Epstein-Barr virus—recent advances

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Epstein–Barr virus is a tumorigenic herpes virus that is ubiquitous in the adult population. The virus is generally spread to and between young children through salivary contact, and only causes clinical illness where primary infection is delayed until adolescence or beyond, when an intense immunopathological reaction leads to the symptoms of infectious mononucleosis in roughly 50% of cases. More than 90% of the world's population carry Epstein–Barr virus as a life-long, latent infection of B lymphocytes. Recent data show that by mimicking B-cell antigen-activation pathways the virus enters the long-lived memory B lymphocyte pool where it evades immune elimination by severely restricting its own gene expression. By influencing B-cell survival mechanisms Epstein–Barr virus may induce tumours such as B lymphoproliferative disease and Hodgkin's disease. Vaccines are being developed to prevent and/or treat these conditions, but an animal model is required to study pathogenesis before a rational vaccine strategy can be formulated.

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Robert Harding

Figure 1. Colourised transmission electron micrograph of the Epstein-Barr virus, x40 000.

Epstein-Barr virus (EBV; figure 1) has co-evolved with human beings over millions of years and during this long association the virus life cycle has become supremely adapted to its human host so that it is one of our most effective parasites. Since its discovery in cultured Burkitt's lymphoma cells in 1964,[1] EBV has been implicated in a wide variety of diseases, both benign and malignant, of either lymphoid or epithelial origin (table 1). Like all herpes viruses, EBV has latent and productive (lytic) phases in its life cycle, the former maintaining the virus long term in its host and the latter effecting virus production and spread. A unique set of latent genes (outlined in table 2) confer EBV with oncogenic potential and the ability to induce immortalisation of B lymphocytes in vitro. Despite this, EBV establishes a harmless life-long infection in almost everyone worldwide and rarely causes disease unless the host–virus balance is upset.

This review concentrates on recent major advances in our understanding of EBV biology and pathogenesis, and addresses topical issues relating to vaccine development for disease prevention. Not all of the EBV-associated tumours are discussed in detail here, but they are listed in tables 1 and 2, and have been reviewed recently.[6]

Epidemiology

More than 90% of adults worldwide have been infected with EBV[7] and carry the virus as a life-long persistent infection, with latent infection of B lymphocytes[8,9] and virus production into saliva.[10,11] In most cases primary infection occurs subclinically during childhood,[12] often by spread between family members via salivary contact.[13,14] Epidemiological studies in the 1970s showed that primary infection occurs early in non-industrialised countries and in low socioeconomic groups,[7,15] whereas in affluent societies seroconversion may be delayed until adolescence, when infectious mononucleosis (IM) develops in between 50% and 74% of cases.[16–19] Changing demography and life styles in the intervening 30 years are likely to have altered this pattern, and new studies are required before a logical vaccine strategy can be planned.

It is commonly assumed that those who remain uninfected throughout childhood generally become infected as adolescents through kissing, and consequently IM is often called the kissing disease. However, there are single reports

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Table1. EBV-associated diseases

Disease	At risk population	EBV association
Lymphocyte origin		
Infectious mononucleosis (IM)	Adolescents/young adults from western societies/ high socioeconomic groups	Majority. IM-like syndromes also occur in cytomegalovirus, HIV primary infection
X-linked lymphoproliferative syndrome (XLPS)	Male offspring of female carriers of XLPS mutation	Majority. A few non-EBV-associated lymphomas occur in children with the mutation
B lymphoproliferative disease (BLPD)	Post-transplant lymphoproliferative disease	^90%
	HIV infection—primary central nervous system lymphoma	<100%
	—peripheral lymphoma	^50%
Burkitt's lymphoma (BL)	African children—endemic BL	97–100%
	HIV infection—sporadic BL	^25%
Hodgkin's disease	Children—developing countries	Overall ^65%
	Young adults—high socioeconomic groups	Mixed cellularity type 80%
	—history of IM	Childhood ^80%
T/NK cell lymphoma	Chronic active EBV	10–100%, depending on histological type
	HIV infection	
Primary effusion lymphoma	HIV infection	70–80%. 100% contain HHV8 DNA
Epithelial cell origin		
Oral hairy leukoplakia	HIV infection	100%
	Other immunodeficiencies	
Nasopharyngeal carcinoma	S Chinese and Inuit races—high incidence	Non-keratinised 100%
	Mayaks, Dyaks, Indonesians,	Keratinised 30–100%
	Filipinos, Vietnamese—moderate incidence	
Gastric carcinoma	Not identified	Undifferentiated carcinoma of naso-pharyngeal type 100%
		Adenocarcinoma 5–15%
Other		
Leiomyosarcoma	HIV infection—mainly children	Not known
	Immunodeficiency—mainly children	
Additional tumours with possible EBV associations		
Salivary gland tumours[2]		
Breast carcinoma[3]		
Hepatocellular carcinoma[4]		
Thymoma[5]		

