EBV-Associated Diseases in Humans and their Animal in vivo Models: Part I

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Epstein-Barr virus (EBV) is one of human herpesviruses and a member of the gamma herpesvirus family (lymphocryptovirus). Infectious mononucleosis, Burkitt’s lymphoma and nasopharyngeal carcinoma are well-known EBV-associated diseases. The range of EBV-associated diseases has recently expanded to include Hodgkin’s lymphoma, T-cell lymphoma, pyothorax-associated or methotrexate-associated B-cell lymphoma, primary effusion lymphoma and lymphoepithelioma-like carcinoma of the stomach, thymus and salivary gland, lymphoproliferative disorders (LPDs) or leiomyosarcomas from immunocompromized host, oral hairy leukoplakia and EBV-associated hemophagocytic syndrome. Animal models of human EBV-associated diseases are essential to elucidate the pathogenesis of EBV-infection and EBV-associated diseases. However, only several reports on the animal models of EBV infection have been reported. Here I review the summary of EBV-associated diseases in humans and those previous animal models using EBV or EBV-like herpesviruses and describe some details on our two newly developed rabbit models of LPD induced by simian EBV-like viruses and a mouse model with murine gammaherpesvirus. These animal models are useful and inexpensive alternative experimental model systems for studying the biology and pathogenesis of EBV, and prophylactic and therapeutic regimens.

Key words: animal model; EBV-associated disease; human; lymphocryptovirus

EBV-associated diseases in humans

Epstein-Barr virus (EBV) is one of eight known human herpesviruses (HHVs) and a member of the gamma herpesvirus family (lymphocryptovirus). EBV was the first human tumor virus identified from cultured lymphoblasts of Burkitt’s lymphoma (Epstein et al., 1964), and its potential role as a causative agent of EBV-associated tumors has been the important subject of such investigation for the last 40 years. EBV preferentially infects human B cells, T cells, natural killer (NK) cells, epithelial cells and smooth muscle cells (Rickinson and Kieff, 2001). Latent EBV infection occurs in the oropharyngeal epithelium, where EBV virions are replicated and released from the epithelial cells to saliva. The EBV-infected cells express a different array of EBV-associated antigens depending on lytic or latent infection (Baer et al., 1984; Rowe...
et al., 1992), and these viral antigens are targeted by EBV-specific cytotoxic T lymphocytes (CTLs) (Catalina et al., 2001). The CTL responses to EBV infections induce a variety of inflammatory systemic symptoms. On the other hand, the lack of CTLs such as in patients with congenital immunodeficiencies or acquired immunodeficiency syndrome (AIDS) or in recipients receiving a potent immunosuppressant, allows EBV-infected cells to proliferate and result in the development of lethal lymphoproliferative diseases (LPDs). EBV is classically associated with infectious mononucleosis (IM), Burkitt’s lymphoma in equatorial Africa and nasopharyngeal carcinoma (Rickinson and Kieff, 2001). The range of EBV-associated diseases has recently expanded to include oral hairy leukoplakia, leiomyosarcoma from AIDS patients, Hodgkin’s lymphoma, T-cell lymphoma, Ki-1 lymphoma, pyothorax-associated B-cell lymphoma, methotrexate-associated B-cell lymphoma, primary effusion lymphoma, LPDs of primary and secondary immunodeficiency, and lymphoepithelioma-like carcinoma of the stomach, thymus, lung and salivary gland (Weiss et al., 1989; Chang et al., 1992; Weiss and Chang, 1996; Anagnostopoulos and Hummel, 1996; Kawa, 2000; Iwatuski et al., 2004) (Table 1, Fig. 1).

**Primary EBV infection and its associated diseases**

**Infectious mononucleosis**

Acute IM is clinically characterized by fever, lymphadenopathy, tonsillitis, pharyngolaryngitis and hepatosplenomegaly, and increased IgM and IgG antibodies to EBV-viral capsid antigens (VCAs) and early antigens (EAs). Acute IM is fairly common in the United States and Western Europe, where a primary infection often occurs during adolescence. Asymptomatic primary infections are common in Asia and developing countries because primary EBV infections occur early in life. EBV virions bearing gp350/220 infect B cells via CD21 (CR2) or a receptor for C3d, and form an episomal EBV in the nucleus (Fingeroth et al., 1984). A complex of gp85(gH)/gp25(gL)/gp42 binds to HLA class II molecules to induce cell membrane fusion in B cells (Molesworth et al., 2000). Binding of the gp42 molecule to HLA class II is essential for virus entry into B cells. Following an incubation period of 2 to 7 weeks, EBV-infected B cells increase in number during acute IM. The EBV-infected B cells, however, are quickly abrogated by cellular immune responses mediated by NK cells, activated T cells and antibody-dependent cell-mediated cytotoxicity (Rickinson and Moss, 1997). CD8+, HLA-DR+ activated T cells increase in peripheral blood, and are defined as “mononucleosis” by hemograms when systemic symptoms manifest. During IM as primary EBV infection, EBV is capable of expressing all viral antigens of lytic cycle: immediate early, early and late proteins, and all antigens of latent infection genes: six EBV-determined nuclear antigens (EBNAs) consisting of EBNA-1, -2, -3A, -3B, -3C and -leader protein (LP), and three latent membrane proteins (LMPs), including LMP-1, -2A and -2B (latency type III, Table 1). All viral antigens of lytic cycle, all EBNAs and LMPs, except for EBNA-1, are target molecules for EBV-specific cytotoxic T cells. Therefore, CTLs directed to these EBV antigens suppress the EBV-infected cells in immunocompetent hosts.

