Epstein–Barr Virus and Cancer

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Epstein–Barr virus is widespread in all human populations, and is usually carried as an asymptomatic persistent infection. In a small minority of infected individuals the virus is associated with the pathogenesis of certain types of lymphoid and nonlymphoid cancers, including Burkitt lymphoma, posttransplant lymphomas, Hodgkin disease and nasopharyngeal carcinoma.

Introduction

Cancer develops as a result of accumulated genetic abnormalities, some of which may be inherited and others acquired. Examples of acquired genetic events include accidents of normal cell development or ageing processes, exposure to radiation or carcinogenic chemicals, or infection by viruses. Viruses are estimated to be involved in the pathogenesis of more than 20% of human cancers worldwide. Furthermore, the epidemiology of some other cancers is suggestive of an infectious aetiology, and ongoing research may extend the number of known tumour viruses.

The first virus to be implicated in the causation of a human cancer was the Epstein–Barr virus (EBV), discovered in 1964 by Epstein, Achong and Barr in a lymphoid cell line established from a biopsy of an African Burkitt lymphoma (Epstein et al., 1964). Burkitt lymphoma is the most common childhood cancer in certain parts of equatorial Africa, and these tumours regularly carry EBV. However, while the evidence is now overwhelming that the virus is a cofactor in the development of this tumour, establishing this fact was complicated when it became apparent that EBV is a ubiquitous virus carried as a persistent infection by 90–100% of adults in all populations worldwide. Thus, the virus clearly requires cofactors to initiate cancer. This phenomenon, where only a small proportion of virus-infected individuals develop the virus-associated cancer, is not unique. Indeed, it is a feature shared by all human tumour viruses identified to date (e.g. Human papillomavirus types 16 and 18, hepatitis C virus, Kaposi’s sarcoma-associated herpesvirus and human T-lymphotropic virus).

One important feature of EBV that is relevant to its association with cancer is that it is the most potent transforming agent known for human cells. Experimental infection of normal resting B cells with EBV in culture regularly leads to the outgrowth of immortalized lymphoblastoid cell lines (LCLs) carrying multiple copies of the viral episomal deoxyribonucleic acid (DNA) genome. The virus carries at least one classical oncogene, the latent membrane protein 1 (LMP-1) gene, whose product mimics a constitutively active receptor of the tumour necrosis factor receptor superfamily. While the isolated LMP-1 gene is oncogenic in experimental models, it is not by itself sufficient to fully transform human cells. Indeed, EBV-induced immortalization of normal B human lymphocytes clearly requires the cooperative functions of at least four other viral genes in addition to LMP-1.

Physiology of Normal EBV Infection

EBV-associated diseases may be regarded as accidents in an otherwise highly successful relationship with the human host. Important clues as to how EBV contributes to disease can therefore be obtained through an understanding of how the virus normally persists in the healthy infected host. Unfortunately, despite years of research since the discovery of EBV and its identification as the cause of infectious mononucleosis, we are still unable to define unequivocally what is the normal physiology of EBV infection.

Primary infection with EBV usually occurs in the mucosal tissues of the oropharynx, but the target cell for primary infection is not known. One possibility is that infection occurs first in epithelial cells, which then enter the lytic virus cycle to produce and release new infectious virions. Circumstantial evidence for the involvement of epithelial cells in normal persistence is provided by the fact that the virus is associated with diseases involving EBV-infected epithelial lesions, e.g. undifferentiated nasopharyngeal carcinoma, and the acquired immune deficiency syndrome (AIDS)-associated lesion, oral hairy leucopla-kiia. However, whereas B cells express the virus receptor, CD21 (complement receptor 2 = FCR2), and are readily susceptible to infection with EBV in culture (Nemerow et al., 1985), epithelial cells do not normally express CD21, and infection in culture is relatively difficult to demon-
strate. While early reports indicated the presence of EBV in exfoliated epithelial cells in throat-washings from healthy infected individuals, and in epithelial cells of parotid glands, a definitive identification of the cell type harbouring the virus was not established. More recent studies suggest that most, if not all, EBV-infected cells within normal epithelial tissues are in fact infiltrating lymphocytes. It is therefore possible that EBV infection of epithelial cells is an ‘illicit’ interaction that rarely occurs in healthy individuals, or is normally abortive, and that rare successful infection of epithelial cells might frequently initiate disease.

There is now growing support for a scenario of EBV persistence in which B cells are both the primary target of EBV infection and also the site of latency. Whether or not epithelial cells are involved in normal persistence, a central role for B cells is undeniable. The main mechanism for expanding the virus-infected B-cell pool is through virus-induced cell proliferation, a phenomenon observed in vitro with EBV-transformed LCLs. In the human host, the potentially pathogenic consequences of this EBV-induced growth transformation are controlled by potent immune T-cell responses, particularly virus-specific cytotoxic T cells (CTLs). Since the virus is never completely eradicated from the infected host, it has to be presumed that EBV growth-transformed B cells are able to revert to a nonproliferating state with a phenotype that enables escape from CTL surveillance.