HHV8=human herpesvirus 8; ^=about.

of EBV detection in male[20] and female[21] genital secretions, suggesting the possibility of sexual transmission. A recent seroepidemiological study on university students lends support to this possibility by showing strong correlations of both EBV seropositivity and history of IM with sexual intercourse and increasing numbers of sexual partners.[22] However, these data are not conclusive because they do not differentiate between direct transmission in genital secretions and spread by practices associated with sexual intercourse such as kissing.

Latent EBV infection of B lymphocytes in the blood of healthy donors affords another potential route of transmission; infection has been documented after infusion of a large volume of fresh blood.[23,24] Transmission from a transplanted organ can also occur with subsequent infection of a previously seronegative recipient,[25,26] and is a risk factor for post-transplant lymphoproliferative disease (PTLD).

It is now known that there are two types of EBV (1 and 2 or A and B) circulating in the community, which show variation in DNA sequence of the latent genes. They show no specific disease association but type 1 is more prevalent in the west, whereas types 1 and 2 are equally prevalent in Africa and Papua New Guinea.[27] Additionally, different EBV isolates can be distinguished by varying lengths of genomic

repeat sequences, and these have been used to track individual isolates through families and in organ donor/recipient pairs.[14,25,26]

EBV life cycle

Our understanding of the virus infectious cycle is based mainly on observations in acute IM, since studies of symptomless primary infection are not often feasible. EBV infects at mucosal surfaces, but whether epithelial-cell infection is part of the normal virus life cycle remains unresolved. In spite of EBV's known tropism for epithelial cells, exemplified by the several EBV-associated tumours/diseases of epithelial origin (table 1), no EBV-infected epithelial cells have been identified in tonsils removed during IM, whereas both latent and lytic infection of B cells can regularly be detected.[28] Since concentrations of EBV in saliva are generally low, it is reasonable to assume that incoming virus from this source requires amplification to establish persistence in the B cell population of its new host. Thus, there could be an initial round of lytic replication in tonsillar epithelium with secondary infection of underlying B cells.[29,30] However, at present the weight of evidence supports direct infection of B cells in the lymphoepithelium of Waldeyer's ring. Here crypt structures

Table 2. EBV latent antigens

EBV antigen	Required for immortalisation	Function known/postulated	Expressed in vivo in							
			PBM	IM	BL	NPC	HD	BLPD	TCL	Gastric carcinoma
EBNA1	+	Genome maintenance	?	+	+	+	+	+	+	+
EBNA2	+	Viral oncogene, transactivates cellular and other latent viral genes	–	+	–	–	–	+	–	–
EBNA3A	+	Activates cellular genes	–	+	–	–	–	+	–	–
EBNA3B	–	Activates cellular genes	–	+	–	–	–	+	–	–
EBNA3C	+	Viral oncogene, increases LMP1 expression	–	+	–	–	–	+	–	–
EBNA LP	+/–	Co-activates EBNA2 responsive genes, increases efficiency of immortalisation	–	+	–	–	–	+	–	–
LMP1	+	Viral oncogene, induces B cell activation and adhesion, protects from apoptosis	–	+	–	(+)	+	+	(+)	?
LMP2	–	Repression of lytic cycle, enhances B cell survival	+	+	–	+	+	+	+	+

(+)=expressed in a proportion of cases; PBM=peripheral blood mononuclear cells; HD=Hodgkin's lymphoma; EBNA=EBV nuclear antigen; IM=infectious mononucleosis; BLPD=B cell lymphoproliferative disease; LP=leader protein; BL=Burkitt's lymphoma; TCL=T cell lymphoma; LMP=latent membrane protein; NPC=nasopharyngeal carcinoma; ?=not known

that dip into underlying lymphoid tissue could allow EBV direct access to B cells.[31,32] In this case, expression of the latent viral genes, which induce B cell proliferation, would serve to amplify the number of virus-infected cells in the body and establish latency before immune mechanisms develop.

