**[Frontiers in Bioscience 7, d268-274, January 1, 2002]**

# PERSPECTIVES IN STUDIES OF HUMAN TUMOR VIRUSES

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## TABLE OF CONTENS

*1. Abstract*
*2. History :Up and down*
*3. Up again and How*
*4. Classes of experimental tumor viruses*
*5. Human tumor viruses*
*6. Conclusions*
*7. References*

## 1. ABSTRACT

Tumor viruses can be found in both the RNA and DNA virus kingdoms. All RNA tumor viruses belong to the retrovirus family. Directly transforming Class I RNA tumor viruses carry cellular oncogenes, picked up by accidental recombination, and usually selected for secondary modifications and high tumorigenicity by the investigator. They are not known to play any role for tumor causation in nature. Class II or chronic RNA tumor viruses do not carry cell-derived oncogenes but they often act by proviral DNA insertion into the immediate neighborhood of a cellular oncogene. Feline, murine, and avian leukemia viruses belong to this category. The human adult T-cell leukemia virus, (HTLV-1) and bovine leukemia virus (BLV) act by expanding the preneoplastic cell population and thereby provides the soil for secondary, cellular changes.

The DNA tumor viruses belong to three very different categories, the papovaviruses, adenoviruses and herpesviruses. Inactivation of the Rb and the p53 pathway by the viral transforming proteins is a convergent feature of the papova- and the adenoviruses. Since all DNA tumor viruses kill their host cell following their entry into the lytic phase, transformation and tumorigenicity are entirely dependent on a non-lytic interaction.

Cells transformed by DNA tumor viruses depend on the continued expression of the virally encoded oncogene. They provide thereby a convenient target for the immune surveillance of the host. Depending on the epidemiological history of the virus in relation to its natural host species, the immune surveillance of the host and the strategy of viral latency and survival can evolve into a truly symbiotic relationship, as best illustrated by the Epstein-Barr virus (EBV). Tumor development occurs only as an accident at the level of the host (immunosuppression) or the cell (specific translocations or other genetic changes).

The list of human viruses presently known to cause or to contribute to tumor development comprise four DNA viruses, namely Epstein-Barr virus, certain human papilloma viruses subtypes, hepatitis B virus, and Kaposi sarcoma herpesvirus (HHV-8); and two RNA viruses, adult T-cell leukemia virus (HTLV-1) and hepatitis virus C.

## 2. HISTORY: UP AND DOWN

Views on the role of viruses in the etiology of cancer have been polarized between two extreme positions during the major part of the last century. The belief that viruses have nothing to do with cancer was as widespread at certain times, as the suspicion that most and perhaps all tumors were virally caused was at other times. The field started with the discovery of Peyton Rous in 1911 that chicken sarcomas could be transmitted with cell free filtrates (1). The tumors arose at the site of inoculation and were of the same histological type as the original sarcoma. This created great excitement: the cancer problem was solved! The enthusiasm subsided rapidly, however, when mouse and rat tumor filtrates failed to induce tumors. We may see this failure in retrospect as the consequence of exaggerated expectations, hasty experiments and a lack of confidence. It led to the conclusion that viruses may have something to do with tumors in birds, but not in mammals.

Two decades later, Richard Shope found that benign warts could be transmitted from the wild cottontail to the domestic rabbit by cell free filtrates (2). Shope's experiments did not change the climate of opinion. The

rabbit was a mammalian but warts were benign papillomas, not cancers. Several important points were overlooked by superficial commentators, however. The initially benign rabbit papillomas could turn occasionally into carcinomas. This was accelerated by the topical application of chemical carcinogens. The term tumor progression was originally coined by Rous to designate this transition, or, in its generalized form, the process whereby "tumors went from bad to worse". Later, Leslie Foulds defined and extended the term (3). It refers to the development of tumors by multiple, stepwise changes in several "unit characteristics". Today we call them phenotypic properties. They are individually variable and reassort independently of each other. It follows that tumor progression can proceed along several alternative pathways, making each tumor a biologically unique individual.

