

Editorial

Themed issue: the biology and pathology of the Epstein-Barr virus

Based on their genomic organisation, tissue tropism, and other biological characteristics, herpesviruses are classified into three categories: α , β , and γ . Of these, the γ -herpesviruses are able to replicate and persist in lymphoid cells and some are capable of infecting other cell types, such as epithelial cells and fibroblasts. The γ -herpesviruses comprise two important genera: the lymphocryptoviruses (also referred to as γ -1 herpesviruses) and the rhadinoviruses (γ -2 herpesviruses).

This themed issue principally concerns the biology and pathological effects of one of the human γ -herpesviruses—the Epstein-Barr virus (EBV)—which is a γ -1 herpesvirus carried by over 90% of the world's adult population as a lifelong asymptomatic infection. EBV is of particular interest to cell biologists, virologists, and pathologists alike because it is epidemiologically, serologically, and directly (by virtue of the detection of the virus genome and gene products in tumour cells) linked to a variety of human cancers. EBV associated cancers include several lymphoid disorders (Burkitt's lymphoma, Hodgkin's disease, post transplantation/human immunodeficiency virus associated lymphoproliferative disease, and some T cell lymphomas) and epithelial tumours (nasopharyngeal carcinoma and gastric carcinoma). All of these tumours are characterised by the presence of multiple extrachromosomal copies of the circular viral genome (episome) in every tumour cell and the expression of EBV encoded latent genes, which contribute to the malignant phenotype. The challenge is to understand the role of this virus in the development of its associated malignancies in the hope that this will provide alternative means to prevent or treat these tumours.

In the opening article in this issue, John Nicholas¹ overviews the organisation of γ -herpesvirus genomes and discusses mechanisms of genomic variation between different virus groups. The γ -herpesvirus genomes are organised into blocks of genes that are conserved across all γ -herpesviruses. These so called “core” genes include those that function as “housekeeping” genes, often encoding proteins that are crucial for infection, virus replication, or virion assembly, which explains why they are so well conserved. Other γ -herpesvirus genes may be regarded as subfamily specific (that is, confined to only γ -1 or γ -2 viruses) or virus specific (that is, unique or partially conserved genes). This latter group includes cellular homologues that have been acquired relatively recently (in evolutionary terms) or ancient genes that have been lost by other members of the subfamily.

Genomic similarities and differences between the γ -herpesviruses are well illustrated by comparing the EBV genome with that of another important oncogenic herpesvirus, the Kaposi's sarcoma associated herpesvirus (KSHV), also known as human herpesvirus 8 (HHV-8), which is a γ -2 virus. KSHV is associated with the development of Kaposi's sarcoma and two rare lymphoproliferative diseases, multicentric Castlemans disease and pleural effusion lymphoma.² The EBV and KSHV genomes have

several genes with sequence/functional similarities. Thus, the EBV encoded nuclear antigen 1 (EBNA1) protein and the KSHV encoded latency associated nuclear antigen (LNA or LANA) are related nuclear proteins that play essential roles in episome maintenance and virus replication. Likewise, the EBV latent membrane protein 2 (LMP2) gene and the KSHV K15 gene are located at similar genomic locations (when the linear genomes are compared alongside each other) and both encode proteins containing 12 transmembrane domains, which function to inhibit B cell receptor signalling and thereby maintain virus latency in B cells.¹ However, although these viruses share genomic similarities there are differences that reflect development along distinct evolutionary pathways. For example, the KSHV genome encodes several cellular homologues not present in EBV.² These include, among others, viral interleukin 6 (vIL-6) and viral cyclin D (v-cyclin D) genes. However, LMP1 is able to induce the expression of both cellular IL-6 and cellular cyclin D genes.^{3–4} Therefore, both viruses have evolved different means (expression of viral homologues in the case of KSHV versus induction of cellular genes by EBV) to produce the same functional endpoints. This tells us that such gene functions are likely to be important for γ -herpesvirus physiology in general.

In fact, much more is now known about the functions of some of the virus genes expressed by EBV during virus latency (see Young *et al*, this issue⁵). Much of the study of latent gene function has focused on EBV infection of B cells because this is the cell type most easily infectable *in vitro* and also the most likely candidate for the natural site of virus persistence in the normal carrier. Of the latent genes, LMP1 has been the focus of particularly intense investigation. LMP1 is a transmembrane protein that has been shown to be transforming in several different situations and, together with several other latent genes, has been shown to be essential for EBV induced immortalisation of B cells. LMP1 has little sequence homology with any known mammalian proteins, although it retains the ability to initiate signalling along several pathways, which include the nuclear factor- κ B (NF- κ B) and JNK (c-Jun NH₂-terminal kinase) pathways—a function reminiscent of other transmembrane proteins of the tumour necrosis factor receptor (TNFR) family.^{6–7} However, unlike normal TNFRs, which require ligand binding for their activation, LMP1 is constitutively activated in infected cells. Blocking the important signalling activities of LMP1 abrogates transformation and testifies to the importance of such events in the transformation process.