Detailed analysis of tonsillar B cells suggests that EBV uses physiological B cell antigen-activation pathways to establish and maintain latency.[33] The hypothesis is that newly infected naive tonsillar B cells, under the influence of the oncogene EB viral nuclear antigen (EBNA) 2, express all the latent viral genes, and proliferate in germinal centres. After this stage an alteration in viral gene expression, with downregulation of EBNA 2, halts proliferation and allows differentiation. Normal B cells require two signals for their survival in germinal centres, where most die by apoptosis. For B cells uninfected with EBV, these signals are delivered by binding to helper T cells, via the CD40 molecule present on the B cell surface, and the B cell receptor (BCR), binding to its specific antigen on dendritic cells. Cells infected with EBV are thought to avoid apoptosis in this environment by expressing EBV latent membrane proteins (LMP) 1 and 2a. These molecules together could provide the necessary survival signals because LMP1 is a CD40 homologue and LMP2a mimics BCR engagement.[34–38] This mechanism would minimise loss of EBV-infected B cells by ensuring their progression into the long-lived memory B cell population, and provide a rationale for the evolutionary conservation of these latent genes. However, although latency in B cells is a successful strategy for survival, it can occasionally allow survival of an abnormal cell with the capacity for uncontrolled proliferation.

After primary infection and the establishment of persistence, EBV can be detected in 0·5–50 in every million B cells in peripheral blood.[39,40] Here EBV gene expression is restricted, possibly to only LMP2a,[41,42] a protein that maintains latency by providing the survival signals, and

inhibiting B cell activation and lytic cycle entry.[43,44] These memory B cells are resting cells with low-level expression of costimulatory molecules, which are thereby assumed to evade immune recognition and elimination.[45] To complete its life cycle EBV must reactivate from latency and infect other susceptible individuals. The molecular details of the reactivation process are unclear, but it is possible that the trigger for memory B cells to enter the lytic cycle is again physiological. Activation by specific antigen would induce homing of infected memory B cells to the tonsil and maturation to plasma cells, a process that has been linked to lytic cycle entry.[46] From this site infectious virus may infect co-resident naive B cells or be shed into saliva. Thus, the persistently infected B cell pool is maintained and the virus life cycle completed.

Effective persistence of the virus despite life-long active immune surveillance by the host suggests effective immune evasion. EBV, like other herpes viruses, has evolved sophisticated mechanisms to safeguard its life cycle. As discussed earlier, during latency viral gene expression is severely restricted, although it is not clear which viral proteins (if any) are expressed. However, expression of EBNA1 is essential during cell division when it mediates binding of viral DNA to cellular chromosomes, ensuring its equal partitioning into daughter cells.[47] This unusual protein contains a glycine/alanine repeat region which confers resistance to degradation by the host cell proteosome.[48] Thus no endogenous peptides are produced for display on the B cell surface for presentation to CD8 T cells. Although this would seem to be an immune evasion mechanism, it may have evolved simply to stabilise the vital EBNA1 protein at a sufficient intracellular concentration to maintain the EBV genome.[49] However, there is no doubt that BL cells, in which EBNA1 is the only known viral protein to be expressed, can proliferate in vivo without invoking effective immune control.

When EBV reactivation occurs, several lytic viral proteins are expressed which actively inhibit immune mechanisms. These include an interleukin 10 homologue that inhibits the costimulatory and antigen-presenting functions of monocytes/macrophages,[50] and several proteins that impair the release of cytokines, particularly interferon (α and γ).[51,52] In addition, a Bcl2 homologue prolongs cell survival by inhibiting apoptosis.[53] These immune evasion mechanisms in effect buy time to allow effective production of infectious virus. However, in healthy individuals a host–virus balance is achieved such that the virus persists and replicates without harming the host.

Infectious mononucleosis

IM, or glandular fever, is one of the commonest causes of prolonged illness in adolescents and young adults in affluent societies. After an incubation period of between 4 and 7 weeks[54] signs of IM typified by fever, pharyngitis, lymphadenopathy, splenomegaly, and hepatocellular dysfunction, sometimes with frank jaundice, may ensue.[55] Splenomegaly in IM is often difficult to detect clinically, although one ultrasound scanning study showed enlargement of the spleen in all 29 cases examined.[56] Rashes, including macular erythema, petechiae, and urticaria occur in about 3% of cases,[57] and concurrent administration of ampicillin results in rash in about 90% of cases[58] (calculated from ref 59).