Analysis of different EBV-positive B-cell lines has shown that the virus can display different patterns of ‘latent’ viral gene expression which correlate with different B-cell phenotypes. It is also evident that in certain circumstances some B cells can support full lytic virus replication, which may occur predominantly at mucosal sites to produce the virus present in the oropharynx and salivaary secretions. It now appears likely that, in normal persistence, viral gene expression is at least in part determined by the normal physiological signals that control B-cell development, selection and terminal differentiation.

Using sensitive polymerase chain reaction (PCR) technology, it has been demonstrated that the EBV-carrying cells in the peripheral blood of healthy individuals are B cells with a nonactivated phenotype (Miyashita et al., 1997). The viral gene expression in these nonproliferating B cells is even more restricted than the ‘latent’ gene expression observed in growth-transformed LCLs, which minimizes the potential targets for immune responses. LCLs express viral genes encoding six nuclear antigens (EBNA-1, -2, -3A, -3B, -3C and -LP) plus three or more latent membrane proteins (LMP-1, -2A and -2B), and the CTL responses are predominantly directed at the EBNA-3 family of proteins. In peripheral blood, it is possible that some infected cells express no viral genes at all, while others express at least EBNA-1 or LMP-2A, which are not by themselves sufficient to induce growth transformation. Even if EBNA-1 were regularly expressed in all infected cells, it would not be a target for virus-specific CTLs because this nuclear protein contains an unusual glycine/alanine sequence domain which has been shown to interfere with processing of antigenic EBNA-1 peptides, thus preventing presentation via major histocompatibility complex (MHC) class I at the cell surface (Levitskaya et al., 1995).

In summary, EBV can establish at least three different types of virus–host cell interactions in normal B cells. The growth-associated state allows expansion of the virus-infected pool of cells, and is characterized by an activated lymphoblast phenotype in which several ‘latent’ viral genes are expressed. These are targets for immune surveillance. A nonproliferating latent infection state is characterized by a nonactivated B-cell phenotype and nonexpression of antigenic latent EBV proteins. Finally, cells in lytic virus-productive cycle probably correspond to terminally differentiated plasma cells and they express more than 80 viral genes, many of which are targets for immune responses.

Pathophysiology of EBV-associated Cancer

In view of the ease with which EBV can transform B cells in culture, it is not surprising that the virus is associated with human cancer, particularly in immunosuppressed patients. Perhaps more surprising is the wide range of types of cancer with different aetiologies with which EBV is associated. Table 1 summarizes the best-characterized EBV-associated cancers.

The presence of EBV in tumours can be demonstrated by a variety of techniques. The most reliable method in routine use is in situ hybridization for detection of the EBV-encoded nonpolyadenylated ribonucleic acid (RNA) species, the EBERs (Wu et al., 1990). The sheer abundance of EBERs allows for much greater sensitivity of detection than is possible by targeting EBV DNA. Furthermore, in contrast to PCR techniques, which are also very sensitive, the use of in situ hybridization allows histological confirmation that the EBV is present in the tumour cells of a biopsy rather than in infiltrating normal lymphocytes. Immunohistochemistry for virally-encoded proteins also allows discrimination between infection of tumour cells and infiltrating normal cells. However, whereas EBERs are abundantly expressed in all types of latent EBV infection, the expression of the individual EBNA and LMP proteins varies according to the type of tumour.

An important tool for investigating the role of EBV in oncogenesis is the analysis of the number of terminal repeat sequences in the viral episome (Raab-Traub and Flynn, 1986). Virions contain linear EBV DNA, which circularizes following infection of a cell, resulting in a characteristic number of repeats where the ends of the
linear DNA are joined. If a monoclonal population of cells is infected with an inoculum of virus particles, analysis of the repeat region will give a polymorphous number of repeats reflecting the multitude of infection events. In contrast, if EBV infection of a single cell precedes monoclonal expansion of a tumour, as expected if EBV has an essential early role in oncogenesis, then the virus will display a monoclonal repeat region. Detection of monoclonal EBV in a lesion reduces the likelihood that the virus is simply a passenger that has infected an already established monoclonal tumour.

**Burkitt lymphoma**

Burkitt lymphoma (BL) is a rapidly growing and poorly differentiated B-cell tumour, with a cellular phenotype corresponding to germinal centre centrocytes, which contains variable numbers of histiocytes that are responsible for the characteristic ‘starry sky’ histological appearance. The lymphoma is ‘endemic’ (i.e. occurs at an incidence of > 5 cases per 100,000 children per year) in certain areas of Africa and Papua New Guinea. Histologically indistinguishable tumours with similar clinical features occur more sporadically throughout the world. EBV can be detected in the tumour cells of almost 100% of endemic BL patients, and the virus is monoclonal by terminal repeat analysis. However, with some interesting exceptions, fewer than 20% of sporadic BL tumours are EBV-positive.

