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Biology and disease associations of Epstein–Barr virus

Dorothy H. Crawford

Division of Biomedical and Clinical Laboratory Sciences, Edinburgh University Medical School, Teviot Place, Edinburgh EH8 9AG, UK (*d.crawford@ed.ac.uk*)

Epstein–Barr virus (EBV) is a human herpesvirus which infects almost all of the world's population subclinically during childhood and thereafter remains in the body for life. The virus colonizes antibody-producing (B) cells, which, as relatively long-lived resting cells, are an ideal site for long-term residence. Here EBV evades recognition and destruction by cytotoxic T cells. EBV is passed to naive hosts in saliva, but how the virus gains access to this route of transmission is not entirely clear. EBV carries a set of latent genes that, when expressed in resting B cells, induce cell proliferation and thereby increase the chances of successful virus colonization of the B-cell system during primary infection and the establishment of persistence. However, if this cell proliferation is not controlled, or if it is accompanied by additional genetic events within the infected cell, it can lead to malignancy. Thus EBV acts as a step in the evolution of an ever-increasing list of malignancies which are broadly of lymphoid or epithelial cell origin. In some of these, such as B-lymphoproliferative disease in the immunocompromised host, the role of the virus is central and well defined; in others, such as Burkitt's lymphoma, essential cofactors have been identified which act in concert with EBV in the evolution of the malignant clone. However, in several diseases in which the presence of EBV has more recently been discovered, the role of the virus is unclear. This review describes recent views on the EBV life cycle and its interlinks with normal B-cell biology, and discusses how this interrelationship may be upset and result in EBV-associated disease.

Keywords: Epstein–Barr virus; primary infection; persistence; Epstein–Barr virus-associated diseases

1. INTRODUCTION

Epstein–Barr virus (EBV) is a member of the ancient and highly successful herpesvirus family. These viruses have coevolved with their respective hosts over millions of years, and during this time have developed sophisticated strategies for lifelong persistence that are beneficial for virus survival. Typically, following a subclinical or trivial primary infection, herpesviruses establish a lifelong latent infection in a specific cellular site. From here continuous low-level virus production or periodic reactivation provides infectious virus to effect spread to further susceptible individuals. This highly successful strategy ensures that the majority of the world's adult population is infected with multiple herpesviruses, and that these infections pose very little threat to the healthy host.

EBV persistently infects over 90% of adults regardless of geographical location. The virus latently infects circulating B lymphocytes, and although this infection is almost always controlled at subclinical levels, under certain conditions EBV is associated with malignant tumours (table 1).

EBV was first discovered in 1964 in B lymphocytes cultured from an African (endemic) Burkitt's lymphoma (BL) (Epstein *et al.* 1964), and the virus is now estimated to be present in around 96% of these tumours (Magrath 1990). Since its discovery, EBV has been found in a variety of other tumour types, in some of which a clear aetiological role for EBV has been established, whereas in others more work is required before a causal association can be made. In addition to BL, lymphoid tumours with an EBV association include B-lymphoproliferative disease in the immunocompromised host (Crawford *et al.* 1980), subsets of Hodgkin's lymphoma (Herbst & Niedobitek 1994) and certain types of T-cell lymphoma (Su 1996). Certain epithelial cell tumours have also been found to be EBV related, including virtually 100% of nasopharyngeal carcinomas (Raab-Traub 1992) and *ca.* 10% of stomach cancers (Takada 1999).

The oncogenic potential of EBV is reflected in its ability to infect and transform B lymphocytes into continuously growing lymphoblastoid cell lines (Pope *et al.* 1968). In depth study of this *in vitro* phenomenon has contributed to our extensive knowledge of the virus life cycle, but the exact mechanisms by which the virus enters, spreads and establishes latency in the normal host, and the pathogenesis of EBV-associated malignancies, remain unclear. This review discusses EBV infection and control in the normal host and outlines possible mechanisms by which disturbance of this balance could lead to EBV-associated disease.

2. EPSTEIN–BARR VIRUS IN THE NORMAL HOST

(a) *Primary infection*

Persistently infected individuals shed EBV either constantly or intermittently into saliva and thereby spread the virus to uninfected individuals through close

Table 1. *EBV-associated malignancies*

tumour	proposed cell of origin	approximate % EBV associations	viral protein expression	postulated cofactors
Burkitt's lymphoma	centroblast	African 96% sporadic 10–70% AIDS 30–40%	EBNA-1[a]	malaria, *c-myc* deregulation, HIV
BLPD[b]	B lymphoblast	90%	EBNA-1, EBNA-2, EBNA-3A, EBNA-3B, EBNA-3C, LMP-1[c], LMP-2A, LMP-2B	immunosuppression, HIV
Hodgkin's disease	centrocyte	40–80%	EBNA-1, LMP-1, LMP-2A, LMP-2B	IM[d]
T-cell lymphoma	T lymphocyte	10%	EBNA-1, LMP-1, LMP-2A, LMP-2B	chronic IM, immunosuppression
nasopharyngeal carcinoma	squamous epithelial cell	100%	EBNA-1, LMP-2A, LMP-2B	genetic and dietary factors
gastric carcinoma of stomach	epithelial cell	10%	EBNA-1, LMP-1, LMP-2A, LMP-2B	not known

[a] Epstein–Barr viral nuclear antigen.
[b] B-lymphoproliferative disease.
[c] Latent membrane protein.
[d] Infectious mononucleosis.

oral contact (Yao *et al.* 1985). Asymptomatic primary infection during early childhood is the rule where standards of hygiene are low, but in industrialized countries with high standards of living, many young children are protected from early infection. Primary infection then often occurs in adolescence when new social contacts are being made. Infection at this time causes infectious mononucleosis (IM) in 30–50% of cases (Steven 1996). The reason for this age-related disease association is not clear, although it has been suggested that virus dose may be a determining factor. Thus the small dose of virus ingested by young children sucking contaminated objects contrasts with the much larger dose which may be ingested by kissing between young adults. In addition, EBV has been detected in secretions from both male and female genital tracts (Israele *et al.* 1991; Sixbey *et al.* 1986) and therefore acquisition by sexual contact may occur, but whether this could cause IM has not been established.

Inevitably our knowledge of primary EBV infection comes from studying the symptomatic infection in IM. Here, following salivary transmission of EBV, infectious virus can be found in saliva indicating a site of productive infection in the oral cavity. However, the exact cellular site of this productive infection is controversial, with some workers proposing initial epithelial cell infection while others favour direct infection of B lymphocytes.