Foulds´ concepts are fully valid today, with one important exception. He regarded the steps of progression as being akin to developmental switches. He attributed them to epigenetic changes in gene expression rather than to changes at the DNA level. It took another decade or two before it was realized that tumors arose by a Darwinian process of largely genetic (i.e. DNA-based) variation and selection (4). Even more recently it was further realized that epigenetic changes by DNA methylation or histone acetylation may also contribute to tumor development and/or progression by the inactivation of tumor suppressor genes or DNA repair genes.

The early work on Shope papilloma was also interesting from the immunological point of view. The virally induced warts that did not progress to carcinoma were simultaneously rejected by a systemically acting host response, mediated by lymphocytes, rather than by antibodies. This was the first example of a tumor rejection response that targeted virally encoded proteins in DNA virus transformed cells.

In the 1930s, John Bittner discovered the milk factor, later called the mouse mammary tumor virus (MMTV) (5). Bittner realized that he has found a tumor virus but he chose to call it the "milk factor", for tactical reasons. According to the dominating opinion at the time, viruses played no major role in the causation of cancer. A "factor" transmitted through the milk that was also referred to as the "maternal influence", and was said to contribute to the high mammary cancer incidence of selectively inbred high breast cancer strains classified the work under genetics, a respected discipline. Its chances of being supported were much greater than if it would have been called a virus.

The discovery of MMTV did not create any major change of opinion. Viewed from our present perspective, this was partly due to the lack of emphasis. The MMTV workers followed a gradual, scientifically solid route of analysis. They interpreted their findings with caution. They pointed out that MMTV could increase the risk of mammary cancer development, but it was neither sufficient nor necessary. Hormonal and genetic factors were involved as well. Extensive analysis at numerous laboratories defined the main genetic factors that have been fixed in the high cancer strains by continuous inbreeding and selection. Some of them were found to influence breast cancer development by supporting or inhibiting the replication of MMTV. Others influenced the hormonal environment, and still others affected the propensity of the normal mammary gland to undergo neoplastic transformation. Removal of the virus from a high cancer strain by foster nursing pups, delivered by Cesarean section, on low tumor strain foster mothers could reduce the breast cancer incidence from 80-90% to 20-30, but not to zero. Conversely, introduction of MMTV into mice of a low tumor strain could raise the tumor incidence from virtually zero to 20-30% but not more.

The role of MMTV as a tumor-susceptibility conditioning factor in inbred mice was readily accepted, but its role as a "tumor virus" remained highly questionable. The notion that the probability of tumor development could be influenced by multiple factors, viruses included, was appreciated, however.

## 3. UP AGAIN AND HOW!

The great paradigmatic shift occurred in the 1950s. It was triggered by the discovery of the murine leukemia virus by Ludwik Gross (6) and the polyoma virus by Sarah Stewart and Bernice Eddy (7). Gross found that cell free filtrates prepared from the "spontaneous" leukemias of the high leukemic AKR strain could transmit the disease to the low leukemia C3H strain. Gross' success in an area where everybody else failed before had three main reasons: the serendipitous use of newborn, less than 24 hours old mice as recipients; the fortuitous choice of C3H, the only low leukemia strain available at the time that happened to be susceptible to the virus carried by the AKR strain, later called the Gross virus; and the dogged persistence of Gross in an area where nobody expected positive results.

The scientific community received Gross´ first report on the successful transmission of mouse leukemia with cell free filtrates with surprise and disbelief. This attitude prevailed for several years; until the originator of the AKR strain, Jacob Furth, took pains to repeat Gross´ experiments under the original conditions and with the same recipient subline (8). He succeeded, in contrast to earlier attempts by other laboratories that were less meticulous in their choice of experimental conditions. This has led to the immediate acceptance of Gross´ findings by the scientific community. Meanwhile, the polyoma virus had been discovered by Sarah Stewart and Bernice Eddy (7). This also stemmed from Gross´work, but in a more indirect fashion. Previously, Gross has occasionally observed the development of parotid tumors in C3H mice inoculated with AKR leukemia filtrate. He realized that they may have been induced by another virus, provisionally referred to as the parotid tumor agent.

