancer [41]. Cigarette smoking is associated with accumulation of a tobacco carcinogen in cervical mucus. Sunlight as well as a genetic defect are cofactors for skin carcinomas caused by HPV5 and 8 in patients with EV. Anal cancer associated with HPV is much more frequent in HIV-infected persons with immune deficiency, especially males. Chronic immunosuppression increases the risk of high grade cervical dysplasia and progression to cancer.

There are no reports that HPV can secondarily alter tumor cell behavior such as invasiveness or aggressivity of tumor cell growth. Genetic instability conferred by the initial HPV infection and accumulating acquired mutations are likely

explanations for the invasive character of late-stage cervical cancer.

Thus HPV is now considered the major causal factor in cervical cancer. Viral genes if not the virus itself have been shown to possess oncogenic properties, and integrated viral genomes or portions of them containing specifically the E6 and E7 genes are regularly present and expressed in the tumor tissue. Associations between the virus and the cancer were not established by sero-epidemiologic surveys unlike in the case of EBV, HBV and HTLV1. Perhaps the most cogent link to cancer is established by the molecular epidemiologic evidence arising from viral genotype analysis in cervical cancer. However, it is important to understand that attempts to link HPV with this cancer would have been obscured without recognition of the high risk genotypes. Simple epidemiologic surveys correlating the virus infection with the likelihood of cervical cancer might have failed because of the prevalence of infection with nononcogenic strains. Finally, the associations with oral and especially skin cancers are much less secure. In short HPV qualifies as an authentic human tumor virus.

5. Polyomaviruses

There are three primate polyomaviruses: JCV, BKV and SV40. There is evidence of a role in neoplasia for each [42,43].

5.1. Oncogenic properties

JCV, a human virus that infects the brain and the kidney, is able to transform cells in culture although not as efficiently as SV40. JCV can transform human fetal glial cells and primary hamster brain cells. JCV-transformed cells exhibit the phenotypic properties associated with transformation including growth in soft agar, serum-independence, morphology, and production of plasminogen activator [44]. The transforming ability of JCV is mostly limited to cells of neural origin, and this property maps to the viral regulatory region at the origin of replication (however, B-cell neoplasms and colon carcinomas have also been linked to JCV). Specifically the JCV early promoter (JCV_E) directs neural cell-specific transcriptional regulation of the large T-Antigen (T-Ag), which is believed to be a major determinant of JCV tropism.

JCV has high oncogenicity in animals. The virus induces multiple types of brain tumors when injected into the brains of newborn Golden Syrian hamsters. JCV is the only polyomavirus that induces solid tumors in nonhuman primates: JCV caused the development of astrocytomas, glioblastomas and neuroblastomas in owl and squirrel monkeys 16–24 months after inoculation intracerebrally, subcutaneously or intravenously; tissues revealed T-Ag expression, but no capsid protein or infectious virus indicating that monkey cells in vivo are non-permissive for JCV replication [43,44]. Transgenic mice expressing JCV T-Ag can develop demyelinat-

ing disease, adrenal neuroblastomas and neural tumors; the latter are of primitive neuroectodermal origin.

BKV is a human virus that infects lymphocytes and cells in the urogenital tract in vivo. Complete BKV genomic DNA or fragments that include the early region can transform human embryonic fibroblasts and cells cultured from the kidney or brain of mouse, rat, hamster, rabbit and monkey [45]. The efficiency of transformation is variable and depends on genetic features of the viral strain. The efficiency of BKV T-Ag-induced transformation of hamster cells is improved by the co-introduction of the human *c-Harvey-ras* oncogene. Transformation of human embryonic kidney (HEK) cells is inefficient and often abortive. However, a fully transformed phenotype can be achieved in co-operation with other oncogenes such as adenovirus E1A, *c-rasA* or *c-myc*. BK virus is highly oncogenic in young or newborn mice, rats and hamsters. BKV is only weakly oncogenic when injected subcutaneously, but gives a high incidence of tumors when injected intracerebrally or intravenously. The types of tumors induced include ependymoma, neuroblastoma, pineal gland tumors, pancreatic islet cell tumors, fibrosarcoma and osteosarcoma, which suggests a tropism of the virus for certain cell types [45]. Transgenic mice containing the BKV early region evince tissue-specific production of tumors including hepatocellular carcinomas, renal tumors, thymomas and thymic lymphomas.

SV40 in its natural host, rhesus monkeys, replicates without producing lesions. However, it is a potent transforming agent for cell cultures from species that are nonpermissive for viral replication including hamster, mouse, rat, bovine and guinea pig [46]. SV40 replicates in human diploid fibroblasts and transforms them; the transformed cells can produce tumors in hamsters. Most early studies on SV40 transformation were with established rodent cell lines such as Balb-3T3 cells, which SV40 can enter and express the T-Ag. The virus does not replicate or express late genes in these cells, which however, undergo numerous cellular and biochemical changes after SV40 transformation [47].

SV40 is also able to induce tumors in laboratory animals. Soon after its discovery, the oncogenic potential of SV40 was demonstrated by induction of sarcomas in newborn hamsters. Tumorigenesis by SV40 depends on the route of injection. In hamsters injected intravenously leukemia, lymphoma, and osteosarcoma develop, whereas in hamsters injected intracranially choroid plexus tumors and ependymomas, and in hamsters injected intrapleurally, mesotheliomas result. In transgenic mice expressing the SV40 T-Ag characteristic tumors localized to the choroid plexus develop.

Although rodent cells do not support SV40 replication, and monkey cells are permissive for SV40, human cells are unique in that they support transformation and low level SV40 (semipermissive) replication. SV40-transformed human fibroblasts exhibit many of the features of transformed rodent cells. After they have undergone “crisis” these cells emerge as rapidly growing transformed cells with integrated viral genomes, but now without viral replication.