**EBV-associated hemophagocytic syndrome**

Hemophagocytic syndrome is an unusual syndrome characterized by fever, splenomegaly, jaundice, pancytopenia, disseminated intravascular coagulation, and features of increased hemophagocytic macrophages in the bone marrow and other tissues (Imashuku, 2002). EBV-associated hemophagocytic syndrome (EBV-AHS) may be associated with acute IM, and EBV-associated LPD, lymphomas, cancers and autoimmune diseases, and commonly occurs in children and adolescents in Asia, but is rarely seen in Western countries. Laboratory examinations reveal pancytopenia, liver dysfunction, coagulopathy, elevated levels of lactate dehydrogenase, ferritin, β2-microglobulin, and serum cytokines including IFN-γ, IL-6, IL-10, sIL-2R, sFas and Fas L (Imashuku, 2002). These cytokines increased in the process of CTL responses directed against EBV-infected CD8+ T cells may induce he-
EBV-associated diseases and their animal models

EBV latent infection is also detected in other lymphocyte subsets of CD4+ T cells, CD16+ NK cells and CD20+ B cells (Kasahara et al., 2001).

**Table 1. Comparative overview of EBV-associated diseases (tumors) in humans and their compatible animal models**

<table>
<thead>
<tr>
<th>Type of infection or disease</th>
<th>EBV-associated diseases</th>
<th>Proposed cell of origin</th>
<th>EBV latent gene expression</th>
<th>Latency type</th>
<th>Animal model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary infection IM</td>
<td>B cell</td>
<td>EBNA-1+, -2+, -3A+,</td>
<td>III +</td>
<td>(monkey, mice)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-3B+, -3C+, -LP+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary infection EBV-AHS/fatal IM (T-LPD)</td>
<td>T cell</td>
<td>EBNA-1+, (LMP-1+),</td>
<td>I/II +</td>
<td>(rabbit)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>LMP-2+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic infection/ LPD X-linked LPD</td>
<td>B cell</td>
<td>EBNA-1+, -2+, -3A+,</td>
<td>III –</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-3B+, -3C+, -LP+,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>LMP-1+, -2+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic infection/ LPD PTLD-like lymphoma (B-LPD) from immunocompromised host (congenital immunodeficiency, transplantation, AIDS)</td>
<td>B lymphoblast</td>
<td>EBNA-1+, -2+, -3A+, -3B+, -3C+, -LP+, LMP-1+, -2+</td>
<td>III +</td>
<td>(monkey, mice, scid mice)</td>
<td></td>
</tr>
<tr>
<td>Chronic infection/ LPD Chronic active EBV infection</td>
<td>T cell or NK cell</td>
<td>EBNA-1+, LMP-1+, -2+</td>
<td>II –</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphoma Burkitt’s lymphoma</td>
<td>B cell (centroblast)</td>
<td>EBNA-1+</td>
<td>I –</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphoma Pyothorax-associated lymphoma</td>
<td>B cell</td>
<td>EBNA-2+, LMP-1+</td>
<td>III –</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphoma Primary effusion lymphoma (co-infection with HHV-8)</td>
<td>B cell</td>
<td>EBNA-1+, (LMP-1+),</td>
<td>I/II –</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphoma Methotraxate-associated lymphoma (LPD)</td>
<td>B cell</td>
<td>(LMP-1+)</td>
<td>I/II ?</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Lymphoma Hodgkin’s lymphoma</td>
<td>B cell (centrocyte)</td>
<td>EBNA-1+, LMP-1+, -2+</td>
<td>II –</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphoma/ leukemia T-cell lymphoma</td>
<td>T cell</td>
<td>EBNA-1+, (LMP-1+), LMP-2+</td>
<td>I/II +</td>
<td>(rabbit)</td>
<td></td>
</tr>
<tr>
<td>Lymphoma/ leukemia Nasal T/NK cell lymphoma</td>
<td>T/NK cell</td>
<td>EBNA-1+, (LMP-1+), LMP-2+</td>
<td>I/II –</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma Gastric carcinoma</td>
<td>Epithelial cell</td>
<td>EBNA-1+, LMP-2+</td>
<td>I/II –</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma Nasopharyngeal carcinoma</td>
<td>Squamous cell</td>
<td>EBNA-1+, (LMP-1+), LMP-2+</td>
<td>I/II –</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Virus replicative lesion Oral hairy leukoplakia</td>
<td>Squamous cell</td>
<td>EBNA-1+, -2+, -3A+, -3B+, -3C+, -LP+, LMP-1+, -2+</td>
<td>III +</td>
<td>(monkey)</td>
<td></td>
</tr>
<tr>
<td>Sarcoma Leiomyosarcoma from immunocompromised host</td>
<td>Smooth muscle</td>
<td>EBNA-1+, -2+, -3A+, -3B+, -3C+, -LP+, LMP-1+, -2+</td>
<td>III –</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