In order to infect the majority of the world's population, EBV must have evolved a highly efficient method of spread. Since most widespread viruses infect and replicate in surface epithelial cells, giving easy access to the outside world and the next susceptible host, many workers assume that EBV also uses this strategy. Thus, in the early 1980s, when it was reported that EBV could replicate in cultured squamous epithelial cells and viral DNA was detected in desquamated tonsillar epithelial cells from IM patients, this seemed to indicate that the virus infects oropharyngeal epithelial cells as a means of entering and exiting the body (Sixbey 1989). An initial round of replication in this cell type would serve to amplify the incoming virus and allow infection of B lymphocytes co-localized in the lymphoepithelium covering the palatine tonsils. However, to date it has not proved possible convincingly to demonstrate EBV infection of, or production by, squamous epithelial cells during primary infection. Study of epithelial cell infection has been hampered by the very low multiplicity of infection in cultured cells, the lack of the classic EBV B-cell receptor molecule (complement receptor 2, CR2) on epithelial cells (Young *et al.* 1989) and the inability to find EBV DNA in epithelial cells in IM tonsils, despite repeated attempts using highly sensitive techniques (Karajannis *et al.* 1997). However, under certain conditions EBV undoubtedly infects epithelial cells since latent infection occurs in the malignant cells of nasopharyngeal carcinoma and lytic replication is found in oral hairy leucoplakia lesions (Greenspan *et al.* 1985) (see §§ 3 and 4(b)).

In a recent study we used an indirect approach in an attempt to define the relative roles of B lymphocytes and epithelial cells in primary EBV infection. We studied the EBV status of six individuals with a rare genetic defect in Bruton's thymidine kinase gene (X-linked agammaglobulinaemia, XLA) which results in a complete lack of mature CR2-expressing B cells. Using a sensitive polymerase chain reaction assay to detect viral DNA, we found that none of the six XLA patients showed evidence of EBV carriage in blood or secretion in saliva. In addition, despite normal T-cell immune responses in XLA, none had EBV specific T-cell immunity, which was easily detectable in all healthy EBV sero-positive controls (Faulkner *et al.* 1999). These results suggest that, in the absence of B lymphocytes, squamous epithelial cells cannot support primary EBV infection.

In contrast to the inefficiency of EBV infection of cultured epithelial cells, *in vitro* infection of B lymphocytes, resulting in activation and continuous proliferation of latently-infected cells as immortalized lymphoblastoid cell lines (LCL), is a highly efficient process. This remarkable event is brought about by the products of nine well

Table 2. *EBV latent viral genes*

gene	gene product	postulated function
BKRF1	EBNA-1[a]	viral genome maintenance essential for *in vitro* immortalization of B cells
BYRF1	EBNA-2	viral oncogene essential for *in vitro* B-cell immortalization
BLRF3-BERF1	EBNA-3A	essential for *in vitro* B-cell immortalization
BERF2a-BERF2b	EBNA-3B	not known
BERF3-BERF4	EBNA-3C	viral oncogene essential for *in vitro* B-cell immortalization
BWRF1	EBNA-LP	not known
BNLF1	LMP-1[b]	induces lymphoblastoid phenotype tumour necrosis factor receptor superfamily member
BARF1/BNRF1	LMP-2A	prevents cell activation and lytic-cycle entry
BNRF1	LMP-2B	not known
BCRF1	EBER1, EBER2[c]	regulates PKC[d] activity upregulates *bcl2* on Burkitt's lymphoma cells
BARF0	BamH1A transcripts	not known

[a] Epstein–Barr viral nuclear antigen.
[b] Latent membrane protein.
[c] Epstein–Barr viral small RNA.
[d] Protein kinase c.

characterized latent genes which are the first to be expressed in the infectious cycle: six Epstein–Barr viral nuclear antigens, EBNA-1, EBNA-2, EBNA-3A, EBNA-3B, EBNA-3C and leader protein (LP), and three latent membrane proteins, LMP-1, LMP-2A and LMP-2B. In addition, two untranslated RNAs (EBER) and a family of transcripts from the BamH1A region of the genome are expressed but their functions are unknown. Our knowledge of the functions of the latent proteins is extensive but incomplete (table 2). EBNA-1, EBNA-2, EBNA-3A, EBNA-3C and LMP-1 are essential for *in vitro* B-cell immortalization and under the pivotal influence of the transactivator protein EBNA-2, they act in concert to drive cells into continuous proliferation, whilst fixing them at the lymphoblastoid stage of differentiation and blocking progression to lytic viral replication in the majority of infected cells (Kieff 1996).

In IM, B-lymphocyte infection at the portal of virus entry has now been convincingly demonstrated. Sections taken from tonsils surgically removed during acute IM, while lacking evidence of epithelial cell infection, contain demonstrable latently and lytically infected B cells (Anagnostopolous *et al.* 1995). Thus at the moment the weight of evidence favours direct EBV infection of B lymphocytes. This event possibly occurs in tonsillar crypts which are branched structures dipping down from the epithelial surface deep into the lymphoid tissue of the tonsil. Here the epithelial lining becomes discontinuous, allowing direct access of incoming virus to underlying lymphocytes (Faulkner *et al.* 2000). Such a mechanism of infection is unlikely to be very efficient, and perhaps to counteract this, the virus has evolved efficient strategies for colonizing the body and establishing latency. First, although latent infection is the usual outcome of B-cell infection, lytic replication has been demonstrated in some tonsillar B cells, and the virus produced can infect further B cells, giving rise to a nidus of infected cells. Second, expression of all the latent genes with the resulting proliferation of lymphoblasts serves to expand the pool of virus-infected cells in the body very effectively. During primary infection, these latently infected B lymphoblasts circulate in the blood (Tierney *et al.* 1994) and lodge in distant organs until they are controlled by the developing immunity. At this stage the natural, subclinical, childhood primary infection probably merges imperceptibly into lifelong persistence, but at an older age the induction of the immune response often heralds immunopathological disease (see § 2(b) below).

(b) *Infectious mononucleosis*

IM usually presents with the acute onset of fever, sore throat, and enlarged and painful lymph glands in the neck. However, the prime clinical feature characterizing the disease, and distinguishing it from other viral and bacterial throat infections, is the severe and debilitating fatigue which accompanies these symptoms and which may last for months after they have resolved. The incubation period of IM is thought to be around 30 days and during this time virus-infected B cells circulate in the blood and infiltrate the tissues (for a review, see Steven 1996).