Denis Burkitt, together with Davies and O’Conor, recognized that the unusually common childhood cancers which presented at various anatomical sites in African children were different manifestations of the same disease. Burkitt also showed that BL was endemic only in particular areas of equatorial Africa where the geographical and climatic features were consistent with the involvement of an insect-borne infectious agent (Burkitt, 1962). Several subsequent studies showed that the geographical restriction actually coincided with areas where infection with the mosquito-borne *Plasmodium falciparum* malaria parasite was holo- or hyperendemic, i.e. where children were subjected to several bouts of malaria infection in early years. The only other location in the world where *P. falciparum* was holoendemic was in parts of Papua New Guinea, and here too BL is endemic. The importance of malaria in the pathogenesis of BL was highlighted by a tenfold reduction in the incidence of BL in cohorts of children where malaria was controlled by distributing chloroquine (Geser et al., 1989).

One of the defining characteristics of BL, whether sporadic or endemic, is the presence in the tumour cells of a chromosomal translocation involving chromosome 8 in the region of the c-MyC oncogene (8q24). Most commonly, the reciprocal translocation involves the immunoglobulin heavy chain locus on chromosome 14 or, less commonly, one of the immunoglobulin light chain loci on chromosome 2 or 22. Although the precise breakpoint varies from one tumour to another, the consequence is always the same: deregulation of c-MyC expression, which drives cell proliferation. Molecular analysis of the translocated genes suggest that the chromosomal aberrations are probably accidents of normal B-cell development, arising during VH DiH or VJ DiH immunoglobulin gene rearrangements or somatic mutations of rearranged V-region genes. Malaria infection may contribute to the generation of these

<table>
<thead>
<tr>
<th>Cancer</th>
<th>Cell type</th>
<th>Frequency of EBV-positive tumours (%)</th>
<th>Other known cofactors</th>
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<tbody>
<tr>
<td>Burkitt lymphoma</td>
<td>B cell (centrocyte)</td>
<td>Endemic: 95–100&lt;br&gt;Sporadic: &lt; 20&lt;br&gt;AIDS-associated: 30–40</td>
<td>Chromosomal translocations causing deregulation of c-MyC oncogene&lt;br&gt;<em>Plasmodium falciparum</em> malaria (endemic Burkitt lymphoma)&lt;br&gt;HIV (AIDS-associated)</td>
</tr>
<tr>
<td>Immunoblastic lymphomas</td>
<td>B cell (lymphoblast)</td>
<td>100</td>
<td>Iatrogenic immunosuppression in posttransplant patients&lt;br&gt;HIV-induced immunosuppression</td>
</tr>
<tr>
<td>Hodgkin disease</td>
<td>Reed–Sternberg cell&lt;br&gt;(B cell?)</td>
<td>Mixed cellularity: 50–95&lt;br&gt;Nodular sclerosis: 10–50&lt;br&gt;Lymphocyte predominant: &lt; 5</td>
<td>?</td>
</tr>
<tr>
<td>Undifferentiated nasopharyngeal carcinoma</td>
<td>Epithelial</td>
<td>100</td>
<td>Carcinogens in diet and environment?</td>
</tr>
<tr>
<td>Sinonasal T-cell lymphomas</td>
<td>T cell</td>
<td>&gt; 95</td>
<td>?</td>
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AIDS, acquired immune deficiency syndrome; HIV, Human immunodeficiency virus.

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Table 1  Epstein–Barr virus (EBV)-associated cancers
molecular accidents by virtue of the mitogenic properties of the parasite causing chronic immune stimulation of B cells and increased germinal centre activity. The synergistic effect with EBV is probably due to increased viral load following malaria-induced T-cell immunosuppression. In addition to inducing cell proliferation, and thus the statistical chance of genetic accidents, EBV may directly promote immunoglobulin gene rearrangements.

No other consistent genetic abnormalities have been identified in BL. The most commonly reported mutations involve the p53 tumour-suppressor gene locus; these have been observed in about one-third of primary BL tumours and in about two-thirds of recurrent BL tumours; therefore, while not a consistent feature, p53 may contribute to tumour progression.

Other risk factors for BL include the plant, *Euphorbia tirucalli*, which is used medicinally, particularly in endemic BL regions of Africa. This plant, as with others that have been proposed as cofactors for BL, contains phorbolesters which increase the efficiency of EBV-induced transformation of B cells.