Confirmation of the diagnosis is usually by serological testing. Heterophile antibody is present in 85% of adolescents and adults with IM,[60,61] but is often absent in young children.[62] The Monospot is a quick slide test for the detection of heterophile antibodies. Although reasonably specific, positive tests are also seen in other conditions including HIV, lymphoma, systemic lupus erythematosus, rubella, parvovirus, and other viral infections.[63–66] IgM to the EB viral capsid antigen (VCA) is both more sensitive and specific, and has almost always developed by the time of clinical presentation. Anti-VCA IgM generally persists for about 1–2 months,[67] and false positives may be caused by rheumatoid factor.[68] IgG to VCA persists for life. Extended serology to detect IgG antibody to early antigens (in the acute phase) or EBNAs (associated with convalescence) may be useful in selected cases.

Death from IM is very rare, although fatal outcomes have resulted from neurological complications, airway obstruction, splenic rupture, myocarditis, cardiac arrhythmia, liver failure, secondary bacterial infection, and thrombocytopenia (table 3).[55] Prolonged fatigue, hypersomnia, and short-lived depressive disorders are common after IM.[69] It is not known whether the fatigue could be ameliorated by a programme of rest and exercise implemented early after the resolution of the acute symptoms, although graded exercise and cognitive behavioural therapy are currently thought to be the most effective treatments for chronic fatigue of unknown cause.[70] High-dose aciclovir reduces virus production in the throat, although it does not significantly alter the duration of individual symptoms.[71–73] Studies of corticosteroids in IM show amelioration of acute symptoms; however, the risks of prednisolone are only justified in severe disease,[74,75] for example where there is incipient airway obstruction,[76] where steroids may reduce the need for surgical intervention to protect the airway.[77]

The lack of efficacy of aciclovir is because symptoms of IM are not directly due to virus infection of B cells, but are immunopathological, resulting from secretion of cytokines by the large numbers of activated cytotoxic (CD8) T lymphocytes (CTL) typically present in peripheral blood and invading the tissues.[78,79] These T cells, which can account for as many as 44% of peripheral blood CD8+ lymphocytes, are directed against lytic, and to a lesser extent latent, viral epitopes,[80] and are thought to control the acute infection by eliminating infected B lymphoblasts. In addition, although less well characterised, natural killer cell and CD4+ T cell responses are also generated in IM, and both these cell types can secrete interferon γ and mediate cytotoxicity.

Chronic active EBV infection

Chronic active EBV (CAEBV) infection is a rare condition, distinct from chronic fatigue, that is typified by severe, chronic, or recurrent IM-like symptoms after a well-documented primary EBV infection in a previously healthy person. The antibody pattern of acute IM is generally retained (high IgG anti-VCA and EA, failure to produce antibodies to EBNA1, and sometimes persistence of anti-VCA IgM). In addition, there is a marked increase of EBV load in peripheral blood, often with infection of T and/or natural killer cells, and evidence of major organ involvement (eg, interstitial pneumonia, bone marrow hypoplasia, uveitis, hepatitis, and splenomegaly).[81,82] CAEBV has a high morbidity and mortality from hepatic failure, lymphoma, sepsis, or haemophagocytic syndrome. The disease is difficult to treat; bone marrow transplantation[83] or adoptive immunotherapy may be helpful.[84]

Table 3. Complications of primary EBV infection

Organ/system	Complication
Liver	Clinical jaundice (5%), abnormal liver function tests (80–90%), fulminant hepatitis (rare)
Respiratory	Respiratory tract obstruction, interstitial pneumonitis (rare)
Neurological*	Encephalitis, acute cerebellar syndrome, aseptic meningitis, Guillain-Barré syndrome, cranial nerve palsy especially VII, transverse myelitis, seizures, mononeuritis, optic neuritis, cerebral haemorrhage
Spleen	Splenic rupture 0·1–0·5% spontaneous or after mild trauma, usually males, splenic infarction
Haematological	Thrombocytopenia, haemolytic anaemia, neutropenia, haemorrhage secondary to mucosal ulceration
Secondary infection	Streptococcal sore throat, sepsis in association with neutropenia
Psychiatric	Depression, anxiety
Renal	Haematuria, interstitial nephritis, glomerulonephritis (rare)
Cardiac	Myocarditis, pericarditis, arrhythmia, electrocardiogram changes
Immunological	Depressed cell mediated immunity