Like LMP1, EBNA2 is also essential for B cell transformation.⁵ This was demonstrated several years ago in experiments showing that EBV strains deleted for the EBNA2 coding regions were non-transforming and that restoration of the EBNA2 sequence to these strains restored their transformation ability. More recent studies of EBNA2 function have provided important insights into its function during infection. EBNA2 mimics the activated

intracellular form (NotchIC) of the Notch receptor. NotchIC is produced by cleavage of the Notch receptor in response to ligand binding, whereupon it translocates to the nucleus and removes the transcriptional repression imposed by the DNA binding factor, CBF1 (also called RBPJk). NotchIC not only diverts cell fate determination but is also associated with the development of malignancy. By targeting CBF1 and mimicking NotchIC, EBNA2 can remove CBF1 mediated transcriptional repression and thereby activate both cellular and viral gene expression, which in turn contribute to the transformation process.⁸

Thus, both LMP1 and EBNA2 interact with and subvert normal cell signalling in favour of alterations in cell physiology that are consistent with virus survival. In fact, the complement of virus gene activities seen during latency could be viewed as a concerted action on the part of the virus to maintain itself and persist in B cells. To achieve this end, EBV has evolved to exploit the normal biology of B cell development, and it is this latent infection in memory B cells, rather than continued expansion via virus replication, that is the hallmark of virus persistence *in vivo*. The fact that this occasionally results in malignancy could be regarded as an accident rather than design because in evolutionary terms it is clearly not in the best interests of the virus to kill the host.

The association between EBV and two epithelial malignancies, nasopharyngeal carcinoma and gastric carcinoma, is reviewed by Gerald Niedobitek⁹ and Kenzo Takada¹⁰, respectively. The involvement of EBV in non-keratinising nasopharyngeal carcinoma was established as early as 1973; however, it was not until 16 years later that EBV genomes were demonstrated in gastric cancers containing prominent lymphoid stroma,¹¹ and later in a proportion of typical gastric adenocarcinomas.¹² EBV is also detectable in some other carcinomas, most notably lymphoepitheliomas of various tissues, including those of the salivary gland, thymus, and lungs. Furthermore, EBV is also associated with oral hairy leukoplakia, a benign disorder seen predominantly in immunosuppressed patients, which is characterised by intense replication of EBV in oral epithelia.

A fundamental question relevant to both epithelial carcinogenesis and to the natural history of EBV infection is how and when EBV infects epithelial tissues. Unlike B cells, epithelial cells do not generally express the EBV receptor, CD21,¹³ suggesting that EBV enters these tissues by other routes. Takada describes two systems for the *in vitro* infection of various human epithelial cells by recombinant EBV containing a selectable marker: either direct contact with high titre virus supernatant or mixed culture with EBV producing Akata Burkitt's lymphoma cells.¹⁰ This work supports a model of EBV infection *in vivo* whereby epithelial tissues may be infected by virtue of their close proximity to lytically infected B cells resident near or within epithelial tissues—for example, adjacent to the sub-epithelial sinus in tonsil¹⁴ or within the nasopharyngeal mucosa.¹⁵ Recent studies have failed to detect the presence of EBV in a variety of non-malignant epithelial tissues, including desquamated oropharyngeal cells and tonsillar epithelium from patients with infectious mononucleosis,^{16–18} and normal epithelium adjacent to EBV positive nasopharyngeal carcinomas¹⁹ and gastric carcinomas,²⁰ suggesting that EBV infection of normal epithelial tissues is not a common event. However, the virus can be detected in preinvasive nasopharyngeal carcinoma²¹ and dysplastic gastric epithelium,²² suggesting that EBV might be an early trigger in the pathogenesis of these lesions, a view supported by the finding that EBV infection of epithelial cells *in vitro* stimulates cell proliferation and induces a malignant phenotype.¹⁰

One of the intriguing findings of Takada's work is that EBV gene expression in artificially infected cells is apparently identical to that observed *in vivo*, being essentially confined to the EBV encoded early RNAs (EBERs), EBNA1, LMP2A, and the BamHI A transcripts.¹⁰ It is interesting that in neither system can expression of the transforming EBNA2 and LMP1 genes be demonstrated. This is analogous to the situation observed in the Akata Burkitt's lymphoma cell line, where reintroduction of the EBERs to EBV negative Akata Burkitt's lymphoma cells restores their capacity for growth in soft agar, tumorigenicity in severe combined immunodeficient (SCID) mice, and resistance to apoptotic inducers—features identical to those observed in the parental EBV positive Akata cells.²² More recent evidence suggests that EBV infection of EBV negative nasopharyngeal carcinoma cell lines promotes their tumorigenicity, despite the fact that neither EBNA2 nor LMP1 are expressed.²³ Overall, these findings suggest that some EBV genes, previously shown to be dispensable for transformation in B cell systems (for example, EBERs and Bam HI A transcripts), might have more important contributions to make in the pathogenesis of some EBV associated malignancies than was first recognised.