5.2. Major malignancies

JCV is associated with brain tumors found in patients with or without progressive multifocal leukoencephalopathy (PML). Of 85 tumors of glial origin 57–83% of tumors were positive for JCV DNA and T-Ag expression depending on tumor type. JCV T-Ag DNA sequences were detected in 11 of 23 pediatric medulloblastomas. Many other studies that have employed PCR-mediated DNA amplification and/or immunohistochemistry of various brain samples provide support for an association of JCV with a variety of tumors of the central nervous system. Other associations include colon cancer and CNS lymphoma [44].

BKV DNA was detected by direct Southern blot hybridization in 19 of 74 brain tumors and in four of nine pancreatic islet tumors. A role for BKV in brain tumors is less well established than for JCV. Investigators have also reported detection of BKV DNA in human tumors by PCR and Southern blotting, including Kaposi's sarcoma, brain and urinary tract tumors [45].

The role of SV40 in the etiology of human cancers is contentious. The first report of SV40 in human cancer was 1974 when the virus and T-Ag were detected in metastases from a malignant melanoma, but there have been no subsequent reports of this association. The numerous reports since then of SV40 in other human tumors have been reviewed recently [46]. SV40 has come to be closely associated with mesothelioma, a rare but aggressive cancer of mesothelial cells. Exposure to asbestos is the major factor identified in the development of mesothelioma. The frequent but not invariable association of SV40 with mesothelioma suggests that the virus can serve as a nonessential co-factor in the development of the malignancy. In cell culture human mesothelial cells are non-permissive for SV40 replication, but are unusually susceptible to transformation in response to infection, and asbestos could serve as a synergistic factor for transformation in these experiments [48]. Human mesothelial cells are reported to be permissive for BKV replication with resulting cytolysis and refractory to infection by JCV [49]. These differences may explain why SV40 rather than the more ubiquitous human polyomaviruses, BKV and JCV, is associated with mesothelioma. SV40 is also associated with some brain tumors [46], osteosarcomas and non-Hodgkin's lymphoma.

Recently more convincing evidence for the association of SV40 with human cancer has emerged that is free from the technical caveats of earlier studies. SV40 DNA integrated into chromosomal DNA was detected by direct Southern blot hybridization in about half of a group of PCR-positive osteosarcoma tumors. Microdissection of frozen mesothelioma tissue revealed the presence of SV40 DNA sequences in the tumor cells, but not in adjacent non-tumorous tissues. Indirect evidence for a link between SV40 and cancer comes from the observations that mesotheliomas from Finland and Turkey (where poliovaccine for some years a source of exposure to SV40 was not administered) are negative for SV40.

Direct evidence of the virus was provided in one report of the isolation of infectious SV40 from a sample of choroid plexus tumor tissue after lipofection of tumor DNA into monkey kidney cells [50]. An adenoviral vector expressing antisense transcript to SV40 early region has been reported to induce growth arrest and apoptosis in SV40-positive human mesothelioma cells growing in culture [51]. In a recent report a meningioma in a scientist at risk of laboratory exposure to SV40 contained SV40 DNA with sequence identical to the laboratory source, as did cerebrospinal fluid. Thus the evidence for the association of SV40 with human cancer continues to strengthen and should no longer be dismissed on the grounds of technical issues regarding PCR and antibody specificity.

5.3. Nature of the association

The association of polyomaviruses with tumors has typically been based on detection of viral sequences by PCR and of viral T-antigen by immunohistochemistry. The findings have been criticized on two grounds: PCR may amplify viral genomes present at less than 1 copy/cell, and antibodies to T-antigen have cross-reactivity among viral species. For JCV, the original evidence for association was based on PCR studies and detection of T-antigen. Further analyses have verified the findings by use of laser-capture microdissection to demonstrate that viral DNA and protein are confined to malignant cells. Careful PCR controls allow the conclusion that JCV (but not BKV or SV40) DNA sequences are present.

For BKV, detection has been by PCR-based Southern blot hybridization and immunohistochemistry. The role of BKV in human malignancy is uncertain and needs verification by, for example, laser-capture microdissection, to verify the presence and specificity of BKV DNA in tumor cells and expression of viral proteins.

For SV40, viral DNA sequences from human tumors have been isolated and compared with those of laboratory strains and monkey isolates with the conclusion that the SV40 DNA found in human tumors differs from the sequence of the reference strain SV40-776 and the other laboratory strains (SV40-B2 and VA45-54). These findings argue against contamination of samples in the laboratory as a source of the SV40 DNA. The promoter region of SV40 isolated directly from monkeys or from human tumors is distinguished by a single copy of a 72-bp sequence that in laboratory strains is duplicated. (This duplication may represent an adaptation to growth in tissue culture.) There is no compelling evidence of human-specific strains of SV40 or for tumor type-specific associations.

5.4. Mechanisms of oncogenesis

Polyomavirus genomes do not encode replication proteins and so to replicate they must drive cells into S-phase when host DNA replication proteins are produced. A major

mechanism by which this is achieved is through the action of the early proteins, large T-Ag and small t-Ag. T-Ag interferes with two tumor suppressor proteins, pRb and p53, that regulate cell cycle progression. The resulting aberrant stimulation of the cell cycle is a driving force for oncogenic transformation. T-Ag can modulate other signaling proteins besides pRb and p53. T-Ag has been shown to bind directly to insulin receptor substrate 1 (IRS-1) and cause it to be translocated to the nucleus where it regulates Rad51 and homologous recombination-directed DNA repair. T-Ag binds directly to β -catenin causing it to translocate to the nucleus where it enhances expression of genes such as *c-myc* and cyclin D1. As well as being able to transform cells by virtue of interacting with cellular signaling proteins, the T antigens of primate polyomaviruses also have mutagenic effects on cellular DNA. SV40 small t-Ag has a mitogenic role in the transformation of cells by binding and inhibiting protein phosphatase 2A. Finally, viral Agnoprotein binds directly to p53. Agnoprotein dysregulates cell cycle progression and DNA repair when expressed in the absence of other viral proteins.