Primary EBV infection induces both humoral and cellular immune responses that control but do not eliminate the infection. Antibodies are generated to both latent and lytic antigens, and those directed against the virus receptor on the viral envelope, glycoprotein 350 (gp350), prevent binding to CR2 on B cells and thereby limit viral spread and prevent reinfection. CD8-positive ($CD8^+$) cytotoxic T-cell responses are key to controlling primary infection, and these cells dominate the picture in IM, being present in the circulation and tissues in very high numbers. Typically a lymphocytosis of up to $15 \times 10^9\,l^{-1}$ is seen, with over 60% of cells being $CD8^+$ cells with an activated phenotype. These cells probably give rise to most of the symptoms and signs of IM (fever, lymphadenopathy, sore throat, hepatosplenomegaly, fatigue) as a result of massive production of cytokines, including lymphotoxin, tumour necrosis factor-α, interleukin (IL)-1β

and IL-6 (Foss *et al.* 1994). The reason for this exaggerated response to primary EBV infection is unclear. A viral superantigen has been proposed, (so called because these antigens elicit a strong primary T-cell response by binding to the T-cell receptor Vβ chain outside the classical peptide binding site), and although some *in vitro* evidence for an EBV superantigen has been presented (Sutkowski *et al.* 1996), this now appears unlikely to explain the T-cell response in IM, since recent studies indicate that the CD8 T cells do not show Vβ restriction and are almost all specific for particular viral peptides (Callan *et al.* 1996). Work using HLA:peptide tetramer staining techniques indicates that CD8 T-cell responses to lytic viral antigens usually predominate in IM, although responses to latent proteins also occur (Callan *et al.* 1998). T cells specific for individual lytic epitopes regularly account for up to 50% of the CD8 T-cell pool, whereas cytotoxic T lymphocytes (CTLs) to the immunodominant latent proteins EBNA-3A, EBNA-3B and EBNA-3C reach only 1–2% (Rickinson *et al.* 2000).

That CD8 T cells are essential for recovery from IM is exemplified by the consequence of primary EBV infection in immunocompromised individuals who are unable to mount the appropriate response. First, a rare genetic abnormality, X-linked lymphoproliferative syndrome (XLPS), which affects 50% of boys in families carrying the abnormal gene, classically results in an inability to control primary EBV infection (Purtilo 1983). Although able to control other herpesvirus infections, children with XLPS usually die of a fulminating IM-like syndrome with hepatic necrosis and bone marrow failure within weeks of acquiring EBV. Some children who survive this initial infection later develop B-cell lymphoma and/or dysgammaglobulinaemia. All tissues are infiltrated by CD8 T cells and contain scattered foci of proliferating EBV-infected B cells. It is postulated that the extensive tissue destruction seen in XLPS is caused by an uncontrolled cytotoxic T-cell response to primary EBV infection and the recent cloning of the XLPS gene supports this theory, although it has not entirely clarified the link with EBV (Sayos *et al.* 1998; Coffey *et al.* 1998).

The XLPS gene (called *SH2D1A* or *SAP*) is located on the long arm of the X chromosome and codes for an SH2-domain-containing protein that has been found to be mutated in the majority of XLPS cases analysed to date. The gene product, SAP (SLAM associated protein) is expressed in activated T cells and binds to the Signalling Lymphocytic Activation Molecule (SLAM) thereby inhibiting its function. Since SLAM is upregulated on activated B and T cells, and enhances their proliferation, an essential role for SAP in controlling T-cell activation in primary EBV infection is envisaged, although not yet proven (Howie *et al.* 2000).

Acquired T-cell deficiencies, particularly the iatrogenic immunosuppression required following solid organ or bone marrow transplantation to protect the graft from rejection, can also lead to problems with primary EBV infection. Although not often manifest as classical IM, presumably because immunosuppressive drugs prevent the development of cytokine secreting T cells to cause the symptoms, primary EBV infection can lead to B-lymphoproliferative disease (BLPD) and lymphoma in this situation (Thomas *et al.* 1990). Since EBV can be transmitted in an organ from a sero-positive donor (Haque *et al.* 1996), sero-negative recipients often become infected early in the post-transplant period when levels of immunosuppressive drugs are high, and so EBV sero-negativity is a risk factor for BLPD development (see § 4(a)).

Finally, in rare cases the symptoms of acute IM persist either continuously or intermittently for years as chronic IM. The cause of this condition is unknown, although it is likely to be immunological in nature. Aside from these rare occurrences, the vast majority of IM sufferers recover fully within six months.

(c) *Persistent infection*

Despite easily detectable immune mechanisms directed against both latent and lytic viral antigens, EBV persists for life following primary infection. In each individual viral load remains fairly constant over time at around 5–500 virus infected cells in every 10×10^6 circulating B cells (Miyashita *et al.* 1995). These cells carry the virus in a latent form, and there is low level continuous or intermittent production of infectious virus into saliva (Yao *et al.* 1985). Thus the virus has evolved a highly successful strategy of immune evasion which is not fully understood at present. Based on their recent work analysing EBV gene expression in B-cell subsets from peripheral blood and tonsil, Thorley-Lawson & Babcock suggest that in order to persist, the virus exploits normal B-cell biology to gain access to the long-lived memory B-cell compartment where it can reside long-term undetected by immunosurveillence mechanisms (Thorley-Lawson & Babcock 1999). They propose that the complex programmes of latent gene transcription patterns first defined by studying EBV-associated malignancies (see table 3), evolved to allow EBV-infected B cells to mirror events that occur when normal B cells are activated by specific antigens, thereby residing long-term in memory B cells.

Antigen-activated B cells enter lymph nodes and proliferate to form germinal centres each containing a clone of antigen-specific centroblasts. At this stage the *c-myc* oncogene is highly expressed and somatic hypermutation of immunoglobulin (Ig) genes occurs. Centroblasts mature into non-proliferating centrocytes that, in order to mature further, require two survival signals. First, the B-cell receptor (BCR) must bind specific antigen on the surface of follicular dendritic cells, and, second, B-cell surface co-receptor molecules, such as CD40, must bind to their ligands on antigen-specific CD4 T cells which deliver T-cell help. Failure to be selected in this way (the fate of the majority of centrocytes) leads to apoptotic death within the lymph node. In contrast, survival in the lymph node is the trigger for maturation into short-lived, antibody-secreting plasma cells or recirculating memory B cells (for a review, see MacLennan 1994).