Further challenges to the dogma surrounding the role of latent genes in EBV associated malignancies have recently come from two studies investigating the association of EBV with two common cancers. In the first of these, Bonnet and colleagues reported the detection of EBV by the polymerase chain reaction (PCR) and EBNA1 immunohistochemical staining in a proportion of classic breast tumours.²⁴ Of particular interest here was the finding that EBV was detected more frequently in breast tumours that were hormone receptor negative and of high histological grade. In the second study, Sugawara *et al* reported EBV detection in a series of hepatocellular carcinomas using Southern blot hybridisation for viral DNA.²⁵ Because this method relies on the presence of relatively high numbers of infected cells in affected tissues to produce a positive result, these findings are unlikely to be accounted for by the detection of rare bystander infected B cells present in the vicinity of the tumour. Furthermore, a single terminal fragment of EBV DNA was identified in these tissues, suggesting that the EBV infected cells in hepatocellular carcinoma represent clonal proliferations. Western blotting and reverse transcription PCR also demonstrated expression of EBNA1 and the BamHI A transcripts. However, in neither study could expression of the EBERs be demonstrated. *In situ* hybridisation for the EBERs is regarded as the "gold standard" for the detection of latent EBV infection in clinical tissues.²⁶ Although the results of these two studies require confirmation, they suggest that tumours displaying novel patterns of EBV latency characterised by absence of the EBERs might be regarded as EBV negative by conventional screens using *in situ* hybridisation to detect these molecules.

In vivo, EBV gene expression in nasopharyngeal carcinomas closely resembles that seen in EBV associated Hodgkin's disease. Both malignancies are characterised by expression of the EBERs, EBNA1, LMP1, LMP2A, and the Bam HI A transcripts, although LMP1 is only expressed in up to approximately 65% of nasopharyngeal carcinoma tumours.^{5–9} Further analysis reveals other similarities between these tumours of diverse cellular origins. Both are characterised by the presence of numerous lymphoid and other inflammatory cells, which surround the tumour cell population. Phenotypic studies suggest important interactions between the tumour cells and the infiltrating lymphocytes (reviewed in Thorley-Lawson²⁷, Niedobitek,⁹ and Flavell and Murray²⁸), which are

apparently facilitated by the expression of immune regulatory receptor–ligand pairs, such as CD40 and CD80/86 on tumour cells and CD40L and CD28 on infiltrating lymphocytes. In vitro, both nasopharyngeal carcinoma and Hodgkin's disease tumour cells are capable of presenting EBV derived peptides in the context of major histocompatibility complex (MHC) class I and are susceptible to lysis by EBV specific cytotoxic T cells (CTLs). Thus, in both nasopharyngeal carcinoma and Hodgkin's disease the ability of the virus infected tumour cells to survive in the face of an apparently competent immune system suggests that in both cases genome positive tumour cells have evolved characteristics that facilitate immune escape. In Hodgkin's disease there is evidence that such immune evasion might be made possible by the production of inhibitory cytokines, such as IL-10, by the tumour cell population.²⁹ The existence of a tumour microenvironment inhibitory to EBV specific CTLs could confound CTL based immunotherapeutic strategies for patients with nasopharyngeal carcinoma and Hodgkin's disease, and suggests that other approaches that target EBV in these tumours might be more fruitful.

It is perhaps fitting, given the growing interest in EBV as a target for antitumour treatments, that the final article of this series by Henri-Jacques Delecluse and Wolfgang Hammerschmidt covers not only the genetic modification of EBV but also the potential use of the virus as a vector for gene therapy. In their review, they describe the development of genetic systems that allow the modification of the EBV genome.³⁰ Such systems, which include the production of genetically modified viruses or plasmids containing all the latent genes that are required for transformation ("mini-EBV" plasmids), have already provided important insights into the function of not only the latent but also some of the lytic cycle genes. Furthermore, they describe the potential to use EBV itself as a gene therapy vector. This would involve the incorporation of therapeutic genes into a transformation defective EBV that could then be used to treat cancers such as B cell lymphomas or leukemias, which might be amenable to natural EBV infection. Is it possible then that we may turn an oncogenic virus into a harmless tool for gene therapy? The answer to this question is obviously some years away. For the time being there is much left to learn about the biology of this virus.

This themed issue highlights the broad impact of EBV research on such diverse areas as epidemiology, pathology, cell biology, and immunology. An increased understanding of EBV latent gene function and virus specific immune responses has had important repercussions for our knowledge of fundamental oncogenic processes and tumour immunology in general. It is hoped that these advances will not only benefit the treatment and prevention of EBV associated tumours, but will also advance attempts to tackle the more common cancers.

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