While *P. falciparum* undoubtedly induces immunosuppression, patients do recover substantial EBV-specific T-cell immunity in between bouts of malaria. Therefore, once BL develops, it has to persist in the face of relatively intact immune responses, and must necessarily have mechanisms for immune evasion. In fact, it has long been recognized that EBV-positive BL tumour cell lines are resistant to EBV-specific CTLs that are able to kill ‘normal’ EBV-transformed LCLs established from the same patients. There are several strands to the immune-evasion strategy of BL. The most fundamental of these is that the tumour cells do not express the antigenic latency-associated proteins, EBNA-2, -3A, -3B, -3C, -LP and LMP-1, -2A, -2B. EBNA-1 remains expressed, but its unique glycine/alanine repeat domain prevents normal processing of the antigenic peptides that are potential targets for CTLs. In addition, the BL tumour cells demonstrate a general defect in the endogenous antigen processing pathway caused by transcriptional downregulation of the transporter associated with antigen processing (TAP)-1 and/or TAP-2 components of the peptide transporter dimer responsible for translocation of cleaved peptide antigens from the cytosol to the endoplasmic reticulum, and of the MHC heavy chains which are required for presentation of peptides at the cell surface. BL cells also display low or undetectable levels of some adhesion molecules and costimulatory molecules involved in the process of antigen presentation. Both the biopsy tumour cells and early passage BL lines established from BL biopsies display surface markers of germinal centre centrocytes, e.g. CD10+, CD77+, CD23− CD30−; however, upon prolonged culture many BL lines show a ‘phenotypic drift’, leading to expression of markers similar to LCLs (e.g. CD10+, CD77+, CD23+, CD30+), and acquiring a sensitivity to lysis by EBV-specific CTLs. This phenotypic drift coincides with a broadening of latent viral gene expression so that all six EBNA-s and the three LMPs may be detected; it is the expression of LMP-1 which is responsible for reversing the defects in antigen processing (Rowe *et al.*, 1995). There is evidence that this phenotypic drift also occurs *in vivo*, as some BL biopsies show a minority of tumour cells expressing LMP-1 and EBNA-2. Even though such cells may have some oncogenic advantage, immune-selective pressures presumably ensure that these lymphoblastoid tumour cell derivatives do not predominate in the original centrocytic tumour. Nevertheless, the very fact that BL cells may be subject to signals that induce normal physiological B-cell development, with concomitant expression of antigenic viral proteins and restoration of antigen-processing functions, may present a novel therapeutic strategy.

Since the viral genes which are known to be absolutely essential for EBV-induced immortalization of normal human B lymphocytes (i.e. EBNA-2, -3A, -3C and LMP-1) are not expressed in BL, how then does EBV contribute to BL? Firstly, EBV-induced growth transformation with full latent gene expression may directly contribute to the generation of the c-myc/immunoglobulin translocation which becomes the predominant mechanism for driving proliferation. Secondly, in the emerging tumour where latent viral gene expression may be restricted by cellular factors, there is evidence to suggest that EBNA-1 and/or the EBERs may continue to contribute to the malignant phenotype. In this context, it is notable that B-cell tumours develop in EBNA-1 transgenic mice (Wilson *et al.*, 1996).

**EBV-associated tumours in immunosuppressed patients**

The fact that EBV is carried as a silent infection in the vast majority of individuals is testimony to the efficiency of the immunosurveillance mechanisms that normally control this potentially oncogenic virus. A breakdown of this immunosurveillance would be expected to allow the development of EBV-induced immunoblastic B cell lymphomas corresponding to the EBV-immortalized LCLs that are established *in vitro* following experimental infection of resting B cells. Indeed, there is an increased incidence of such tumours in patients where there is a general impairment of T-cell functions, as in organ transplant patients following iatrogenic immunosuppressive therapy and in patients with AIDS. However, only a minority of immunosuppressed patients develop EBV-associated tumours. This suggests that the potency of normal EBV-specific immune responses is such that, even in long-term iatrogenically immunosuppressed transplant patients, there is often sufficient residual surveillance to
curtail the development of posttransplant lymphoproliferative disorders.

Posttransplant lymphoproliferative disorders

Posttransplant lymphoproliferative disorders (PTLDs) comprise a spectrum of disorders ranging from polyclonal hyperplastic to monoclonal neoplastic lymphoid proliferations that are usually of B-cell origin and contain EBV. EBV-negative recipients of EBV-positive bone marrow transplants are at particular risk of developing multifocal lesions of immunoblastic lymphoma shortly after transplantation. In contrast, a smaller minority of patients who were EBV-positive prior to organ transplantation will develop EBV-induced complications, and the latency period in these cases is often longer. PTLDs appear to be EBV-driven lymphoproliferations that would normally be controlled by the immune response. Indeed, relaxation of immunosuppression can lead to rapid progression of the tumours (Starzl et al., 1984).