Stewart and Eddy started out on the assumption that Gross´ leukemia virus experiments were correct. Since his virus was apparently quite weak, however, they wished

to amplify it by adding the leukemia filtrates to embryonic mouse fibroblast cultures first, followed by culturing and later inoculation of the filtered supernatants into newborn mice. The mice developed a wide variety of different solid tumors, but no leukemia.

*The pendulum swings.* After the discovery of the murine leukemia and polyoma viruses the climate of opinion shifted to the opposite extreme. During the 60s and for at least another decade, most discussions on cancer, including chemical, radiation induced and spontaneous tumor development, circled around or at least touched on the possibility that the activation of latent viruses may have been responsible. This was not only based on the murine leukemia and polyoma viruses. After the discovery of polyoma virus and Furth´ confirmation of Gross´ experiments, new tumor viruses were isolated from rodent, feline, simian and fowl tumors of many different kinds. What was deemed impossible during many years was now readily feasible.

How could it happen now, why did it not happen before?

The keyword is confidence. Trusting a positive outcome leads to perseverance.

Another interesting question is why the isolation of the murine leukemia virus had so much greater impact than the earlier discovery of MMTV. The two systems are not very different. Leukemia induction by the Gross virus is as dependent on host factors as MMTV-assisted mammary carcinogenesis. It only works if the genetic constitution of the host is favorable for leukemia virus replication, permitting the development of viremia. This explains why the choice of the recipient strain was so crucially important for Gross´ success.

The difference between the impact of MuLV and MMTV was thus mainly due to a difference in emphasis, as already mentioned. Bittner always stressed the complexity of his system. Gross had more categorical views, based on his firm conviction that most, if not all tumors are caused by viruses. With the added discovery of the polyoma virus, the presumed viral etiology of cancer was overstated once again and the field was carried away from one extreme to the other. Both were equally wrong. This will appear from a consideration of the different tumor virus categories.

## 4. CLASSES OF EXPERIMENTAL TUMOR VIRUSES

The viruses so far mentioned fall into three major categories. Rous sarcoma virus belongs to the acute or class I RNA tumor viruses. The murine leukemia and the mammary tumor virus fall into the category of chronic or class II RNA tumor viruses. The Shope papilloma and the polyoma virus are DNA tumor viruses.

Some interesting generalizations can made on the basis of this and later experimental work that has identified many additional viruses in all three categories.

*All RNA tumor viruses belong to the retrovirus family.* They carry their genetic information in RNA. Following their entry into a susceptible target cell, the virally encoded reverse transcriptase turns their RNA into proviral DNA that can integrate into cellular DNA at random. When virus production is activated again, the proviral DNA is transcribed into RNA. This is followed by viral RNA replication, the production of new viral proteins, the assembly of new viral particles, and their release by budding, but it is not accompanied by any cytopathic effect. Virus production is therefore compatible with cell proliferation.

Activation and transcription of the integrated provirus is an error-prone process. Adjacent cellular DNA may contribute to the RNA sequences carried by the derived viral particle. In the vast majority of the cases, this has no adverse consequences but occasionally the incorporated cellular sequence may originate from a gene whose activated product can stimulate the entry of the cell into the S-phase. Virus particles that carry such sequences may cause cell proliferation when they infect new recipient cells. The probability that this happens is very low, because every step in the process, from the integration of the virus into the “right place”, through the production of the appropriately (in frame) fused viral - cellular messages, the release and the replication of competent virus and the subsequent new infection of a susceptible cell, are all low probability events. A tumorigenic virus variant is usually generated by the purposeful and often prolonged selection for tumorigenicity by the investigator. The potency of tumorigenic strains is increased by animal passage. This requires great persistence on the part of the investigator. Following the early discovery of the Rous sarcoma virus, it took four decades before new acute or class I RNA tumor viruses could be identified on the basis of their ability to induce tumors at the site of inoculation and to transform normal into tumor cells in vitro. Following the revival of viral oncology in the 1950s, some 40 such viral strains, carrying about 20 different cellular oncogenes, were isolated in rapid succession from fowl, rodent, feline and simian tumors.