*Pharyngitis, lymphadenopathy may be absent

X-linked lymphoproliferative syndrome

X-linked lymphoproliferative syndrome (XLPS or XLP) is a rare, familial, fatal form of IM that has been recognised for almost 30 years.[85,86] Typically XLP affects young males who are clinically well before primary EBV infection, but when infected most rapidly succumb to fulminant IM. Death results from hepatic necrosis secondary to massive CTL infiltration and cytokine release, aplastic anaemia or pancytopenia, or in some cases superimposed bacterial or fungal infection.[87,88] Haemophagocytosis is often a prominent feature.[87,88] Agammaglobulinaemia and/or B cell lymphoma may occur either before EBV infection or later in those that survive IM.[89] The acute syndrome is very difficult to treat, but etoposide may have some effect.[88,90,91] Recently a few patients have undergone bone marrow transplantation with a reported success rate of 50%; however the long-term survival rate is unknown.[92]

The underlying genetic abnormality in XLP was identified in 1998.[93–95] This discovery rapidly led to development of diagnostic tests and the recognition that the syndrome consists of a spectrum of clinical manifestations encompassing dysgammaglobulainaemias[96] (including some cases of common variable immunodeficiency),[97,98] lymphoid vasculitis with aneurysm formation,[99] and, rarely, non-EBV-related lymphoma.[100]

The defective gene product in XLP is a small, src-homology 2 (SH2) domain-containing cytoplasmic protein named SH2D1A or SAP (signalling lymphocytic-activation molecule [SLAM] associated protein). SAP is expressed in T and natural killer cells and is upregulated by cell activation. SAP interacts with several signalling molecules, the best characterised being SLAM (CD150), which is expressed on activated B and T cells and induces a Th1 phenotype with interferon γ secretion.[101] SAP binding to SLAM inhibits signal transduction initiated by SLAM, and thereby controls T cell activation. In natural killer cells SAP binds to 2B4 to mediate cytotoxicity, which is impaired in XLP.[102] In XLP it is probable that the functional absence of SAP allows uncontrolled T cell activation and dysregulated cytokine production. However, it is not clear how this relates to EBV because activation of the SAP/SLAM pathway is not specific to primary EBV infection (H Williams, University of Edinburgh, personal communication).

XLP patients are a difficult group to study, and results of the limited number of immunological investigations undertaken before EBV infection are contradictory, some showing mild immune abnormalities but others not. Combined B and T cell defects are invariably present after EBV infection, although no consistent abnormalities in immune control of EBV have been reported.[103] Since it is now clear that lymphoma can result from a mutation in the SAP gene in the absence of EBV infection, it is hypothesised that XLP is a more generalised immunodeficiency that can be initiated by several virus infections, rather than an abnormality restricted to immune regulation of EBV. However, the exact nature of the immune deficit remains unknown.

B cell lymphoproliferative disease

EBV-associated B cell lymphoproliferative disease (BLPD) occurs in immunodeficiency states with impaired T cell immunosurveillance, where a lack of EBV-specific CTLs allows proliferation of latently infected B cells.[104,105] This is a common life-threatening complication of transplantation when immunosuppressive drugs are used to prevent graft rejection. In the context of organ transplantation, BLPD is often known as PTLD. Risk factors for PTLD include high levels of immunosuppressive drugs and primary EBV infection during therapy. Clinical presentations are varied and can mimic graft-versus-host disease, graft rejection, or more conventional infections. Presenting features may resemble an IM-like illness or an extra-nodal tumour, commonly involving the gut, brain, or the transplanted organ.[106] IM-like presentations typically occur in children, within the first year of transplant, and are often associated with primary EBV infection after transfer of donor virus from the grafted organ.[26,107,108] By contrast, extra-nodal tumours are commonly seen in EBV seropositive recipients several years after the transplant. These varied presentations make the diagnosis of PTLD difficult and consequently predictive markers have been sought. Many studies have investigated EBV load in this context,[109] and high concentrations of EBV DNA in peripheral blood have often been reported in PTLD. However, large fluctuations in viral load are common, and concentrations as high as those seen in PTLD can be seen in healthy transplant recipients.[110–113] At present there is no standardisation of the technique for viral load estimation between different laboratories; however, with the availability of new rapid, quantitative techniques, continuous monitoring is feasible and may give a clearer indication of those at risk.