Thorley-Lawson & Babcock (1999) suggest that during primary EBV infection infected lymphoblasts reach the germinal centre and switch from full latent gene expression to a programme where EBNA-2, which drives B-cell proliferation and inhibits differentiation, is turned off, and only EBNA-1 and the LMPs are expressed. This allows EBV to exploit the B-cell maturation pathways, with proliferation as centroblasts under the influence of cell-cycle progression genes such as *c-myc*. In EBV-infected centrocytes, the virus provides the two survival

Table 3. *Patterns of EBV latency*

type of EBV latency	EBV gene expression	tumours showing latency type
I	EBNA-1[a], EBER[b], BamH1A transcripts	Burkitt's lymphoma
II	EBNA-1, LMP-1[c], LMP-2A, LMP-2B, EBER, BamH1A transcripts	Hodgkin's lymphoma nasopharyngeal carcinoma
III	EBNA-1, EBNA-2, EBNA-3A, EBNA-3B, EBNA-3C, LP, LMP-1, LMP-2A, LMP-2B, EBER, BamH1A transcripts	lymphoproliferative disease

[a] Epstein–Barr viral nuclear antigen.
[b] Epstein–Barr viral small RNA.
[c] Latent membrane protein.

signals by the expression of the viral-coded LMP-1 and LMP-2A, which have operational functions similar to CD40 and BCR, respectively. Thus infected cells survive to emerge as plasma cells or memory B cells.

In normal sero-positive individuals virus carrying cells in peripheral blood are small resting memory B cells with a very restricted virus gene expression (Miyashita *et al.* 1997). To date there is no consensus on the latent viral genes expressed in these cells, although most studies report detection of LMP-2A transcripts (Qu & Rowe 1992), with others additionally detecting EBERs, BamH1A transcripts and/or EBNA-1 (Chen *et al.* 1995; Chen *et al.* 1999). How these cells survive long-term in the face of cytotoxic T cells specific for the latent proteins is not understood. An EBNA-1-only phenotype is an attractive hypothesis because EBNA-1 is the only viral antigen required for maintenance of the viral genome (Yates *et al.* 1985). In addition, the protein contains a glycine–alanine repeat region which prevents the molecule from processing in the proteosome. Thus no EBNA-1 peptides are presented at the cell surface and no CD8 T-cell response is generated (Levitskaya *et al.* 1995). However LMP-2A is immunogenic, so an alternative hypothesis suggests that, in an analogy with herpes simplex latency in neurons, LMP-2A transcripts may be present, but no EBV-coded proteins are expressed in resting memory cells. Alternatively, since these resting cells do not express the required co-stimulating molecules to interact with CD8 T cells, it is possible that they go unrecognized.

In order for the EBV life cycle to be completed, the latent infection in memory B cells must be activated to lytic replication in sites where the virus can spread to other individuals in saliva. Antigen activation of resting memory B cells leads to their homing to lymph nodes and proliferating to form a small secondary germinal centre with the production of antibody-secreting plasma cells and replenishment of the memory pool. Thus from time to time EBV-carrying memory B cells will become activated by antigen, and proliferate into germinal centres.

In lymphoblastoid cell lines plasma cell differentiation is linked to entry into the viral lytic cycle (Crawford & Ando 1986). If this is also the case *in vivo*, then EBV-infected plasma cells, which preferentially home to epithelial sites, could produce virus particles which would infect new B cells and/or spread via saliva to other individuals. At this stage both the cell proliferation, and the production of virus with the potential to infect more B cells, would serve to replenish the latently infected memory cell pool.

3. EPSTEIN–BARR VIRUS INFECTION IN THE IMMUNOCOMPROMISED HOST

There is an increasing number of individuals in whom T-cell function is impaired either iatrogenically, in order to treat chronic disease or retain grafted organs, or as a result of human immunodeficiency virus (HIV) infection. It is well documented that in the immunocompromised host the interplay between EBV replication, latency and immune control, which is so well balanced in the healthy host, can be deranged. Decreased EBV-specific cytotoxic T-cell activity allows increased virus replication and production in the oropharynx, and increased numbers of circulating latently infected B cells (Haque *et al.* 1997). Generally a new balance is set which is stable and causes no disease. However BLPD occurs in a small proportion of immunocompromised individuals (for a review, see Thomas *et al.* 1991*a*) and it is therefore important to understand the changes which predispose its development. In addition interesting lessons can be learnt about EBV persistence and control in the healthy host by studying a situation where lack of control may cause exaggeration of the normal picture.

We have recently carried out a prospective study measuring levels of EBV DNA in peripheral blood mononuclear cells (viral load) in a cohort of cardiothoracic transplant recipients regularly over a three-year period. In agreement with other smaller studies, we found viral loads raised above the normal range in around 50% of transplant recipients, with levels correlating with cumulative dose of immunosuppressive drugs.

Raised viral loads during immunosuppressive therapy could either be explained by proliferation of the resident virus-carrying B cells, or by increased lytic replication with infection of bystander B cells. The former would occur if CTL activity is essential in the healthy host to eliminate cells expressing latent genes, the latter if T cells are paramount in controlling lytic replication. To address this issue we analysed EBV gene transcription patterns in peripheral blood of our study participants. Despite high viral loads, around half of the recipients retained the restricted transcription pattern seen in healthy EBV carriers (EBERs, LMP-2A) throughout the study period. Since the viral genes responsible for cell proliferation (EBNA-2, LMP-1) are not expressed in these cells, it is unlikely that cell proliferation could account for the raised viral load. Rather, it is more likely that increased numbers of EBV-infected cells gain access to the B-cell

memory compartment. This could result from reduced T-cell control of lytic replication, leading to increased shedding of virus and more infection of B lymphocytes at that site. In line with this interpretation, we found transcripts for the late lytic membrane protein gp350 in combination with EBERs and LMP-2A transcripts in peripheral blood mononuclear cells of 26% of recipients (P. A. Hopwood, L. Brooks, R. Parratt, B. J. Hunt, M. Bokhari, J. A. Thomas, M. Yacoub and D. H. Crawford, unpublished data). Thus in these patients T-cell control of lytic replication was reduced sufficiently to allow virus production in circulating B cells.

Lytic replication and virus production in peripheral blood with infection of naive B cells in the circulation, would give rise to cells expressing all the latent viral genes and a lymphoblastoid phenotype. In contrast to other recent studies (Babcock *et al.* 1999) we detected full latent gene expression in peripheral blood mononuclear cells from 17% of recipients. This occurred sporadically, at a time when levels of immunosuppressive drugs were high, and its occurrence correlated with detection of lytic transcripts. In the model of EBV persistence outlined above, these lymphoblasts are a transient stage in the progression to viral persistence in the memory pool. They home to lymph nodes, proliferate in germinal centres and emerge as virus-carrying memory B cells or virus-producing plasma B cells. Since all the recipients who demonstrated full latent viral gene expression in peripheral blood in our study remained healthy and did not develop post-transplant lymphoproliferative disease (PTLD) during the study period, we suggest that this sequence of events is normally self-regulatory. However, if at any stage during these events EBV transcription became fixed in a pattern which drives cell proliferation rather than differentiation, then PTLD may result. Alternatively, the increased cell proliferation in the lymph node predisposes to additional genetic events occurring, which may again favour PTLD outgrowth (see § 4(a)).