The lesions in PTLDs typically resemble the LCLs that can be established by EBV infection of resting B cells in vitro, both in terms of cell phenotype (i.e. activated lymphoblasts) and viral gene expression (EBNA-1, -2, -3A, -3B, -3C and -LP, plus LMP-1, -2A and -2B). However, some lesions display a more restricted pattern of EBV gene expression, lacking one or more of EBNA-2, -3A, -3B, -3C, -LP or LMP-1, -2A, -2B. No specific karyotypic abnormalities are associated with PTLD, but alterations in oncogenes or tumour suppressor genes (e.g. c-myc, N-ras, bcl-6 and p53) have been reported in a proportion of lymphomas. Interestingly, the more malignant lymphomas of PTLD often show a more restricted pattern of latent gene expression than the LCL-like pattern usually detected in the polyclonal proliferations; this would present the emerging tumour with an immunological advantage at the expense of loss of some of the EBV-induced growth-transforming functions.

A picture emerges of EBV infection of B cells not usually being sufficient by itself to cause lymphomas, even when patients are immunosuppressed; however, following immunosuppression, there is an increased likelihood of EBV-driven polyclonal B lymphoproliferations, which, in time, may acquire further genetic aberrations to generate lymphomatous PTLD.

AIDS-associated malignancies

Human immunodeficiency virus (HIV)-induced immunosuppression in AIDS patients also leads to an increased incidence of EBV-associated diseases. These include oral hairy leucoplaikia, which is a nonmalignant epithelial lesion of the tongue and comprises multiple foci of EBV-replicating cells; this lesion was originally thought to be unique to HIV-infected patients, but has since been described in immunosuppressed posttransplant patients as well. As might be expected, AIDS patients are also prone to developing immunoblastic lymphomas resembling those that can arise in posttransplant patients. In contrast to HIV-negative immunosuppressed posttransplant patients, small noncleaved lymphomas of the Burkitt type with c-myc/immunoglobulin gene translocations are also relatively frequent in HIV-infected individuals. Generally, the BL tumours appear as early, rather than late, manifestations in AIDS. In other words, AIDS-associated BL tumours tend to arise in patients who are less immunosuppressed than those AIDS patients who develop immunoblastic lymphomas.

While the AIDS-associated immunoblastic lymphomas are almost invariably EBV-positive, only 30–40% of cases of AIDS-associated BL are EBV-positive. Therefore, the frequency of EBV association with BL in AIDS does exceed that expected for sporadic BL, but is less than that observed with endemic BL. This suggests that HIV synergizes with EBV to promote an elevated incidence of BL in a similar manner to malaria and EBV in endemic BL, albeit that HIV is less efficient at generating the c-myc/immunoglobulin gene translocations. All AIDS-related B-cell lymphomas are consistently negative for HIV proviral DNA, thus ruling out a direct role for this retrovirus in the lymphomagenesis. While HIV probably contributes similarly to malaria by chronically stimulating the immune system, the c-myc/immunoglobulin gene translocations predominantly involve the switch region of the immunoglobulin gene in AIDS-associated BL, rather than the VH-DJH region. This may indicate fundamentally distinct mechanisms by which HIV and malaria promote the genetic accidents, or it may reflect the tendency for a later age of onset for AIDS-associated BL.

Leiomyosarcoma

There is increasing evidence that EBV is also associated with the pathogenesis of leiomyosarcoma, a rare smooth muscle lesion that develops in some immunosuppressed children. It is not clear what the role of immunosuppression is in this tumour, nor how EBV infects these cells.

Hodgkin disease

Hodgkin disease (HD) is characterized by the presence of malignant Reed–Sternberg cells within a tumour mass that is largely comprised of reactive lymphocytes, plasma cells, histiocytes, eosinophils and fibroblasts. The origin of the Reed–Sternberg cell has been the subject of much speculation, but there is now substantial evidence supporting the view that this cell type is derived from germinal centre B cells (Foss et al., 1999). EBV has long been suspected to be involved in HD, as a past history of infectious mononucleosis constitutes a recognized risk factor. However, a direct involvement of the virus only gained general acceptance once the EBV was shown by in situ hybridization to be present in the malignant Reed–
Sternberg cells of a substantial proportion of cases (Wu et al., 1990). Previously, the concern was that detection of viral DNA might reflect the presence of EBV in normal B cells within the reactive lymphocyte infiltrate. Interestingly, EBV is preferentially associated with certain histological subtypes, most notably the mixed cellularity type of HD in which 50–95% typically display EBV-positive Reed–Sternberg cells. The nodular sclerosis type of HD shows a lower association with EBV, typically 10–50%, while the lymphocyte predominance type of HD is generally EBV-negative. To a large extent, the variability in the overall proportion of EBV-positive cases of HD in different cohorts reflects the relative proportions of the different histological subtypes.