The major *cofactor* for BKV and JCV infection is immune suppression of renal transplant recipients and in AIDS patients, respectively. For SV40 it is exposure to asbestos. However, asbestos may be considered to be the primary carcinogen for mesothelioma and SV40 the cofactor.

5.5. Tumor modifiers

It is difficult to ascertain whether the association of a polyomavirus with human tumors is causal or incidental. The virus might initiate tumorigenic events, or the tumors might offer a microenvironment that favors viral replication. However, polyomaviruses have a very potent oncogene, T-Ag, and are highly tumorigenic in cell cultures and animals, so it would be surprising if they were associated with tumors merely by chance. Since there is expression of T-Ag in some tumors and a consequence seems likely, perhaps the virus provides a secondary “hit” in the process of malignant progression for some cancers rather than an initial causative role.

5.6. Causation

All three of these polyomaviruses (JCV, BKV and SV40) have a possible role in the etiology of human malignancies. Studies on the molecular biology of these viruses and their effects on cultured cells and in animal models form a coherent picture with what is known about the viruses and their associations in a clinical setting. However, it remains to be proven stringently that they have a causal role in human neoplasia. Problems include the following: the viruses are ubiquitous in nature, but the associated cancers are rare. The incubation period between infection and appearance of cancer is long. The initial viral infection is usually subclinical making it difficult to establish when it oc-

curred. The human viruses JCV and BKV do not productively infect animal models. Environmental co-factors (e.g., co-carcinogens) or host factors (e.g., immune status) modulate pathogenesis. These considerations make it difficult to apply Koch’s postulates to the polyomaviruses. Zur Hausen has proposed alternative criteria for defining a causal role for an infection in cancer [52]. There is good evidence that the JCV, BKV and SV40 polyomaviruses fulfill criteria for oncogenicity. These viruses transform cells in culture and produce tumors in inoculated animals or transgenic mice with patterns consistent with their putative role in human tumorigenesis. The presence of viral DNA and viral gene expression in a subset of several human tumors has been established. However, appropriate epidemiologic studies that might confirm the links between these viruses and associated diseases have not been done. JCV and BKV viruses infect a very high percentage of most human populations, which poses a challenge for standard epidemiological studies. For SV40, seroepidemiologic studies would permit an assessment of SV40 prevalence in the population and may allow a correlation between SV40 seropositivity with disease. Since individuals who were never exposed to contaminated polio vaccine appear to have been infected with SV40, this virus may have established itself as a human pathogen [53].

6. HBV and HCV

Hepatocellular carcinoma (HCC) is among the most common cancers in the world. Most HCC cases are due to hepatitis B virus (HBV), but the number of hepatitis C virus (HCV)-associated cases is still growing in many countries. The role of HBV as a major etiological agent of HCC has been firmly established; the lifetime risk of developing HCC is estimated to be 10- to 25-fold greater for chronic HBV carriers compared with noninfected populations, placing HBV in the first rank among known human carcinogens [54–56]. Another link of HBV to liver cancer comes from animal hepadnaviruses that produce chronic infections of the liver and HCC [57]. The epidemiology of HCV is less established, but estimates of the lifetime risk of HCC in patients chronically infected with HCV are between 5 and 20% [56,58]. Viral hepatitis B and C account for about 1 million deaths per year, including cirrhosis and HCC-related deaths. The two hepatotropic viruses are quite distinct in genome structure and biological properties, but there are striking similarities between the pathogenesis of chronic HBV and HCV infections. Neither virus can transform cells in culture. However, HBV produces HCC in transgenic mice.

6.1. Epidemiology of HBV and HCC

Epidemiological studies have clearly demonstrated that chronic HBV infection is a major etiological factor in the development of primary liver cancer.

1. The incidence of HCC and the prevalence of HBV serological markers follow the same general geographical pattern of distribution. HCC is common in regions where HBV is endemic, but is much less common than other types of cancer in regions where HBV infection is uncommon [59].
2. In patients with HCC serological evidence of HBV infection is detected in about 70% in Africa and in more than 90% in mainland China, as compared with 10–20% of the total population in the same areas. In regions of low endemicity such as Western countries, chronic HBV carriers still represent 15–20% of HCC patients [60].
3. In prospective studies, a marked increased risk (10- to 100-fold) of HCC has been demonstrated for hepatitis B surface antigen (HBsAg) carriers compared with noncarriers in different ethnic or social groups [56].

6.2. Epidemiology of HCV and HCC

Less than 20% of HCC cases in low incidence regions such as North America, Europe and Japan can be attributed to HBV despite the increasing incidence of HCC. HCV, a human RNA virus related to Flaviviridae and Pestiviridae, is increasingly implicated in HCC in these regions (as well as in countries where HBV infection is highly endemic, e.g., China). More than 150 million persons in the world are estimated to be infected with HCV [58], 70–80% become chronic carriers, and most have mild disease with slow progression. Use of HCV markers has produced epidemiological evidence of association between HCV, cirrhosis, and HCC [61]. HCV genotype affects the risk of developing HCC especially HCV type 1 [62]. Infection by genotype 1 has emerged as an independent risk factor for developing HCC in HCV-infected cirrhotic patient, and HCV 1b is the most prevalent type in HCC without cirrhosis.

6.3. Mechanisms of oncogenesis

The exponential relation between HCC incidence and age indicates that, as in other human cancers, multiple steps are required, probably involving independent genetic lesions. The long latency period between HBV or HCV infection and HCC may signify indirect action of these viruses, perhaps through long-term toxic effects of the immune response against infected hepatocytes that trigger chronic inflammation, continuous cell death, and consequent cell proliferation [63], and potentiate the action of exogenous carcinogenic factors, such as aflatoxins and alcohol.