Our findings suggest that control of lytic replication is key to controlling latent infection by regulating the number of new infection events which occur in the body. Since new infection events produce cells which transiently express the lymphoblastoid phenotype and therefore have the capacity to proliferate, these cells are potentially dangerous to the host. Thus the recent findings that CTLs directed against lytic antigens are immunodominant in both acute primary and persistent EBV infection (Rickinson *et al.* 2000) may be explained in evolutionary terms as the first line of protection from the potential for B-cell proliferation. CTL to latent antigens would act as a second line of defence by attacking the proliferating lymphoblasts.

Immunosuppression can also lead to the benign epithelial cell disease oral hairy leucoplakia (OHL) (Greenspan *et al.* 1985). This white corrugated plaque-like lesion is usually found on the lateral margin of the tongue in HIV-positive people.

The lesion is painless and self-limiting. Histological features include intracellular oedema of the spinous layer and superficial hyperparakeratosis. OHL is a focus of EBV replication in the superficial layers of the squamous epithelium covering the tongue, thereby demonstrating that EBV can infect and replicate in non-malignant epithelial cells. EBV DNA and lytic-cycle antigens are detected in the lesion, but are confined to the superficial, more differentiated epithelial cells (Thomas *et al.* 1991*b*). These findings suggest that the lesions arise by continual virus production and reinfection within the superficial epithelial layers, with no evidence of epithelial cell persistence or latency. However, whether this represents an amplification of the normal life cycle of EBV due to reduced T-cell surveillance, or infection of an aberrant cell type related to HIV infection, is not clear.

4. PATHOGENESIS OF EPSTEIN–BARR VIRUS-ASSOCIATED MALIGNANCIES

Although EBV infection is common, the associated malignancies are rare, and despite virus infection being ubiquitous, many of the malignancies are geographically restricted. These facts indicate that, like most virus-associated tumours, additional factors are involved in tumour development. So although EBV may by essential for tumorigenesis, it is not generally sufficient on its own. Consequently much work has been devoted to understanding the role of EBV in tumour formation and the cofactors required for tumour outgrowth. EBV-associated malignancies clearly identify the dual tropism of the virus for cells of lymphoid or epithelial origin (table 2).

This review speculates on the pathogenesis of each tumour by considering how the cell phenotype might have arisen from the persistent infection outlined above.

(a) *Epstein–Barr virus-associated lymphoid malignancies*

EBV is associated with three lymphoid tumours: Burkitt's lymphoma, B-lymphoproliferative disease in the immunocompromised host and Hodgkin's lymphoma. Although these are all of B-cell origin, they differ markedly in their clinical presentation, immunohistological features and cofactor dependence.

(i) *Burkitt's lymphoma*

Burkitt's lymphoma (BL) was first identified as a tumour of the jaw occurring in children in equatorial Africa where it is the commonest childhood cancer (Burkitt 1958; for a review, see Magrath 1990). BL is often multifocal and almost always arises in extra-nodal sites. In addition to the jaw, other common sites include the ovary, mammary gland, liver, intestine and kidneys. BL is a fast-growing tumour which is rapidly fatal if untreated, although chemotherapy can give good long-term survival. In contrast to the high incidence of BL in equatorial Africa, the tumour only occurs sporadically and at low incidence worldwide.

Histologically BL consists of rapidly proliferating, small, non-cleaved B cells infiltrated with large, pale staining histiocytes which give the classic 'starry sky' pattern. Although the tumour arises in extra-nodal sites, phenotypically the cells resemble germinal centre B cells.

BL development shows the classic multistep process typical of virus-related tumours, with at least three identifiable factors cooperating in tumour outgrowth. These

include malaria, EBV and constitutive expression of the *c-myc* oncogene.

Burkitt's lymphoma and Epstein–Barr virus

Although EBV DNA is present in tumour cells of around 96% of African BL, the association with the non-African (sporadic) BL is less constant. A figure of around 20% is quoted for North American and European tumours, with intermediate values (50–70%) reported for South America, North Africa and Russia (Sixbey 2000). The EBV genome is present in BL cells as multiple nuclear episomes, and although there is some evidence for chromosomal integration of whole or part genomes in some BL lines, this is not always the case (Delecluse *et al.* 1993). Examination of the terminal repeat sequences of the EBV genome in BL tumour tissue shows that it is clonal, suggesting that virus infection is an early event occurring before the expansion of the malignant clone (Raab-Traub & Flynn 1986). Analysis of the viral antigen expression in malignant B cells reveals only EBNA-1 (Rowe *et al.* 1986), although EBERs and BamH1A transcripts are also present. Since EBNA-1 is the minimum requirement for maintenance of the genome in dividing cells (Yates *et al.* 1985), and is not recognized by cytotoxic T cells (Levitskaya *et al.* 1995), this limited gene expression clearly enables cells to evade immunosurveillance mechanisms. The sole expression of EBNA-1 in BL has cast doubt on the role of the virus in tumour outgrowth, but since EBNA-1 transgenic mice have recently been shown to develop lymphoid tumours, the protein may have an oncogenic function *in vivo* (Wilson *et al.* 1996). In addition, recent *in vitro* evidence suggests that EBER transcripts induce the anti-apoptotic gene *bcl-2* (Komano *et al.* 1999) and thus give the cells a survival advantage.

Phenotypically BL cells resemble centroblasts, the antigen-activated rapidly proliferating cells in lymph nodes which form germinal centres. These cells are CR2 positive and it is therefore possible that this cell type is infected by EBV *de novo*. However it is probably more likely that the EBV-infected centroblasts of BL are derived from newly infected tonsillar B cells which have undergone virus-driven blast transformation and homed to lymph nodes (see § 2(c)). Proliferating centroblasts are normally subject to hypermutation events which serve to increase the affinity of the BCR for antigen, and effects their survival within the germinal centre. The recombinase activating genes *RAG1* and *RAG2* play a vital part in this process, and these molecules are upregulated by EBNA-1 (Srinivas & Sixbey 1995). Thus EBV infection could predispose to genetic mistakes occurring during B-cell maturation.

Genetic abnormalities in Burkitt's lymphoma

All BL tumours, whether EBV associated or not, show one of three chromosomal translocations (8:14, 8:2 or 8:22) which brings the *c-myc* oncogene on chromosome 8 under the influence of the Ig heavy chain on chromosome 14, or light chain (kappa on chromosome 2, lambda on 22) genes and cause its constitutive expression (Zech *et al.* 1976). *c-myc* is a cellular oncogene which drives the cell to continuous proliferation and inhibits differentiation—thus this translocation is thought to be pivotal in BL development. Many BL tumours examined also show mutations in the tumour suppressor gene *p53* (Farrell *et al.* 1991). This is the commonest gene mutation seen in human cancers, and is generally thought to be a late event in tumour evolution.