*Class I RNA tumor viruses* are not known to play any tumorigenic role in nature. This is understandable, because most of them are defective, due to the replacement of essential viral genetic information by the inserted cellular genes. They produce crippled virus particles that can only multiply in the presence of complete, but non-transforming “helper virus”.

*Chronic or class II RNA tumor viruses* have no transforming activity in culture. They do not induce tumors at the site of inoculation and carry no cellular oncogenes. Insertion in the immediate neighborhood of a cellular oncogene is the most frequent mechanism whereby they contribute to the tumorigenic processes. Since the proviral DNA integrates at random, the likelihood of such an insertion is low. A very high level of virus production, leading to viremia, is usually a precondition for tumorigenicity. This is the reason why only some mouse strains that can support virus replication and/or are

deficient in their immunological responsiveness to the virus, are susceptible to the tumorigenic effect of murine leukemia virus or mammary tumor virus, as already mentioned.

Insertion in the neighborhood of a cellular protooncogene is not the only mechanism whereby an RNA tumor virus can initiate tumor development, but other alternatives are less well documented. HTLV-1, or adult T cell leukemia (ATLV) virus, the only RNA tumor virus known to contribute to human neoplastic disease is an example of this. It is believed to stimulate the expansion of preneoplastic cell populations, paving the way to cellular changes that may be more directly involved in the tumorigenic process (9).

In conclusion, the RNA tumor viruses have provided a wealth of new information about virus-cell interactions in tumorigenic processes and have led, indirectly, to the discovery of a gamut of cell division regulating cellular genes, the oncogenes. They can be regarded as a model of what can happen, but they give us very little information about what does actually happen in the genesis of human neoplasia.

*The DNA tumor viruses* provide a very different picture. They belong to several unrelated virus groups. In contrast to the RNA tumor viruses that can replicate in growing cells without killing them, the DNA tumor viruses kill the cells in which they replicate. Their proliferation and their tumorigenic potential depend entirely on the blocking of the viral life cycle. This occurs either in non-natural host cells that are non-permissive for the lytic cycle or in special, non-permissive cell types of permissive hosts.

All DNA tumor viruses carry their own transforming genes as part of the viral genome. The number of virally encoded transforming genes varies between one (SV40LT), two (adeno- and papillomaviruses) and six (EBV). The virally encoded transforming proteins are immunogenic, as a rule. The challenge of massive viral transformation is met by the immune surveillance of the host. Tumors that can be related to these viruses occur in unexpectedly high frequencies in immunosuppressed hosts. They represent the major part of the "opportunistic tumors" that arise exclusively or predominantly in congenitally, iatrogenically (as after organ transplantation) or virally (e.g. by HIV) immunosuppressed persons.

It is a common denominator of all DNA tumor viruses that they target both the *Rb* and the *p53* pathways. This is an important point for the understanding of viral strategy. But it is also entirely consistent with the evidence from cancer genetics, showing the central importance of crippling these two pathways, involved in the control of the cell cycle and of apoptosis, for tumor development.

The transforming proteins of SV 40, the adeno- and the papillomaviruses inactivate Rb and p53 in different ways, but they all do it with their transforming proteins. Transformation itself is a byproduct of latency. Establishment of latency requires the induction of DNA replication in the recipient cells that can be seen as another common feature of the DNA tumor viruses. This carries the risk of malignant proliferation. The host inhibits the progressive growth of the transformed cells through its immune response, however. All strong cases of immune surveillance against potentially neoplastic cells come from the field of the DNA virus related tumors. While the immunocompetent hosts reject potential tumor cells, the virus goes into hiding. It stays in non-proliferating cells where it is not "seen" by the immune response. The study of EBV provides a particularly interesting "success story "that favors to the survival of both the virus and the host.

## 5. HUMAN TUMOR VIRUSES

Four of the six presently known viruses that have been identified as being involved in the causation of human cancer in a direct or a contributory capacity (EBV, HPV, HBV, HHV-8) are DNA viruses while the remaining two (HTLV-1 and HCV) are RNA viruses.