A role for viral proteins in HCC oncogenesis might be sensitisation of liver cells to mutagens. In transgenic mouse models unregulated expression of the HBV X and S proteins are associated with hepatocarcinogenesis [64,65]. The HBx protein behaves as a promiscuous transactivator of cellular genes such as oncogenes, growth factors and cytokines, binds and inactivates p53 and interacts with the DNA re-

pair protein DDB1, which may affect repair functions and allow the accumulation of genetic changes. HBx activates calcium-dependent signalling events that might account for induction of apoptosis [66]. Rearrangement of integrated HBV sequences in HCC may lead to abnormal expression of the S gene protein. Specific activation of c-Raf-1/Erk2 signaling by the truncated preS2S protein results in an increased proliferation rate of hepatocytes.

Transgenic mice carrying the complete HCV genome or the HCV core develop liver steatosis and have high rates of HCC [67,68] perhaps through interaction with cellular proteins required for the control of cell growth. The HCV core protein induces oxidative stress in transgenic mice. The NS5A protein of HCV can sequester p53 in the cytoplasm, downregulate p21, activate STAT3, and inhibit TNF α -mediated apoptosis as well as alter intracellular calcium levels and induce oxidative stress. Thus, persistent stimulation of cellular stress responses by accumulation of viral proteins within hepatocytes may predispose the cell to genetic alterations and play an important role in HCC. Whether HCC once established becomes independent of the expression of viral genes is unknown.

Hepadnavirus replication does not require viral DNA integration in host chromosomes, which, however, is detected in ~80% of HCC [69]. HBV DNA integration occurs at early stages in acute infection. Because of the absence of complete genomes in virtually all HBV inserts, these sequences cannot serve as a template for viral replication. Highly preferred integration sites have been mapped in the HBV genome within the “cohesive ends” region. Integration can take place at multiple sites on various chromosomes [69]. Evidence for direct insertional mutagenesis was first provided in two independent HCCs, with retinoic acid receptor β (RAR β) and cyclin A as target genes. HBV frequently targets cellular genes involved in cell signaling, some of which may represent preferential target sites for viral integration: 15 of 22 cellular genes targeted by HBV were key regulators of cell proliferation and viability [70] including recurrent HBV DNA integration into the hTERT gene encoding the catalytic subunit of telomerase. Thus, the sites of oncogenic viral integration are nonrandom, and genes at those sites may provide a growth advantage to a clonal cell population in which additional mutations accumulate.

The woodchuck hepatitis virus (WHV) acts mainly as an insertional mutagen, activating *myc* family genes (*c-myc* and *N-myc*) in woodchuck HCC [71] and producing insertional events with high frequency (>90%).

6.4. Cofactors

Independent or cooperative factors have been implicated, such as prolonged exposure to dietary aflatoxins, notably aflatoxin B1 (AFB1) in South Africa and in southern provinces of Mainland China [72]. Diabetes mellitus (DM) has been reported to increase the risk of HCC in the presence of HCV, HBV or alcoholic cirrhosis [73].

Despite striking similarities between the pathogenesis of chronic HBV and HCV infections, large studies of genetic alterations in HCC have outlined significant differences between tumors associated with different etiologies [74]. HBV DNA integration and the HBx regulatory protein might promote genetic instability. HBV-related tumors harbor a high rate of p53 mutations and rare β -catenin mutations, whereas the inverse situation is found for HCV-related HCCs [74]. Moreover, genome-wide studies of gene expression in HCC and in chronic hepatitis by microarray screening have revealed distinct patterns in HBV- and HCV-related tumors. These observations suggest that HBV and HCV might play different oncogenic roles.

7. *Helicobacter pylori*

Once established in a host, *H. pylori* colonizes the stomach for decades, most often for the individual's entire lifespan, unless removed by antimicrobial treatment [75]. *Helicobacter pylori* has colonized the human stomach since time immemorial [76]. Colonized hosts have an extensive and complex response to the organism that enhances risk for adenocarcinoma of the (non-cardia) stomach [77], as well as for gastric non-Hodgkin's (MALT) lymphoma.

7.1. Oncogenic properties

Helicobacter pylori colonization evokes tissue responses involving epithelial, lymphoid, neuroendocrine, and phagocytic cell populations in the gastric mucosa [76]. Co-incubation of *H. pylori* with cells in tissue culture induces a substantial change in host cell signal transduction, with pleomorphic phenotypes, which parallel in vivo observations. Similarly, in the gastric mucosal inflammatory infiltrate are B and T cells that are directly or indirectly driven by *H. pylori* antigens. The epithelial cell and lymphoid responses interact, and the phagocytic and neuroendocrine cells may have local and distant effects, respectively. It is the nature of these persistent interactions with the host, affecting cell cycle properties, among others, that determines cancer risk.

7.2. Major malignancies with which *H. pylori* is associated

The stomach may be divided into two anatomical compartments related to *H. pylori*-associated cancer risk: the cardia, and the remaining major part of the stomach (non-cardia). It is now clear, from a variety of sources of information, that *H. pylori* colonization is associated with increased risk of non-cardia cancer, whether of the intestinal or diffuse histological type. The data for cardia cancers are less consistent, in part because of an issue of classification. *Helicobacter pylori* colonization is inversely associated with esophageal adenocarcinomas, and the classification of "cardia" cancers may in fact represent a mixture of

adenocarcinomas arising in the esophagus, cardia, and gastric fundus (non-cardia) [77]. *Helicobacter pylori* also is strongly associated with gastric MALT lymphomas.