Burkitt's lymphoma and malaria

Burkitt first noted clustering of BL cases, and on the basis of their occurrence in low-lying equatorial areas that coincide with the geographical limits of holoendemic malaria, he postulated that malaria was a cofactor for BL development (Burkitt 1969). This has since been substantiated by the finding that where the incidence of malaria has fallen due to mosquito eradication programmes there has been a parallel fall in the incidence of BL. Furthermore, the resistance to malaria infection afforded by the sickle cell trait also protects from BL. However the exact role of malaria in BL development is unclear.

In Africa, almost all children are persistently infected with EBV by the age of two years, and during an attack of malaria children show high viral loads in peripheral blood which return to normal levels on recovery (Lam *et al.* 1991). This increased viral load could be due to proliferation of existing virus infected cells or increased lytic replication with infection of bystanding B cells. Both these events could be caused by the immunosuppressive effect of malaria that have been shown to decrease the levels of EBV-specific CTL during an acute attack (Whittle *et al.* 1984). Additionally, certain malaria parasite antigens cause polyclonal activation of B cells (Greenwood & Vick 1975) with hyperplastic lymph nodes seen in chronically infected cases. This effect may also activate EBV-infected memory B cells causing expansion of the infected B-cell pool. It is postulated therefore that children living in holoendemic malaria areas, who have several attacks of malaria every year until solid immunity is established at around adolescence, have an imbalance in the control of their persistent EBV infection.

Pathogenesis of Burkitt's lymphoma

Taking the above facts into account, the following steps can be postulated in the development of BL.

(i) Primary EBV infection at an early age with establishment of viral persistence in circulating B cells.
(ii) Recurrent bouts of malaria during which immunosuppression leads to increased lytic viral replication with virus production, EBV infection of naive B cells, blast transformation and germinal centre formation.
(iii) Polyclonal B-cell activation by malaria antigens causes blast transformation of EBV-infected resting memory B cells with secondary germinal centre formation.
(iv) EBNA-1 expression in proliferating centroblasts (with highly active *c-myc* genes) enhances and prolongs *RAG* expression and predisposes to mistakes occurring during the hypermutation process.
(v) Recriprocal chromosomal translocation between the *c-myc* and Ig gene loci giving constitutive myc expression, enhanced cell proliferation with blocked cell differentation.
(vi) Outgrowth of EBNA-1-positive malignant cells invisible to immune mechanisms.

Table 4. *Histological classification of post-transplant lymphoproliferative disease (PTLD)*

early lesions including infectious mononucleosis-like reactive plasma cell hyperplasias
polymorphic PTLD, which may be polyclonal or monoclonal
monomorphic PTLD of diffuse large B cell lymphoma (immunoblastic, centroblastic or anaplastic) or Burkitt's type
T-cell lymphoma
other, including Hodgkin's disease, plasmacytoma-like lesions and myeloma

(vii) Additional genetic abnormalities such as *p53* mutations in tumour cells.

BL also occurs in HIV-positive people (Ziegler *et al.* 1982) where it is recognized as an AIDS defining illness. Here BL constitutes one of a variety of histologically diverse lymphomas, only a minority of which are EBV related. In the above postulated stepwise progression to BL, HIV replaces malaria in the pathogenesis (Lenoir & Bornkamm 1987) since it is known to cause both immunosuppression and polyclonal B-cell activation (Lane *et al.* 1983). In this regard it is interesting to note that BL occurs relatively early in the evolution of HIV disease, when immunosuppression is minimal. At this stage HIV-induced B-cell activation causes a florid reactive hyperplasia in lymph nodes (recognized clinically as progressive generalized lymphadenopathy) which could predispose to a chromosomal translocation between the *c-myc* and Ig loci.

(ii) *B-lymphoproliferative disease*

B-lymphoproliferative disease (BLPD) occurs in up to 10% of transplant recipients, and rarely in other immunocompromised patient groups (for a review, see Hopwood & Crawford 2000). Predisposing factors include high dose immunosuppressive therapy and primary EBV infection. Thus those who are EBV negative prior to transplant and undergo primary infection while on high dose immunosuppression are particularly at risk of BLPD. Since the virus can be transferred from donor to recipient in the donor organ (Haque *et al.* 1996), BLPD associated with primary EBV infection tends to occur early after transplant. Also, as young people are most likely to be EBV negative, the disease is more common in children than adults.

BLPD presentation depends on the site of the tumour, but often includes prominent non-specific symptoms such as a persistent febrile illness with lymphadenopathy which may be misinterpreted as opportunistic infection or graft rejection. The tumour is more often nodal than extra-nodal, although the brain, gut and transplanted organ are also common sites.

Diagnosis of BLPD is made on histological appearances of biopsy material, where a variety of patterns are seen, covering a spectrum from polyclonal polymorphic lesions to monoclonal, monomorphic B-cell lymphoma (table 4). EBV association is assessed by *in situ* staining for EBER mRNA (the most abundant RNA in latently infected cells), and immunocytochemical staining for latent viral antigens. Around 90% of tumours are EBV positive, and the majority expresses a lymphoblastoid phenotype with full latent viral gene expression. In most tumours a few cells support lytic viral replication. More restricted viral gene expression patterns have also been described, either in individual tumour cells (Oudejans *et al.* 1995) or in the tumour as a whole (Cen *et al.* 1993). These include a BL-like EBNA-1-only phenotype, and these tumours sometimes show *c-myc* oncogene rearrangements (Hunt *et al.* 1996). Other chromosomal changes have been described, but no single abnormality is characteristic of the disease.

If left untreated BLPD is generally fatal, and even with treatment the mortality can be high (Armitage *et al.* 1991). First-line treatment is reduction of immunosuppressive therapy which leads to tumour regression in the majority of early cases, particularly at the polyclonal stage of the disease (Starzl *et al.* 1984). However, except in the case of renal transplantation, where the graft can be lost without fatal consequences, this line of treatment is difficult to maintain because of the consequent graft rejection. Relapses are common and resistance develops as the disease progresses to the more malignant forms. Other types of therapy are required, but there is no consensus, with some transplant centres using chemotherapy and others preferring more recently introduced immunotherapeutic methods. The latter include antibody therapy directed at B-cell specific surface molecules (CD20,21) (Fischer *et al.* 1991; Cook *et al.* 1999), and infusion of EBV-specific cytotoxic T cells (Rooney *et al.* 1995). These new approaches are in the early stages of assessment, but both show promise as non-toxic alternatives to conventional lymphoma therapy.