In the former group, EBV is most directly involved in the causation of immunoblastomas that arise in immunodefective persons, such as transplant recipients, certain congenital immunodefectives and in HIV-infected persons. EBV may also play a role in Burkitt lymphoma (BL) and nasopharyngeal carcinoma (NPC), as indicated by the regularity of its association with these tumors, but the nature of the viral contribution is not fully understood.

Special subtypes of the human papilloma viruses are known to contribute to the genesis of cervical carcinomas and of skin tumors. Human herpesvirus no.8 (HHV8) is associated with Kaposi sarcoma, Castelman´s disease and body cavity lymphoma. Hepatitis virus type B and C contribute to the genesis of primary liver cancer. The evidence for these virus-tumor associations is epidemiological and molecular, but the relative role of the virus and of cellular genetic changes has not been fully clarified.

Comparison of the potentially tumorigenic herpesviruses in different species provides some important lessons. EBV can be regarded as a very ancient human herpesvirus because all Old World primates carry closely related viruses that interact with and transform B cells like human EBV. New World primates do not carry EBV-like viruses. When experimentally infected with EBV, they develop the same type of fatal lymphoproliferative disease as immunodefective humans. *Herpesvirus saimiri* and *ateles* are indigenous to New World primates. They do not induce tumors in their natural host, the squirrel and the spider monkey, where they are ubiquitous. They may cause fatal lymphoproliferative disease in other New World primates that do not normally encounter these viruses in nature. This shows the paramount importance of previous viral exposure for the ability of a species to oppose the tumorigenic effect of a herpesvirus. I call this "immunological anticipation".

Marek's disease (MD) virus in chickens is not ubiquitous in its natural host species. It is highly infectious

and causes epizootic lymphomas in previously uninfected chicken flocks. This is the only example where a virus causes tumors in an epizootic fashion. There is now a preventive vaccine against MD, but earlier it was the major economic problem for the poultry industry. There are resistant flocks, however. Resistance is often linked to MHC.

Since the transforming and potentially tumorigenic effect of EBV is best known, it is reviewed in the next section.

*Epstein Barr Virus:* n high endemic Burkitt lymphoma (BL), the virus is present in 98% of the cases. In sporadic BL the incidence is much lower (25%). AIDS-associated BLs are EBV positive in 30-40% except for those that start in the brain and are 100% positive. Post-transplant immunoblastomas carry the virus in nearly 100%. The frequency of EBV carrying Hodgkin's lymphomas is about 50%. EBV-carrying T cell lymphomas are rare except lethal midline granulomas that are always EBV-carrying. It is not understood how the virus gets into the Hodgkins and the T cell lymphomas and what it does there.

Among non-neoplastic epithelial tissues, oral hairy leukoplakia, (OHL) is a lytic, EBV-producing focus in AIDS patients. EBV can thus replicate lytically in oral epithelia. This facilitates virus spread to the outside world. The OHL lesions may be cured by acyclovir. The undifferentiated or anaplastic form of NPC carries EBV in 100%. Unlike the incidence of NPC itself that is an ethnically related tumor, its EBV carrying status is not geographically or ethnically variable. Anaplastic salivary gland tumors may also carry EBV, as also some gastric cancers and leiomyosarcomas (for review see 10).

*EBV-B cell interaction.* EBV is the most highly transforming known virus. Nevertheless, its interaction with humans is largely apathogenic. Tumor development is always a biological accident of immunosuppression or of cellular changes. EBV transformed B blasts are highly immunogenic for T cells of the same donor. Autologous mixed lymphocyte cultures generate cytotoxicity at a comparable level as the most incompatible MHC class differences. They behave more like allogeneic mixed lymphocyte cultures, even though they are autologous. Both paradoxes can be resolved by the understanding of two relationships: between the virally transformed cells and the immune system, and between the regulation of viral gene expression and the host cell phenotype.