7.3. Nature of the association

For gastric adenocarcinoma, the association with *H. pylori* is based on serological studies, observational studies in individuals with single (removed) cancers, treatment studies, and animal models. In 1994, IARC gave the association of *H. pylori* and gastric adenocarcinoma a class I designation (strong evidence of association), and in the last decade the data have become increasingly strong, specific, and consistent [78]. An important confounding factor is that in the course of development of gastric adenocarcinoma (intestinal type) most, if not all, individuals develop atrophic gastritis. *Helicobacter pylori* colonization becomes markedly reduced or eliminated. Thus, prospective studies in which the interval between ascertainment of *H. pylori* status and clinical development of gastric cancer is greater than 10 years have the strongest associations [78]. In such circumstances, the odds ratios range from about 5 to 12 for the association of *H. pylori* and non-cardia gastric adenocarcinoma. The presence of replicating *H. pylori* appears necessary for the development of the pre-malignant lesions, but as discussed above, may not be present at the time of cancer diagnosis.

For gastric MALT lymphoma, the presence of replicating *H. pylori* is almost universally present. Treatment studies, in which elimination of *H. pylori* changes the natural history of the tumor, leading to "cure," indicates the importance of *H. pylori* persistence for these malignancies.

7.4. Mechanisms of oncogenesis

Persistent bacterial colonization appears critical for the development of atrophy and intestinal metaplasia which lead to malignancy. As part of persistence, there is pro-inflammatory activity and changes in epithelial cell proliferation and apoptosis. Essentially all adenocarcinomas occur in persons with *cag*⁺ *H. pylori* strains. These strains possess a 35–40 kb chromosomal "pathogenicity" island that is required for injection of the *H. pylori* CagA protein into epithelial cells, where it undergoes tyrosine phosphorylation and changes epithelial cell signal transduction pathways [75,77]. Another highly interactive molecule is VacA, which suppresses T-cell responses. This local immunosuppression also may affect oncogenesis, as the strains of particular *vacA* genotypes that have high VacA activity are associated with enhanced cancer risk. Thus, the pro-inflammatory, immunosuppressant, pro-proliferative, and anti-apoptotic effects of *H. pylori* colonization each may contribute to the enhanced cancer risk.

The gastric MALT lymphomas are antigen-driven. These are B-cell lymphomas, and the presence of the organism and its associated inflammatory response appear necessary for the active proliferation of the malignant clone(s).

When host pro-inflammatory cytokine genotypes have been combined with *H. pylori* genotypes, large risk differentials have been uncovered. Individuals colonized with *cag*⁺ strains of *vacA* S1 genotype, and who have particular alleles in the IL-1 β or TNF α locus, have from 20- to 80-fold increased gastric cancer rates compared with persons of the low cancer genotypes and *cag*⁻ *vacA* S2 strains [81]. Improving ability to stratify risk based on host, bacterial, and interaction co-factors suggests it will soon be possible to define persons at the highest gastric cancer risk.

For gastric MALT lymphoma, the data that eradication of *H. pylori* with anti-microbial agents markedly changes the natural history of the malignancy provides important confirmation of its pathogenetic role. However, whether all of the histologic processes, termed MALT lymphoma, are in fact true malignancies, or whether some may reflect benign hyperplasia (monoclonal or polyclonal) remain to be determined.

7.5. Cofactors

Host genotype appears to affect *H. pylori* gastric cancer risk. Individuals with particular polymorphisms in the genetic loci that regulate the pro-inflammatory cytokines IL-1 β , TNF α , or that affect IL-1 RA, or IL-10, have enhanced gastric cancer risk [77–79]. The cytokine milieu affects gastric acid secretion as well as inflammation-related epithelial cell events. Events in childhood when *H. pylori* is acquired probably also play a role in the pathogenesis of the inflammatory process. Individuals born of large sibships and/or at later birth order are at increased gastric cancer risk [80].

7.6. Tumor modifier

None is known for adenocarcinoma, but *H. pylori* presence is clearly a modifier for MALT lymphoma.

7.7. Causation

In summary, a wide body of data based on epidemiologic, clinical, treatment trials, and experimental animal models indicates that the presence of *H. pylori* is a strong risk factor for adenocarcinoma of the (non-cardia) stomach. For particular combinations of strains and hosts, the risk relation parallels that for smoking and lung cancer.

For gastric MALT lymphoma, there also is a strong relation, but more work is needed to determine the boundary between benign and malignant disease.

8. HTLV-1

HTLV-1 is the first and still the only human retrovirus discovered in the context of malignancy, namely, certain acute T-cell leukemias (ATL) that are endemic in southern Japan

where HTLV-1 causes ATL in 3–5% of infected persons over their life-time [82]. Curiously, unlike the many animal retroviruses that cause cancers in animals and gave rise to the discovery of oncogenes and the concept of protooncogenes, HTLV-1 does not contain a classical oncogene. However, the virus can induce expression of cellular protooncogenes. In nonendemic regions including the USA, England, and in the Caribbean Islands, parts of South America and Africa, the virus is also associated with ATL as well as some T-cell lymphomas and forms of mycosis fungoides. HTLV-1 also causes a progressive myelopathy (HAM) in 1–5% of infected persons. World-wide 10–20 million people are estimated to be infected with the virus. A second retrovirus, HTLV-2, was isolated from a case of hairy-cell leukemia, but the virus has remained an agent without an established disease association.

8.1. Oncogenic properties

The virus is T-cell tropic, does not produce an obvious cytopathic effect, but is found in transformed CD-4-positive T cells. HTLV-1 can immortalize such cells in culture. HTLV-1 is also found in CD8+ T cells. HTLV-1 infects B and T lymphocytes, dendritic cells, fibroblasts and rodent cells. One to five percent of mononuclear cells contain integrated proviral DNA in peripheral blood in asymptomatic carriers.