PTLD arises as an opportunistic tumour in the setting of intense T cell immune suppression, which allows latently infected B cells to survive and proliferate in vivo. Tumour cells generally express all the latent viral genes, and this pattern is assumed to be sufficient to induce a tumorigenic phenotype. Early lesions are often polyclonal with later progression to a monoclonal lymphoma.[114] However, the fact that lesions are commonly single suggests that only rare cells have the capacity to form tumours, and the predilection for certain body sites possibly indicates a requirement for specific external stimuli, such as cytokines. No consistent genetic abnormalities have been reported, but recently examination of immunoglobulin heavy chain gene rearrangements in these tumours shows that they often arise from post-germinal-centre B cells with non-functional immunoglobulin gene rearrangements.[115] Such cells would normally be eliminated in germinal centres, but are probably rescued by EBV infection, as a consequence of the ability of EBV to affect B cell survival and maturation processes.

First-line treatment for PTLD is reduction of immunosuppressive therapy, which allows recovery of CTL activity and leads to tumour regression in most cases, but risks rejection of the transplanted organ.[116] However, PTLD often recurs, and becomes progressively resistant to this conservative form of treatment. Despite the use of conventional lymphoma chemotherapies, the death rate remains at over 50%.[117] Novel forms of immunotherapy have

been tested in PTLD, including both antibody and cell-mediated approaches.

Rituximab is a humanised mouse monoclonal antibody that targets the CD20 molecule on the surface of all mature B cells.[118] It is currently licensed only for treatment of CD20-positive diffuse large-cell non-Hodgkin lymphoma (NHL), in conjunction with chemotherapy or alone for follicular lymphoma if chemotherapy has failed. Use in PTLD has shown some favourable outcomes[119,120] although tumour recurrences have been reported.[121] A cytokine release/tumour lysis syndrome has been associated with some fatalities.[122] Additionally, some instances of fulminant infections have been reported, although whether there is any causal association is unknown.[121,123,124] At present results are anecdotal and controlled trials are needed to assess the long-term outcome.

Infusions of cultured EBV-specific CTL have been used effectively for both prevention and treatment of PTLD in bone marrow transplant recipients where the donor is available to provide HLA-matched T cells.[125] However, the production of these donor CTL for individual patients is too expensive and time-consuming for wide application. A related strategy is to develop a bank of HLA-typed CTL that can be used to treat PTLD patients on a best-HLA-match basis. Encouraging results from a pilot study suggest that this may be a more feasible option.[126] A controlled trial is now underway, and if successful, this form of therapy could be applicable for other infections and tumours in the immunocompromised setting.

Hodgkin's disease

A causative association between EBV and Hodgkin's disease has long been suspected on the grounds of raised concentrations of antibodies to EBV antigens for months or years before onset of Hodgkin's disease,[127,128] and the increased incidence of Hodgkin's disease in the 5 years following IM.[129]
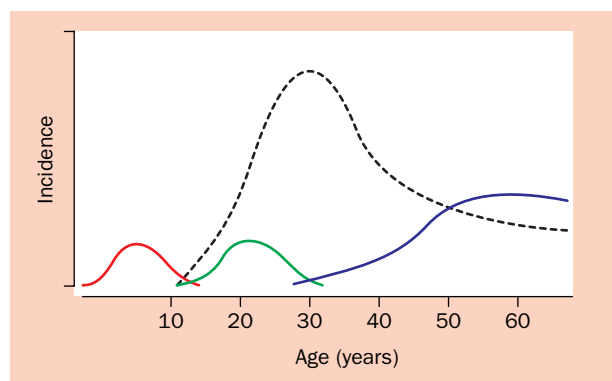

Figure 2. Incidence of Hodgkin's disease by age. Red line=EBV-associated cases; green line=non-EBV-associated cases. A four-disease model is postulated, three peaks are associated with EBV infection—(1) Childhood cases associated with primary infection. Peak age <10 years; this accounts for most childhood cases in developing countries. (2) Young adult age group, 15–34 years associated with delayed primary EBV-infection. (3) Older adult group, accounts for most cases over 55 years. (4) Non-EBV-associated Hodgkin's disease, this is the largest group and accounts for the young adult peak in developed countries. The height of each peak will vary depending on the country in which the data are generated. Based on data included in reference 131. Courtesy of Ruth F Jarrett.