While it now seems most likely that EBV is actively involved in the pathogenesis of a significant proportion of HD, the precise mechanism remains unknown. Reed–Sternberg cells express LMP-1, LMP-2 and EBNA-1, but none of the other EBNAs. Thus, while Reed–Sternberg cells do not express the full gamut of latent genes known to be necessary for transformation of normal resting B cells, they do express exceptionally high levels of a known viral oncogene, LMP-1. One of the many functions of the LMP-1 protein is to activate the nuclear factor-κB (NFκB) transcription factor. HD Reed–Sternberg cell lines, whether EBV-positive or EBV-negative, regularly show a constitutively high level of NFκB activity, which has been shown to be necessary for enhanced cell survival of Reed–Sternberg cell lines subjected to apoptotic stimuli, and for their growth as xenotransplants in severe combined immunodeficient (SCID) mice. In this context, it is interesting to note that, while no consistent genetic aberrations have been identified for HD, mutations of the 1κBα gene have been identified in a small proportion of cases (Cabannes et al., 1999). These mutations result in functionally deficient proteins that are unable to repress NFκB, causing constitutively high activation of the transcription factor.

One puzzling aspect of EBV-positive forms of HD is why they are not eliminated by immune responses, as they do express at least two viral proteins (LMP-1 and -2) that are known targets for EBV-specific CTLs, and there is no evidence of impaired endogenous antigen processing in Reed–Sternberg cells. Interestingly, HD that arises in the context of immunosuppression in posttransplant patients or AIDS is almost invariably of the mixed cellularity type and EBV-positive, and relaxation of immunosuppression has been observed to cause regression of the HD. Furthermore, while EBV-positive CTLs have been isolated from the reactive lymphocyte populations of EBV-negative cases of HD, they are more difficult to detect in EBV-positive cases (Frisan et al., 1995). It is possible that the microenvironment in EBV-positive HD tumours serves to protect the malignant cells from immune elimination. In this context, LMP-1 is known to induce expression of a number of cytokines, including interleukin 10 (IL-10) which might serve both as an autocrine stimulus for the Reed–Sternberg cells and as a negative immunomodulator of effector T cells.

Nasopharyngeal carcinoma

Poorly differentiated or undifferentiated carcinoma of the nasopharynx (NPC) is derived from epithelial tissues of the nasopharynx, particularly from the fossa of Rosenmüller, and typically shows an extensive lymphoid infiltrate. NPC is endemic amongst southern Chinese populations in southeast Asia, and the tumour cells almost invariably harbour EBV. A role for EBV in the pathogenesis is strongly implicated by seroepidemiological studies; in particular, serum immunoglobulin A (IgA) antibody titres to several EBV antigens are elevated in individuals prior to the onset of disease. These antibody titres decrease during remission following successful treatment and may rise again before relapse. It is striking that antibody titres to many lytic cycle antigens are elevated when the tumour itself generally displays a latent pattern of EBV gene expression. This phenomenon is not understood, but the implication is that the antibody responses reflect excessive lytic virus replication in nonmalignant cells, possibly at an unusual anatomical location or in an ‘illicit’ cell type.

Infection with EBV in southeast Asian populations usually occurs in young children, while NPC most frequently arises after the age of 40 years. This strongly implicates other cofactors. The unusual susceptibility of southeast Asian populations, and the anthropologically related Eskimo people, suggests a possible heritable predisposition. There is a body of evidence consistent with the possibility that a gene closely linked to the human leucocyte antigen (HLA) locus on chromosome 6 may influence susceptibility to NPC; however, environmental and/or dietary factors appear to be dominant cofactors, as migrant Chinese populations in low-risk geographical locations, such as North America, show a reduced incidence of the cancer with successive generations. Of the environmental factors, occupational exposure to smoke or dust is a significant risk. With regards to dietary carcinogens, one of the best-studied candidates for southeastern Chinese and Greenland Eskimo populations is salted fish, which contains high levels of volatile nitrosamines and is fed to children at an early age. A deficit of fresh fruit and vegetables in the diet of Chinese infants is also a risk factor.

Beyond the known risk factors, very little is understood about the mechanisms of NPC pathogenesis. Systematic cytogenetic analyses on fresh biopsy material has not yet been undertaken, but there does not seem to be a consistent gross cytogenetic abnormality in NPC. Nevertheless, areas of loss of heterozygosity have been detected on several chromosomes. One of these deletions results in loss of the p16 gene, which regulates cell cycle progression. Mutations of the p53 gene locus are detected in a small proportion of
metastatic NPC tumours but have not been found in primary NPC tumours. This suggests that, while p53 is not generally involved in the pathogenesis of primary NPC, it may trigger metastasis.

The most consistent feature of NPC is the presence of EBV in tumours. Furthermore, the viral episomes are clonal, indicating a single infection event prior to clonal expansion of the carcinoma (Raab-Traub and Flynn, 1986). The malignant cells of most NPC patients display a pattern of latent gene expression similar to that observed in HD, i.e. EBERs, EBNA-1, LMP-1 and LMP-2. While the LMP-1 oncogene product is not detected in 35–50% of cases, there is evidence that it may be expressed in all premalignant lesions. A critical role for LMP-1 is therefore implicated in the development of NPC. It is not clear why LMP-1 expression is lost in a proportion of established tumours, although, as NPC patients are generally immunocompetent, immune-selective pressures may be involved. As with HD, the question arises: why are the malignant cells of NPC not eliminated by EBV-specific CTLs? Again, perhaps cytokine secretions in the microenvironment of the tumour may cause a deficiency of functional CTLs among the lymphocyte infiltrate.