8.2. Mechanism of oncogenesis

After infection Reverse Transcriptase (RT) in the virion using genomic RNA as template, synthesizes proviral DNA that is then integrated into the host cell genome by virally encoded integrase. Replication of virus is directed from these integrated viral genomes. The U3 region of the 5' LTR serves as the viral promoter and is instrumental in determining whether an infected cell is permissive for viral replication. Typical retroviral genes are encoded by the genome (*gag*, *pro*, *pol*, *env* and *IN*), but in addition there are six functional proteins encoded within the *px* region of the genome that are unique. HTLV-1 proviral DNA integrates in a common chromosomal site in all ATL cells in a given patient, producing a state of "clonal integration" [82]. However, the integration site is not unique but differs in different cases of ATL, and it does not produce insertional mutagenesis. Unlike many other retroviruses, but like bovine leukemia virus, HTLV-1 does not contain an oncogene derived from a cellular protooncogene. Rather the *trans*-acting factor *tax* encoded within the *px* region is essential for cellular transformation and induces and interacts with specific sets of cellular genes. *Tax* binds to factors that regulate these genes, which are important for disease pathogenesis by the virus. *Tax* activates the IL-2 receptor and several cytokines involved in T-cell growth as well as other genes, in part by destabilizing I κ B and activating NF κ B. *Tax* also dysregulates cellular gene expression through CREB/CRE. *Tax* can induce Bcl-XL and resistance to apoptosis. *Tax* interferes with the DNA

polymerase α component of DNA repair mechanisms and inactivates p16 INK4A, an inhibitor of cyclin-dependent kinases 4–6 [82]. Finally by causing mislocation of hsMAD1 and hsMAD2, Tax can produce loss of mitotic checkpoint.

The viral genome persists in cells as a DNA copy or proviral genome in CD4⁺ T cells. HTLV1 infection also leads to chromosomal instability caused by tax. The virus itself, in contrast to HIV, is genetically stable because the HTLV1 proviral genomes are replicated in their cellular context by cellular polymerase α , not by reverse transcriptase, which is error-prone, and is used for replication of virus.

8.3. Epidemiology

The epidemiology of HTLV1 infection was crucial in establishing the virus as the cause of ATL. It is estimated that 20 million people are infected with the virus world-wide, most concentrated in regions of endemic infection. In Japan over a life time the risk of ATL in male carriers of HTLV1 is 6.6 and 2.1% in women. The virus is transmitted from mother to child via breast milk or transplacentally and by sexual and intravenous routes – all by passage of HTLV1-infected T cells, not free virus, which probably accounts for its low levels of transmission to contacts. ATL occurs in infected persons but only after an extraordinarily long latent period ~60 years. During this interval acquired genetic and epigenetic changes are thought to come together in a relatively few infected persons to promote disease expression by the virus, presumably also in the context of genetic susceptibility factors.

8.4. Cofactors

Certain HLA alleles increase the risk of ATL. Identification of specific viral strains that increase the risk of ATL has been unsuccessful. The route of exposure may also determine outcome. Mucosal exposure may lead to an impaired immune response that may affect pathogenesis of the disease and further leukemogenesis.

8.5. Causation

HTLV1 is considered the sole causal agent for ATL whether it occurs in endemic areas or in sporadic cases. The viral genome persists in a proviral DNA form for life, for the most part asymptotically and without replication of virus, and therefore transmissibility which occurs through transfer of latently infected CD4-infected T cells is relatively restricted (even within families), and disease occurs in only a fraction of infected persons. The virus is able to transform target T cells in vitro, but there is no animal model of leukemogenesis. Sero-epidemiologic studies initially showed that ATL occurs only in infected persons, as indicated by presence of HTLV1 antibodies and subsequently underscored by demonstration of HTLV1-infected CD4 T cells in subjects. Expression of the tax viral gene,

a transactivator of cellular proliferative genes, is central to the mechanism at least of initiation of ATL. Certain haplotypes predispose to likelihood of ATL in infected persons. The virus also causes HTLV1-associated myelopathy (HAM). HTLV1 fulfills all criteria for a human tumor virus and indeed may be considered the first virus so established through its consistent association with ATL, its oncogenic properties and its epidemiology.

9. Discussion

Best established as human oncogenic agents are HPV (cervical cancer), EBV (B-cell lymphoproliferative diseases), KSHV (Kaposi's sarcoma and primary effusion lymphoma PEL), HTLV1 (T-cell leukemia), HCV and HBV (hepatocellular carcinoma) and *H. pylori* (gastric cancer). However, the criteria accepted as establishing causality differ in degree of completeness for the various malignancies. For HPV and cervical cancer, although the epidemiologic evidence was mustered later than the molecular, all criteria are met. For EBV and B-cell lymphoproliferative disease the case rests mainly on consistency of association in vivo, the immortalizing properties of EBV when it infects B cells in vitro and an understanding of the importance of immune deficiency in the disease, not on epidemiologic studies. In the case of NPC in which the universal presence of latent viral genome makes for a compelling association, and the molecular and epidemiologic evidence are impressive, there is still hesitation in calling the virus the etiologic agent, mainly because the case is obscured by the evident need for cofactors (most likely environmental and genetic), at least in the endemic regions, that have not been decisively identified. Most accept that KSHV is the primary cause of KS and PEL, based on both molecular and epidemiologic evidence. Similarly the consistent finding of HTLV1 in leukemic cells in regions endemic for ATL seemed to be enough to establish a causal relation between virus and disease even before demonstration of transforming ability of the virus and despite lack of animal models; in this case discovery of the agent and compelling seroepidemiology were sufficient. Although HTLV1 antibodies were found in many normal persons especially in endemic areas or affected families, they were detected in all cases of ATL and became a *sine qua non* for the diagnosis. For HCV seroepidemiology has provided the leading evidence for causality of HCC along with demonstration of the role of virus infection not in directly transforming cells, but in causing tissue injury leading to malignancy, uncovered later. HBV provides the classic example of causality established essentially by a seroepidemiologic study based on correlation of the prevalence of a viral antigen in the blood and high risk of HCC versus low risk in persons without the antigen. Helicobacter as principal cause of gastric cancer was established both by the consistent detection of the carcinogenic strain (*cag*⁺) of the organism in association with the tumor or pre-malignant tissue, the recognition of a