A firm association was finally established by the identification of EBV DNA in the malignant Hodgkin and Reed Sternberg (HRS) cells, albeit in a subset of Hodgkin's disease tumours.[130] Overall 65% of Hodgkin's disease tumours contain EBV DNA, with the mixed cellularity type being most commonly EBV-associated (table 1).

The age of onset of Hodgkin's disease typically shows a bimodal distribution with one peak in childhood and another at over 50 years of age; however, this varies according to geographical location. The childhood peak occurs later in affluent (15–35 years) than non-affluent (5–10 years) societies. This age difference is reminiscent of primary EBV infection and development of IM, which suggests that Hodgkin's disease might be a non-typical response to primary EBV infection. Although the findings in non-affluent societies seemed to fit this hypothesis, with most Hodgkin's disease developing before the age of 10 being EBV-associated, this is not the case in more affluent societies. Surprisingly, here non-EBV-associated, nodular sclerosing Hodgkin's disease predominates in the adolescent/young adult peak. A recent population-based study in the UK showed non-EBV-associated cases having a unimodal age distribution as above, and a flatter, but probably bimodal, age distribution for the EBV-positive cases (figure 2).[131] It is generally accepted that EBV has an causative role in the pathogenesis of Hodgkin's disease, although the exact mechanistic details are still unclear.

Characterisation of the viral gene expression in HRS cells showed a restricted form of latency with EBNA1, LMP1, and LMP2A expression (figure 3).[132] LMP1 is known to induce cellular activation and proliferation pathways and inhibit apoptosis, and LMP2 expression enhances cell survival and inhibits lytic cycle activation.[133] Viral monoclonality in HRS cells[134] suggests that EBV infection is an early event in tumour development, and taken together these findings are consistent with an oncogenic role for EBV.

The cellular origin of HRS cells has long been controversial, but recently this has been definitively identified as germinal centre B cells that contain functional immunoglobulin gene rearrangements but show defective immunoglobulin transcription.[135] This finding, like that in PTLD, suggests that survival of these atypical cells may be an accident of the EBV life cycle in B lymphocytes. The fact that HRS-like cells have also been found in IM lymph nodes[136] and PTLD[137] draws parallels between IM and these tumours.

The finding of a viral cause for a subset of Hodgkin's disease tumours opens up the possibility for immunotherapy directed at viral antigens displayed on the tumour cell surface. Although LMP1 and 2 do not contain immunodominant T cell epitopes,[138] pilot studies are underway to assess CTL therapy for Hodgkin's disease in cases that do not respond to conventional chemotherapy.[139]

EBV in HIV-infected people

Most HIV-infected people are persistently infected with EBV, and with progressive immunodeficiency numbers of EBV-infected B cells in the circulation increase[140] and opportunistic lymphomas may develop. The risk of NHL is increased 60-fold compared with the general population,[141] and its presence is an AIDS-defining illness. However, these NHL are not as
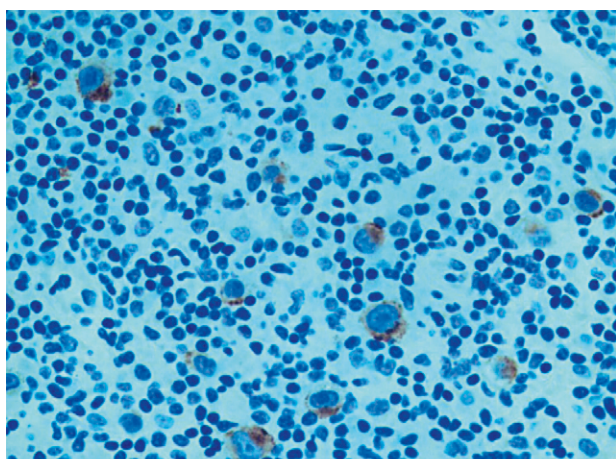
Figure 3. Photomicrograph of mixed cellularity Hodgkin's disease stained for LMP1 expression. There is strongly positive staining in the malignant Hodgkin cells (original magnification x400). Courtesy of C Bellamy.

uniform as PTLD in their EBV-association and histological type.