Pathogenesis of B-lymphoproliferative disease

Most BLPD tumours consist of lymphoblastoid cells which express all the latent viral genes, including the known viral oncogenes EBNA-2 and LMP-1. Thus there is little doubt that EBV plays a key role in driving cell proliferation and tumour development in this case. In the model for EBV persistence previously described (see § 2(c)), cells of this phenotype are generated in lymph nodes where they mature into memory B cells. Thus the lymph node site of origin of BLPD fits with the model. However, we have detected virus-infected B cells in the circulation of 17% of healthy transplant recipients (see § 3), indicating that even in the absence of adequate T-cell control these cells do not proliferate in an uncontrolled manner but proceed down the defined maturation pathway. What then is required to turn an EBV-infected cell into a tumour cell? Any genetic or epigenetic event which interrupts the normal B-cell maturation pathway and fixes the cell at the lymphoblastoid stage of differentiation would allow continued cell proliferation. In this regard it has been suggested that an imbalance of cytokine production may be critical for initial outgrowth of BLPD. This hypothesis is based on findings in the severe combined immunodeficient (SCID) mouse model for BLPD. SCID mice lack mature B and T cells and therefore accept grafted tissue from other animal species

(Ansell & Bancroft 1989). When peripheral blood mononuclear cells from normal EBV-positive individuals are inoculated into such mice, they grow into BLPD-like tumours in a proportion of cases (Mosier *et al.* 1988). Although of B-cell origin, this tumour outgrowth entirely depends on the presence of CD4 T cells in the inoculum (Johannessen *et al.* 2000). We believe that these T cells produce cytokines essential for tumour outgrowth, which, once the tumour is established, the tumour cells produce for themselves in an autostimulatory manner. Evidence for this comes from the detection of transcripts for IL-2, IL-4, IL-6, IL-10 and interferon gamma in SCID mouse-passaged tumour material and their localization to the tumour cells using *in situ* techniques. In the human situation BLPD tumours contain a heavy infiltrate of CD4 Tcells (Perera *et al.* 1998), and tumour cells display the same cytokine profile as in the SCID mouse-passaged lesions (M. Ashgar, S. Howie & D. H. Crawford, unpublished data). This initial cytokine dependence could partly explain the distribution of BLPD since lymph nodes and gut submucosal lymphoid aggregrates are rich in CD4 T cells, and transplanted organs are often the site of rejection events mediated primarily by CD4 lymphocytes.

BLPD is also common in HIV-positive people, particularly in the late stages of AIDS when CD4 counts are low and immunosuppression is intense. The most common site for AIDS BLDP is the brain, and almost all of these tumours are EBV positive (MacMahon *et al.* 1991). At present it is not understood how, and at what stage in the disease, these EBV-infected B cells enter the brain. It is assumed that they grow there preferentially because it is an immunoprivileged site, although it is not clear why this is necessary in the face of intense immunosuppression.

(iii) *Hodgkin's lymphoma*

Hodgkin's lymphoma (HL) shows characteristic pathological, epidemiological and virological features which distinguish it from other lymphomas. The tumour accounts for around 20% of all lymphoma in the western world, is the most common lymphoma in young people, and has a rising incidence. HL shows a bimodal age-incidence curve with one early peak and another in middle to old age. The early peak varies geographically, involving children where the standard of living is low and young adults in Westernized societies (for a review, see Mueller 1997).

Histopathologically HL is typified by the presence of large multinucleate Reed–Sternberg cells (RSC) and Hodgkin's cells which, although in a minority in the tumour tissue (1–2%), together constitute the malignant element. The origin of these cells has long been disputed, but it is now clear from studying their Ig genes that they are derived from B lymphocytes and are clonal. RSC are surrounded by a heavy infiltrate of reactive mononuclear cells which make up the bulk of the tumour tissue. The nature of this cellular infiltrate classifies HL into five histological subgroups: lymphocyte predominant; mixed cellularity; nodular sclerosing; lymphocyte rich classical; and lymphocyte depleted. Of these, the lymphocyte predominant form of the disease is now considered a distinct (non-EBV associated) entity and the following discussion refers to the remaining subgroups, which together constitute 'classical' HL.

Hodgkin's lymphoma and Epstein–Barr virus

An association between EBV and HL has been recognized for many years, based on the finding of a three-fold increase in risk of HL with a history of IM (Gutensohn & Cole 1980) and an altered pattern of serum antibodies to EBV antigens before tumour development (Mueller *et al.* 1989). However, it was not until the late 1980s that the association was confirmed by the demonstration of clonal EBV DNA in a subset of HL (Weiss *et al.* 1987). The EBV genome was subsequently localized to RSC (Wu *et al.* 1990), which were also shown to express high levels of the LMP-1 protein in the absence of EBNA-2 (Pallesen *et al.* 1991).

It is now clear that these cells display a restricted form of EBV latency with only EBNA-1, LMP-1 and LMP-2A protein expression in addition to EBER and BamH1A transcripts detected.

EBV is most commonly found in early childhood and old age HL and, though seen in tumours across the histological spectrum, shows the strongest association with the mixed cellularity subtype. Overall EBV is detected in approximately 50% of HL in Western societies, with higher incidences reported from developing countries, even approaching 100% in studies from South America. In addition EBV is overrepresented in HL occurring in the immunocompromised setting, particularly in HIV infected individuals.

Pathogenesis of Epstein–Barr virus-related Hodgkin's lymphoma

It has long been recognized that the epidemiology of HL is consistent with that of a disease caused by a common infectious agent which in most communities is acquired in childhood but which in the developed world may be acquired in adolescence or later. Thus the identification of EBV DNA in a proportion of HL appeared to be precisely in line with the above prediction and to reinforce even more strongly the apparent association between the young adult HL in Western countries and IM. However, the situation is not as first expected since HL among young adults actually shows the lowest EBV association. In addition, no clear-cut correlations with EBV status have yet been found, either in HL tumour cell phenotype or at the level of prognosis and treatment outcome. Larger prospective studies are required to assess these associations adequately.

At the cellular level, RSC are now recognized as B cells with hypermutated Ig genes, indicating that they have traversed the germinal centre and are arrested at the centrocyte or post-centrocyte stage of B-cell differentiation. Centrocytes are normally resting cells, which are prone to apoptosis but EBV infection with expression of LMP-1 is presumably key to providing growth and survival signals. The role of LMP-2A in the malignant phenotype is less clear. Based on the findings that Ig gene mutations in classical HL are non-productive, Thorley-Lawson and Babcock suggest that LMP-2A provides the BCR signals required for survival of the malignant cells (Thorley-Lawson & Babcock 1999).