Table 1
Oncogenic infectious agents, their properties and malignancies caused or associated with them

Infectious Agent	Transforms Human Target Cells	Tumors in Animal Model	Present in Tumor Tissue	Integrated or Episomal	Cofactors	Epidemiology	Malignancy	Causative
HPV	+	+	100%	+	genetic, immunodef.	+	cervical cancer	yes
BKV	+	+	20%	+	?	ND	brain tumors	?
JCV	+	+	50–80%	+	?	ND	gliomas, medulloblastoma	?
SV40	+	+	+	+	asbestos*	ND	mesothelioma	?
SV40	+	+	?	?	?	ND	brain tumors	?
EBV	+	+	100%	+	genetic, environmental	+	NPC	probable
EBV	+	+	100%	+	immunodef.	ND	lymphoproliferative dis.	yes
EBV	+	–	98% (endemic regions)	+	malaria	+	Burkitt's lymphoma	no **
EBV	+	–	40–50%	+	?	+	Hodgkins disease	no
EBV	+	–	15%	+	?	ND	gastric carcinoma	no
KSHV	+	+***	>95%	+	immunodef., genetic	+	Kaposi's sarcoma	yes
KSHV	–	–	100%	+	immunodef.	ND	primary effusion lymphoma	yes
HTLV1	+	?	100	+	genetic	+	T-cell leukemia	yes
HCV	–	–	±	–	aflatoxins	+ ?	hepatocellular carcinoma	yes
HBV	–	+	100%	+	aflatoxins	+	hepatocellular carcinoma	yes
Helicobacter	–	–	±	–	genetic	+	gastric cancer	yes
Adenovirus	+	+	0	+	NA	–	none	NA

*Asbestos may be primary etiological agent, and SV40 a cofactor.

**EBV a cofactor.

***Simian rhadinovirus.

ND - Not Done.

NA - Not Applicable.

gradient of colonization of the stomach that correlated with distribution of the tumor, and later by inferring that antibiotic treatment reduced the risk of development of the cancer.

Thus it is clear that in deciding on causality use of the generally applied criteria is selective, and the criteria may be weighted differently.

Equally instructive are the malignancies in which causality is not considered to be established. Despite the fact that EBV was discovered in BL and linked tightly to the malignancy not only by seroepidemiologic studies in endemic regions, but also by the earliest examples of molecular hybridization analyses for viral genomes in BL tissue applied systematically to a virus-associated malignancy [83,84], EBV nowadays is considered to be at most a contributing cofactor. The two reasons for this loss of etiological status were the discovery that EBV is not always present in BL tissue, especially in sporadic cases in nonendemic regions [85,86], whereas any one of three characteristic chromosomal translocations that in all instances leads

to dysregulated expression of *c-myc*, whether in endemic or sporadic cases, is the molecular lesion that has come to be accepted as the sole primary cause of BL, with EBV and perhaps holoendemic malaria as cofactors. In fact *c-myc* overexpression has never been duplicated in vitro in human B-lymphocytes to produce BL-like cell lines, nor has a functional lesion that affects *c-myc* expression been shown to arise in EBV-transformed lymphocyte lines cultured in multiple passages over long periods.

In Hodgkin's disease although the EBV episome is detected in Reed-Sternberg cells in a clonal form, the association, while it may point to as yet undefined pathologic subtypes of HD, is inconsistent. Therefore EBV is likely not to be causative, but the virus may contribute to oncogenesis, perhaps specifically tumor progression. Finally the concept is emerging that a tumor virus need not be considered only from an etiologic perspective. Additionally or alternatively in some cases EBV may modify tumor phenotype. EBV LMP-1 induces MMP-9, COX2, and through this pathway VEGF, FGF-2 protein and its release into extracellular

medium and HIF1 α . Thus, LMP-1 is not only the principal EBV oncoprotein, it also induces a constellation of cellular invasion and metastasis as well as angiogenic factors. These observations may help to define a role for EBV in malignancies with which the virus is inconsistently associated, such as Hodgkin's disease and breast cancer. Perhaps the role of the virus in such conditions may be to modify tumor cell behavior so that it becomes more aggressive and has an enhanced malignant phenotype. Recently new evidence for the role of EBV in the oncogenesis of HD has been published [NRT].

In the case of the human papovaviruses (in which it seems SV40 may now be included) while BKV and JCV have oncogenic properties both in cell culture and experimental animals, the detection of viral genomes in tumor tissues is inconsistent, thus at this point precluding a causative role for these viruses in these more recently emerging associations with brain tumors. Moreover, appropriate epidemiologic studies that might produce additional lines of evidence have not been attempted. Interestingly in the case of SV40 epidemiologic surveillance of cohorts of persons exposed to SV40 infection from contaminated Salk and Sabin vaccines carried out for decades led to the conclusion that SV40 was not oncogenic in human beings, which it seems now may not necessarily be concluded. SV40 is clearly implicated in mesothelioma, probably as a cofactor, and perhaps in brain tumors and non-Hodgkin's lymphomas, for which the evidence is not as strong. It is instructive to consider why these associations of virus and tumors have been vexatious issues. The first problem was that the associations were built initially solely on PCR data and dismissed as due to contamination with SV40 from the testing laboratories. However, since the presumptive "humanized" strain of SV40 detected differed specifically in sequence from the laboratory strain this concern has been deflated. Another problem was the low copy number of SV40 genomes in tumor specimens: <1 copy/cell on average. However, subsequently microdissection of SV40-positive mesotheliomas, which are characterized by heavy stromal tissue, showed that the tumor cells plus some immediately adjacent presumably precancerous cells contained SV40 DNA, whereas most of the stromal tissue making up the bulk of the specimens was negative for the virus. Thus the viral genome copy number would be higher for the tumor cell population itself. Also infection of mesothelioma cell lines by SV40 in vitro produced an enhanced transformation phenotype.