While the evidence implicating EBV in the pathogenesis of NPC is compelling, the actual role of EBV is far from understood. Furthermore, the mechanism of infection of epithelial cells lacking CD21 expression is unknown, although there is increasing evidence to support the possibility of transmission of EBV via cell-to-cell contact. Alternatively, it has been proposed that cell-free EBV may gain entry via IgA antibodies to virus membrane antigens, levels of which are elevated prior to the onset of NPC, and which may mediate virus entry to epithelial cells via IgA receptors. A scenario for the pathogenesis of NPC that is consistent with currently available evidence is that the unusual IgA serology preceding NPC reflects unusual infection of mucosal epithelial cells, which predominantly show lytic or abortive infection. This increased viral activity may precipitate infection of a premalignant epithelial cell, which by chance supports latent gene expression and subsequent LMP-1-dependent transformation to a fully malignant phenotype.

**EBV-associated nasal T-cell lymphomas**

Sinonasal T-cell lymphomas are relatively common in Japan and other southeast Asian populations. Little is known about the mechanisms of EBV involvement in these tumours, although the monoclonality of the viral genome implicates a role early in oncogenesis. While EBV infection of T cells in healthy individuals is probably very uncommon, a number of in vitro studies have demonstrated that certain immature T-cell populations can be infected. Interestingly, the viral genome remains in a linear form in these experimentally infected T cells, suggesting that latent infection is not a normal feature of EBV-infected T cells. Therefore, EBV-positive T-cell lymphomas may derive from a rare illicit virus–host interaction that allows persistence of the viral episome.

**Other EBV-associated malignancies**

EBV is frequently detected in other non-Hodgkin T-cell lymphomas, although in many cases the virus is detected in only a subpopulation of the tumour cells within the lesion. In such cases, it is possible that the virus is a secondary infection of an already neoplastic cell and may contribute to tumour progression, similarly to the situation seen with p53 gene mutations in follicular lymphomas and also some BL and NPC tumours. EBV has also been detected in a high proportion of gastric lymphoepithelial carcinomas, and in a proportion of undifferentiated carcinomas from a variety of other anatomical locations.

**Frequency and Clinical Features of EBV-associated Cancer**

**Burkitt lymphoma**

BL accounts for 30–70% of childhood cancers in equatorial Africa. In the endemic areas, the annual incidence of BL has been reported to be as high as 22 per 100 000 boys aged 5–9 years, and 10 per 100 000 girls. In the United States and in western Europe, the incidence is less than 0.3 cases per 100 000 children. Clinically, BL is multifocal. In equatorial Africa and Papua New Guinea, BL most frequently presents as jaw tumours, often affecting more than one jaw quadrant. Jaw involvement is more common in younger children; in 1970 Burkitt reported about three-quarters of Ugandan BL patients below 5 years of age, but only one-quarter of BL patients over 14 years old, showed jaw involvement. Young children with no obvious jaw tumours will often show orbit involvement. About half of endemic BL cases show involvement of the abdominal viscera, but involvement of bone marrow, lymph nodes or spleen is rare. In contrast, sporadic cases of BL in the United States or western Europe rarely present as jaw tumours but frequently involve lymphoid or abdominal tissues.

**Non-Hodgkin lymphoma in immunosuppressed patients**

While skin and lip cancers are the most common neoplasms in posttransplant patients, PTLDs constitute the next most common type and account for about 25% of neoplasms in transplant patients. The incidence of PTLDs typically varies between 0.5 and 20% of transplant patients, and the
vast majority of PTLDs are EBV-positive B lymphoproliferations. Several factors affect the incidence, including the type of transplant, the age and EBV-status of the recipient, and the method or severity of immunosuppression. At one end of the spectrum, bone marrow or renal transplant recipients show a lower incidence of PTLD (typically < 2%), while at the other end of the spectrum, the highest incidence (5–20%) is observed in heart or intestinal transplant recipients.

EBV-positive PTLDs typically arise soon after transplantation, within a median of 3–5 months; thereafter there is a small but steady annual risk of PTLD. Clinically, the presentation of PTLD is varied. It may present as an infectious mononucleosis-like illness, or as a tumorous form in which extranodal tumours outnumber nodal tumours by 2:1, and may involve single or multiple organs. The extranodal tumours frequently involve the lungs, central nervous system or gastrointestinal tract. Allograft involvement in PTLD varies according to the type of allograft. In lung transplants allograft involvement can be as high as 80%, while in heart transplants cardiac involvement occurs in less than 1% cases; other types of transplant typically show allograft involvement in 30–40% of cases.