(iv) *Epstein–Barr virus and T-cell lymphoma*

EBV DNA was first demonstrated in T-cell lymphomas from two cases of chronic IM in 1988 (Jones *et al.* 1988) and since then, the virus has been found associated with

a variety of T-cell lesions (for a review, see Su 1996). These include nasal and extranasal angiocentric T–NK cell lymphoma, angioimmunoblastic lymphadenopathy, and peripheral T-cell lymphoma. EBV-associated tumours may show a CD4 or CD8 cell phenotype, and the malignant cells generally display a similar pattern of latent viral gene transcription to that seen in HL, i.e. EBER, EBNA-1, LMP-1 and LMP-2A, although at the protein level LMP-1 expression is often weak and heterogeneous within the tumour cell population.

Activated T cells express the EBV receptor CR2 (Fingeroth *et al.* 1988) and *in vitro* EBV infection of certain T-cell lines has been achieved (Watry *et al.* 1991). In addition, recent studies have demonstrated EBV-infected T cells *in vivo* in IM and healthy EBV carriers (Su 1996), but at the present time it is unclear how these rare cells relate to the malignancies, and the role of EBV in tumour production.

(b) *Epstein–Barr virus-associated epithelial cell malignancies*

EBV has classically been associated with undifferentiated nasopharyngeal carcinoma. This is an unusual tumour, often termed a lymphoepithelioma because of the intense infiltration of lymphocytes that surround and outnumber the malignant epithelial cells. Similar lymphoepitheliomas occasionally occur in other tissues, such as salivary gland, lung, thymus and stomach, and these have also been linked to EBV (Niedobitek & Herbst 1994). Little is known about the involvement of EBV in the pathogenesis of these tumours and they will not be discussed further in this review. However, a recent more general association between EBV and gastric cancer will be considered briefly.

(i) *Nasopharyngeal carcinoma*

Nasopharyngeal carcinoma (NPC) is a tumour of the squamous epithelium of the post-nasal space, which shows marked geographical restriction. The tumour occurs at very high incidence in southern China, where it is the commonest malignancy in men and second commonest in women. NPC also has a high incidence in the Inuit in North America and Greenland, Malaysians, Dyaks, Indonesians, Filipinos and the Vietnamese; a moderately high incidence in North Africa, Sudan and Kenya; and is rare in the rest of the world. NPC generally occurs in middle or old age, but an additional younger age peak is also found in North Africa (for a review, see Raab-Traub 1992).

NPC arises from squamous epithelial cells and is classified according to the degree of differentiation shown by the tumour cells. 70% are undifferentiated with the remainder being either non-keratinizing or squamous cell carcinomas. The undifferentiated and non-keratinizing forms usually contain a large infiltrate of non-malignant T cells. The primary tumour metastasises early to cervical lymph nodes, and node enlargement is the presenting symptom in around one third of cases. Other cases present with nasal obstruction, bleeding, discharge, deafness, tinnitus or cranial nerve palsy caused by the primary tumour. Diagnosis is made on histological examination, and the treatment of choice is radiotherapy which gives a five-year survival of 60% in early stage disease.

Nasopharyngeal carcinoma and Epstein–Barr virus

The association between EBV and NPC was first made on serological grounds in 1966 (Old *et al.* 1966) and this was later confirmed by the demonstration of EBV DNA in the malignant epithelial cells (Wolf *et al.* 1973). 100% of undifferentiated NPC is EBV-associated regardless of geographical location and some studies suggest that the more differentiated tumours are also EBV related (Raab-Traub *et al.* 1987), although this remains controversial. The malignant epithelial cells of NPC show a restricted pattern of latent EBV gene transcription, qualitatively similar to that seen in HL, with EBNA-1, LMP-1, LMP-2A and LMP-2B protein expression as well as EBER and BamH1A transcripts (Brooks *et al.* 1992). Again, as in some EBV-positive T-cell lymphomas, LMP-1 protein levels range from those of LCLs to undetectable. A minority of cells in NPC support early lytic replication (Martel-Renoir *et al.* 1995).

Pathogenesis of nasopharyngeal carcinoma

The 100% association between EBV and undifferentiated NPC is persuasive evidence of an aetiological role for the virus in NPC development. However tumour outgrowth is clearly a complex process in which the virus is only one contributing factor. The viral oncogene LMP-1 is known to inhibit the differentiation of squamous epithelial cells in culture (Dawson *et al.* 1990) and to induce a tumorigenic phenotype in animal models (Nicholson *et al.* 1997). Since in the majority of NPC the tumour cells express LMP-1, it is generally assumed that this contributes to the malignant phenotype.

Other factors which go some way to explaining the geographical distribution of NPC include a genetic predisposition, part of which maps to an as yet unidentified region in the MHC locus (Lu *et al.* 1990) and several putative environmental and/or dietary factors such as the use of snuff made from traditional Chinese herbal remedies and the ingestion of salt fish dishes containing carcinogenic nitrosamines (Armstrong *et al.* 1998).

(ii) *Gastric carcinoma*

Gastric carcinoma is a common tumour which occurs throughout the world. Reported EBV associations vary between studies from different geographical locations from 4% to 18% of all gastric cancers. Also the EBV association varies between the histological types of gastric carcinomas with over 80% of the rare lymphoepithelioma-type tumours being EBV positive and virtually none of the intestinal and diffuse types (Takada 1999). In EBV-associated tumours viral DNA is found as a non-integrated episome that is clonal and present in all the tumour cells. Viral gene transcription in such tumours is closer to BL than to NPC with EBNA-1 only, or EBNA-1 with LMP-2A mRNA present in a proportion of cases, but no detection of LMP-1 or LMP-2B (Imai *et al.* 1994). The BamH1A and EBER transcripts are always expressed. It is not clear how EBV infects gastric epithelial cells since CR2 is not expressed. However, CR2-negative carcinoma cell lines can be infected with EBV *in vitro* and the resultant viral gene expression mirrors that of the *in vivo* tumour described above (Takada 1999). The relative roles of

EBV and of related cofactors, such as *Helicobacter pylori* infection and genetic abnormalities, remains to be elucidated.

5. CONCLUDING REMARKS

EBV is a highly successful human parasite. It persists harmlessly in over 90% of the world's population, but under certain conditions can cause fatal disease. Unravelling the mechanisms of harmless persistence and malignant cell transformation is an enormously complex task. However, recent advances in technology have produced the sensitivity required to examine EBV persistence in detail, and our knowledge has expanded accordingly. Although the role of EBV in the wide spectrum of its associated diseases is still far from clear, the very strong circumstantial evidence linking this virus to various forms of human cancer is a considerable spur to the further development of a vaccine. A vaccine to prevent EBV infection would not only have the potential to relieve the world of a substantial cancer burden but could also provide definitive proof of an essential role for the virus in human tumour aetiology.

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