Three principal types of NHL are recognised in the HIV setting (table 1), with Burkitt-like tumours developing relatively early in the disease process, whereas primary central nervous system lymphoma (PCNSL) and peripheral NHL occur at a late stage. All three types may be EBV-associated, the strongest association being with PCNSL where almost 100% of tumours contain EBV DNA and express latent viral genes.[142] EBV DNA can be detected in the cerebrospinal fluid in most of these cases, occasionally preceding a visible mass on imaging and forming the basis of a useful diagnostic test.[143] The incidence of NHL in HIV, particularly that of PCNSL, has fallen since the introduction of highly active antiretroviral therapy (HAART)[144] and there is some evidence that introducing effective antiretroviral therapy prolongs survival after tumour diagnosis.[145]

Hodgkin's disease, although not an AIDS-defining illness, is also more prevalent in people with HIV infection, and in this setting EBV-associated mixed cellularity and lymphocyte depleted types are most common.[146,147]

In addition to lymphomas, EBV is also associated with oral hairy leukoplakia (OHL), which is common in late HIV infection. This is a painless, white, corrugated lesion that was first recognised on the lateral margins of the tongue in AIDS patients,[148] but has now been seen in other immunosuppressed groups.[149–151] The lesion contains EBV replicating in squamous epithelial cells but without malignant transformation or the establishment of latency.[152,153] It is unclear whether this represents an amplification of the normal EBV life cycle or infection of an aberrant cell type. OHL is usually symptomless, but can be treated with aciclovir if required.[154]

Prospects for a vaccine

At the recent International Workshop on EBV and associated Diseases (Cairns, Australia, July 2002), a discussion session was dedicated to EBV vaccines. After many years in production, there are now two candidate vaccines ready for trial, but there was no consensus on how and when these should be used. As often happens when commercial companies are involved, there is a conflict between financial and health priorities. Whereas scientists and clinicians believe that EBV-associated tumours in developing nations (nasopharyngeal carcinoma in China, Burkitt's lymphoma in Africa) should be the main priorities, a financially viable vaccine would have to be used in the west for prevention of non-life-threatening cases of IM. Other minor markets would include XLP families and seronegative transplant recipients.

The predominant EBV envelope glycoprotein gp340 has been developed as a vaccine because of its ability to induce neutralising antibody.[155] However, it is unlikely that sterile immunity, which may be necessary for prevention of EBV-induced tumours, will be elicited with this vaccine. Nevertheless, it is possible that this vaccine could prevent IM by moderating the initial viral replication and spread during primary infection, thereby curtailing the massive CTL response to lytic antigens that invokes the immunopathology giving rise to symptoms.

Using our now extensive knowledge of EBV-specific CTL epitopes, a peptide-based vaccine has recently been designed to induce specific cellular responses.[156] To overcome the problem of HLA specificity of viral CTL responses, the extensive allelic variation of human HLA genes, and variation in epitopes between EBV strains, a "polytope" vaccine containing multiple epitopes to which over 94% of the population should respond has been formulated.[157] Again, this vaccine is not designed to induce sterile immunity but it is hoped that it will be effective in the prevention of disease states. An alternative exciting future approach would be to generate therapeutic vaccines in the form of genetically engineered constructs designed to generate or enhance specific immune responses to the latent viral gene products known to be expressed in individual EBV-associated tumours.

Of the many unresolved issues relating to a vaccine for IM, our lack of information on susceptibility remains critical. EBV-seronegative teenagers form the susceptible population, but only around 50% of susceptible people would naturally develop symptoms of IM on seroconversion. Furthermore, without up-to-date studies in a range of social groups and geographical locations, it is not clear how large this population is.

The rationale for prevention of symptoms in IM by neutralising incoming virus and/or reducing the replication of virus after infection depends on the assumption that initial viral dose determines the severity of the disease. This was not

upheld by a recent study, which showed viral loads of equal magnitude in IM and two out of three cases of subclinical seroconversion.[158]

The vaccination of healthy individuals against an infection not perceived as being serious demands a very safe and effective vaccine. Therefore, updated epidemiological data and more basic research on pathogenesis of IM are needed, and for this a good animal model is urgently required. The cotton-topped tamarin has been useful in the past for studies on tumour prevention, but is not a good model for IM. The recently discovered EBV-like lymphocryptovirus, naturally endemic in Rhesus monkeys, looks more promising, since oral infection induces an IM-like syndrome, followed by latent infection in B cells and oropharyngeal secretion.[159] The tumorigenic potential of the virus is under investigation, but it is hoped that experiments in this animal model will assist in unravelling the intricacies of EBV infection in health and disease.

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