**Hodgkin disease**

HD occurs worldwide but is particularly common in the developed western countries where it is the most frequently occurring malignant lymphoma, with an annual age-adjusted incidence of about 2.5 per 100 000. In such populations, HD shows a bimodal age incidence: it is rare in children and the incidence rises to a first peak at about 25–35 years, with a second rise in incidence beyond 45 years. In developing countries, childhood HD is more common. When the histological subtypes of HD are analysed, the most common form, nodular sclerosing, shows a unimodal age incidence corresponding to the young adult peak in developed countries, while the incidence of the other subtypes generally increases with age. In locations where childhood HD is relatively common, the mixed cellularity subtype predominates in this age group.

The clinical features of HD vary with geographical location. In developed western countries the disease most commonly presents as a unifocal lesion in cervical lymph nodes. However, as many as 75% of patients experience no classical symptoms, and the disease is often found as an abnormality on chest X-rays performed for nonspecific symptoms. The tumour subsequently spreads to adjacent lymph nodes, with palpable enlargement. As the disease becomes more aggressive, other organs become involved, including the spleen, distant lymph nodes, liver and kidneys.

**Nasopharyngeal carcinoma**

Numerically, in world health terms, NPC represents the most significant of the EBV-associated cancers. It is the most common tumour of men and the second most common tumour of women in southern China. In most low-risk locations throughout the world, the age-standardized incidence is typically less than 1 per 100 000 per year, whereas in southeast Asia it varies from around 2 per 100 000 in northern China to almost 80 per 100 000 in the southernmost Gaungdong province. In all geographic locations, the male:female ratio ranges from 2:1 to 3:1. The incidence increases with age, tending to peak at around 45–55 years.

Irrespective of the geographical location or ethnic origin of the patient, NPC most commonly arises in the lymphoreticular tissues of fossa of Rosenmüller, but may also arise in other areas of the nasopharynx. Metastasis is a common feature, with more than half of NPC patients showing involvement of cervical lymph nodes, giving rise to the associated symptoms of nosebleeds and nasal obstructions. Tinnitus and hearing loss may follow tumour involvement of the eustachian tube. Neurological symptoms may also result from intracranial invasion.

**Approaches to Management**

**General therapies**

Chemotherapy is the standard treatment for BL, while NPC is generally treated by radiotherapy. In both cases, treatment can be very successful. Remission of endemic BL can be achieved in about 80% of cases, but treatment may be hampered by lack of facilities or inability to follow up in rural areas of Africa. The prognosis for sporadic cases of BL is not as good. For NPC, a 5-year survival rate of around 80% of cases can be achieved where the tumour is confined to the nasopharynx; in cases where the tumour has metastasized, the survival rate may not reach 20%.

The choice of treatment for HD is complex, based on the type and stage of the disease, but the overall survival rates can exceed 90%. Generally, radiotherapy is usually employed for early-stage HD, but the treatment of choice in later stages or in patients who relapse following radiotherapy is combination chemotherapy.

In PTLD, reduction of immunosuppression is the primary intervention. Cytotoxic chemotherapy may also be effective for PTLD where patients are unresponsive to withdrawal of immunosuppression. Radiotherapy is less effective but may be the procedure of choice when there is central nervous system involvement. The rapid growth of some tumours may require surgical intervention.
Antiviral therapies

The involvement of a virus in the aetiology of a cancer presents various additional options for treatment. For EBV-associated cancers, acyclovir and ganciclovir can be used to inhibit lytic virus replication; however, while these drugs clearly reduce the viral load, and might theoretically reduce the likelihood of tumour development, there is no reason to suppose that they would have any effect on established tumours that show latent EBV gene expression. Prophylactic use of acyclovir and ganciclovir has been reported to decrease the frequency of PTLD.

Vaccines have been developed based on the gp350 viral glycoprotein, which is the most abundant component of the virion envelope, and the dominant target for neutralizing antibody. Several options are available for gp350 vaccines, including purified protein and recombinant adenoviral vectors, and clinical trials are in progress; however, these vaccines have the same drawback as acyclovir and ganciclovir, in that they target the virus rather than latently infected cells. An alternative strategy being developed is to use synthetic peptides as CTL-epitope vaccines. This approach is designed to stimulate CTL responses to EBV latent gene products expressed in EBV-infected tumours.

Adoptive transfer of EBV-specific CTL is another approach that has been actively pursued in recent years, and there are several reports of successful regression of PTLD following CTL infusion. One of the major limiting factors in CTL-based immunotherapy is the generation of CTLs for transfusion, but considerable advances are being made to minimize this problem. Adoptive CTL therapy may prove beneficial both prophylactically and for treatment of established tumours; however, at least in PTLD, there is a hazard that regression of the lesions in response to CTL therapy can result in severe and life-threatening local tissue damage.

References


Further Reading