The virus modulates the expression of its latently persisting genomes, depending on the phenotype of its carrier cell. It uses the transcription factor flora of its host to control its gene expression. Two important interactions are called "Latency I " and "Latency III". Latency III is only found in virally transformed immunoblasts. Here the virus expresses six nuclear proteins (EBNAs) and three membrane proteins (LMPs). The six EBNA mRNAs are spliced from a single giant message that is initiated from a number of alternative promoters, in the Bam H1 C or W region (WpCp program). Latency I was discovered in Burkitt lymphoma, but is also found in small lymphocytes that carry latent virus in normal individuals where the virus is hiding with no pathogenic effects. BL is a germinal center cell derived neoplasia. The normal B cell that harbors the virus is probably a germinal center derived memory cell.

In latency I, a single virally coded protein, EBNA1, is expressed from the Qp promoter, generating a monocistronic message. The EBNA1 protein binds to the origin of latent viral replication (OriP). This binding is essential for the maintenance of the viral episomes. Without EBNA1, the viral episomes are lost. It is therefore understandable that EBNA1, alone among the nine proteins, is expressed independently of the phenotype of the cell.

Latency II is a subtype of Latency I. Every EBV carrying cell expresses EBNA1. Non-B cells such as T cells, HL, and NPC cells, express EBNA1 and the LMPs. The difference between Latency I and Latency II is due to the fact that the LMP promoters are repressed in B cells unless EBNA2 is expressed. EBNA2 overrides this repression. In non-B cells, there is no such repression and LMP expression is constitutive.

Switching between Latency I and III can occur in both directions. The switch from I to III is very well known. BL type I cells use latency I in vivo and when freshly explanted, also in vitro. They express germinal center (GC) markers such as CD77 and CD10. When EBV-carrying BL lines are propagated *in vitro*, they often switch to a more immunoblastic phenotype. Type III (BL) resembles normal lymphoblastic cell lines. Type I lines express only EBNA1, whereas Type III lines express all EBNAs and also the LMPs.

The probable EBV B cell scenario in primary infection can be described as follows: The virus attaches to CD21 on B cells. Its entry leads to B cell transformation, followed by blast proliferation that involves release of cytokines that can function as B cell growth factors. I call this the "run uphill" phase. This elicits an immune rejection response; hereafter the virus goes into hiding. The virus carrying immunoblasts are killed by CTLs, but the virus persists in resting memory type B cells that express EBNA1. These cells are not recognized by the immune system.

We have recently obtained evidence for the opposite switch. It appears that resting B cells with type I latency are generated from immunoblasts. The switch from proliferating blasts to latently infected small B cells appears to be akin to the generation of memory cells after the antigen dependent B-cell activating complex fades away.

Using the RNA-track method, we have shown with Anna Szeles that LCL cells express the full immunoblastic (Type III) program but a small fraction (less than 5 %) of the cells give positive signals only with a Bam H1K probe, corresponding to the structural EBNA1

message, but not with a W probe that detects the immunoblast associated type III message (11).

Recently we have found that the type III to type I switch can be promoted by exposing LCLs to CD40 ligand-expressing but not to control L cells. CD40 ligand is one of the normally interacting ligands that B cells confront in the lymph node germinal centers (12).

How is it that EBNA1, a viral protein that induces specific antibodies in all healthy EBV-carriers and whose derived peptides can generate specific CTLs in mice, does not induce a CTL response in BL patients, nor in healthy individuals? It may be added that EBNA1 is the only virally encoded protein that is expressed in a cell phenotype independent fashion.

Maria Masucci in Stockholm and her co-workers have done a crucial experiment that explains the immunologically privileged position of EBNA-1 (13). They showed that the characteristic glycine-alanine repeat of EBNA 1 prevents its ubiquitin–proteasome dependent processing and subsequent association of its peptides with MHC class I molecules. They are thereby prevented from serving as CTL targets.

## 6. CONCLUSIONS

Viruses do not evolve to cause cancer but to propagate their own genome. Viral contributions to tumor development can be regarded as biological accidents. The predominant accidents are different for the RNA and the DNA tumor viruses.