In general, however, PCR-based associations are not sufficient for a credible case, although Southern blot hybridization and sequencing, real time PCR and detection of expression of transforming viral genes can strengthen the evidence. In the end in situ methods for detection of viral gene products in tumor cells are essential. The gold standard would be detection of viral genomes and specific viral RNAs by in situ cytohybridization together with corresponding viral proteins detected by immunohistochemistry in the tumor cells.

Use of the latter technique by itself often leaves open the question of whether positive results might be due to cross reaction of the viral antiserum with a cellular antigen.

As to the negative epidemiologic studies of SV40, in retrospect their methodology was seen in all cases as inadequate or flawed [87,88]. Also it seems likely that the power of the studies to detect an increase in incidence of rare tumors was probably insufficient. Clearly more refined epidemiologic studies, but based on use of discriminative markers for detection of SV40, are needed.

Finally, the case of adenovirus is instructive. It is a virus that can transform human cells in culture and insert integrated viral genomes into such cells, and the virus can produce tumors in experimental animals. Moreover, the virus can disable both p53 and Rb by mechanisms similar to those used by HPV and the human Polyomaviruses for oncogenesis. Yet there is no substantiated association of adenovirus and human malignancy. The question remains whether this attractive candidate tumor virus may have a link to cancer that could be discovered by a more targeted search with modern methods of detection and discrimination of oncogenic strains among the many types of adenovirus, in contrast to the (then advanced) methods employed by Green et al. decades ago that gave negative results. However, the conservative conclusion at this point must be that even a cogent set of oncogenic properties does not equate to an oncogenic virus.

As to criteria for establishing causality Hill made some useful generalities many years ago. Hill's criteria are based on strength and consistency of association, specificity, temporality, biologic gradient, plausibility, experimental evidence and analogy, but Hill did not consider them conclusive and believed that not every criterion would be required for every cancer implicated. However, these criteria were essentially epidemiologically based and could not anticipate the great power that what are now understood to be common molecular mechanisms of oncogenesis would bring to arguments for causality.

What then are we to conclude are the minimal criteria that establish causality? First certainly all would accept consistency, indeed invariable, of association between agent and malignancy. The association might be established on epidemiologic evidence alone provided a specific viral marker is exploited for evidence of infection. However, clearly in some cases etiologic status is attained without epidemiologic evidence which while often powerful is not indispensable. Second, finding of integrated or episomal viral genomes and gene products in the tumor cells is the hallmark of the malignancies caused by most viruses. Third these viruses have directly oncogenic properties and can transform cells in culture and produce tumors in experimental animals. However, not all agents operate in this fashion. For HCV and HBV and HCC, as well as helicobacter and gastric cancer, the etiological relation might be indirect, caused by persistent replication of the agent and chronic tissue injury, rather than by inherent oncogenic properties of the agent.

In short, confirmation of a viral etiology of a given tumor does not constitute a major challenge in the case of a number of oncogenic viruses consistently associated with specific tumors. The strength of such associations may lead to interventions to prevent or reduce the occurrence of these cancers. For example, in the case of KSHV, aggressive treatment of HIV infection has reduced the risk of KS. Development of anti-KSHV drugs may eventually be effective in reducing the incidence of non-AIDS associated KS. Vaccination against HBV is effective preventing liver cancer [89], and HPV vaccine promises to be able to prevent cervical cancer.

Fundamentally, if viruses could be said to have a mission it is to replicate and spread both in the infected organism and in the human population in the face of strong resistance by the host. To replicate, viruses requisition host factors that may then cause imbalances in host cells. With rare exceptions, viruses block cellular mechanisms (e.g., pro-apoptotic pathways) designed to commit the cell to suicide in response to the imbalances. Viruses that are unable to persist in some kind of harmony with the cells they infect and which ultimately kill the cell are potential oncogenic agents only in the rare cases of infection with a defective virus or in a cell in which the full range of viral gene expression is precluded by the host. A scenario likely to occur more frequently is the imprinting of modified cell cycle control and predisposition to more extensive changes in cellular gene expression in the case of viruses that persist in the cells they infect. In either case, the assessment of the role of viruses in the etiology of cancer is important for two reasons. First, as lifestyles change or new therapeutic modalities take hold, the etiologic role of viruses in human cancer may change. Examples are the incidence of KS in the AIDS era (and diminution with more effective anti-HIV therapy) and the association of EBV with lymphomas in recipients of organ transplants as well as in patients with AIDS.

Second and perhaps equally important is identification of the myriad ways in which the human cell can be imbalanced to the point where its growth is uncontrolled and becomes invasive, as revealed by viruses, which serve two functions in this regard: they are highly specific, reproducible and controlled probes and actuators of cell function, and identification of viral gene products involved in oncogenesis makes it feasible to monitor cancers in which viral etiology is not established.

The events of the past 20 years have shown that both the incidence and etiology of human cancers are likely to change with time as a consequence of the discovery and entry of new viruses into the human population as well as with new appreciation of the oncogenicity of common infectious agents. Advances in medical practice and in ability to detect and analyze viral gene products are also likely to implicate more viruses in cancers of human beings, and therefore reexaminations such as this one are likely to be needed in years to come.

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