*RNA tumor viruses.* Potentially tumorigenic accidents are relatable to the retroviral life style, particularly the transcriptional roundtrip from RNA to DNA and back to RNA, the random integration into the genome of the host cell, and the highly error-prone excision and replication of the viral genome that entails the risk of incorporating host cell DNA into mainly crippled virus particles. The latter mechanism has given rise to all the class I or acute RNA tumor viruses that have brought us the first information about the oncogenes, but appears to play no role in the causation of naturally occurring tumors. They can be regarded as a highly informative artifacts of laboratory experimentation. Chronic or class II RNA tumor viruses can assist tumorigenesis by accidental juxtaposition to cellular protooncogenes, accompanied by the constitutive activation of the latter. This mechanism has been well investigated in the laboratory, particularly in relation to murine leukemia and mammary carcinoma. Choice of the host strain is critical for tumorigenicity in these cases. This can be related to host permissiveness for viral replication. The probability of juxtaposition into the immediate neighborhood of a cellular oncogene is enhanced by viremia.

In naturally occurring tumors, insertional oncogene activation by retroviruses was demonstrated in fowl and cat leukemia. In humans, a single retrovirus, HTLV-1, is known to contribute to the genesis of adult T cell leukemia. This is due to a different mechanism: expansion of preleukemic cells, followed by cytogenetic changes.

*DNA tumor viruses.* Although they belong to different virus families (papova, adeno, herpes) they share two features. They all stimulate DNA synthesis in their growth transformation-susceptible target cells. Their transforming proteins inactivate both the Rb and the p53 pathway. Stimulation of an S-phase is probably necessary for the integrating viruses to insert into the cellular DNA, and, for the episomal viruses to establish the correct chromosome-episome balance. Inactivation of the Rb and p53 pathway decreases the risk of growth arrest and/or apoptosis.

It may be noted that the development of non-viral tumors has similar requirements. Multistage carcinogenesis involves oncogene activation that drives the cell towards the S-phase and also inactivation of both the Rb and the p53 pathways to avoid growth arrest and apoptosis.

In contrast to the spontaneously evolving tumors, virally transformed tumor cells are immunogenic for the host. Immunosuppression is one of the important accidents that can permit the malignant proliferation of virally transformed cells that would be otherwise rejected. It is therefore not surprising that transplant recipients, congenitally immunodefective persons and HIV-infected immunodefective persons are prone to develop EBV-carrying immunoblastomas.

The papillomavirus carrying skin and cervical tumors that appear particularly frequently in the immunosuppressed patients is another case in point, and so is Kaposi sarcoma, related to HHV-8.

The DNA virus host relationship is thus characterized by three interactive strategies: Stimulation of cell proliferation, immune rejection and withdrawal of the virus into hiding. They are best known in the EBV-B cell system. Primary infection induces blast transformation in B cells, followed by DNA synthesis and rapid proliferation. This is driven by six of the nine EBV encoded, transformation associated proteins. The role of the proliferative phase is to bring the number of virus carrying cells to a certain ceiling level, before immune rejection sets in. Eight of the nine growth transformation associated proteins are highly immunogenic. Due to our very long history of coexistence with this virus, we have a surprisingly large number of specific T cells, capable of recognizing one or the other of the eight proteins, depending on the HLA class I equipment we carry. The immune rejection that is most clearly seen in mononucleosis, leads to the annihilation of the proliferating blasts. A small proportion of the blasts switch, however, to a germinal center type phenotype, the normal counterpart of the Burkitt lymphoma cell. In these cells, the viral program is down-regulated, so that they express EBNA1 only, the protein required for the maintenance of the viral episomes. EBNA1 carries a long glycin-alanine repeat that prevents the ubiquitin-proteasome associated processing of

the protein. For that reason, it cannot provide a target for killer T-cells.

The latent virus is thus carried in nonproliferating germinal center memory B cells, unrecognized by the immune response. This results in a perfect equilibrium between the virus and the host, to the benefit of both.

The EBV scenario is a good example of a non-pathogenic virus-host equilibrium that a potentially tumorigenic virus can establish with its host.

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**Key Words:** Virus, Tumor, EBV, RNA tumor viruses, DNA tumor viruses, Review

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