This table was modified from the tables in the textbook (Rickinson and Kieff, 2001).

EBV, Epstein-Barr virus; EBV-AHS, EBV-sassociated hemophagocytic syndrome; HHV-8, human herpesvirus-8; IM, infectious mononucleosis; LPD, lymphoproliferative disease; PTLD, post-transplant lymphoproliferative disease.
**EBV-associated LPD or leiomyosarcomas in immunocompromised individuals**

In patients with congenital immunodeficiencies or AIDS or in recipients of organ transplantations receiving a potent immunosuppressant, the lack or suppression of CTLs caused the EBV-associated B-cell LPD with latency type III infection of EBV. EBV is also involved in patients with methotrexate-associated LPD. Leiomyosarcomas may also develop in the immunocompromized hosts, although mechanism of EBV infection to smooth muscles has not been clarified (Rickinson and Kieff, 2001).

**Chronic and latent EBV infection and their associated diseases**

**X-linked lymphoproliferative syndrome (Duncan disease)**

In patients with X-linked lymphoproliferative syndrome, an inherited immunodeficiency characterized by increased susceptibility to EBV and B-cell LPD (Nichols et al., 1998), the presence of small deletions and mutations in DSHP/SH2DIA/SLAM-associated protein (SAP) gene was detected.

**Chronic active EBV infection**

Chronic active EBV infection (CAEBV) is a disease of CD4+ T-cell or NK cell LPD characterized by chronic or recurrent infectious mononucleosis-like symptoms persisting over a long time and by abnormally high titers of anti-EBV antibodies and increased levels of EBV-DNA in the peripheral blood (Okano, 1991). CAEBV is also characterized by a high mortality and high morbidity with life-threatening complications, such as virus-associated hemophagocytic syndrome, interstitial pneumonia and malignant lymphomas several years after disease onset. Patients with T-cell CAEBV had a shorter survival time than those with NK-cell type of disease (Kimura et al., 2001). Latency type II infection of EBV expressing EBNA-1 and LMPs proteins is demonstrated in cases with CAEBV. However, patients with CAEBV may have congenital or acquired immunological defects in CTL responses against EBV antigens, which may allow the survival of EBV-infected NK or T cells.

**EBV latent infection and tumor development**

Most EBV-associated LPDs are of B cell lineage, but T cell neoplasms and Hodgkin’s lymphoma may occur. Latent EBV infection plays a pivotal role in the occurrence of African Burkitt’s lymphoma, pyothorax-associated lymphomas (PALs), Hodgkin’s lymphoma (Figs. 1a and b), primary effusion lymphoma induced by HHV-8 coinfection, and various types of B cell lymphomas (Iwatsuki et al., 2004). The association of latent EBV infection with NK/T cell lymphomas is less common than B cell lymphomas. Extranodal NK/T-cell lymphoma, nasal type is more prevalent in Asia, Mexico and Central and South America, and a disease characterized by histological features of angiocentric and angiodestructive infiltration of lymphoma cells with severe ulceration or necrosis in nasal cavity. All those LPD except PAL have latency type I or II, whereas latency type III is detected in PAL. PAL is considered to have a localized immunodeficiency in pyothorax lesions.

EBV-associated carcinomas such as nasopharyngeal carcinoma and stomach carcinoma (Figs. 1c and d) show latency type I/II. Entry of EBV into epithelial cells that do not express CD21 or HLA class II is mediated by gp85(gH)/gp25(gL) complexes without gp42 (Borza and Hutt-Fletcher, 2002). The restriction of EBV gene expression such as latency type I (or II) showing only EBNA-1 (or LMPs) allows EBV-infected cells to evade immune surveillance, and persists throughout latent infection. EBNA-1 is essential for replicating EBV episomes during latency by binding to OriP, a cis-acting element of the EBV genome, and by promoting the replication of viral episomes by host cell DNA polymerase. LMP-1 shows oncogene activity with the upregulation of cellular gene expression in various cell types, and prevents apoptosis by the induction of bcl-2 (Rickinson and Kieff, 2001).

EBV-AHS, CAEBV and NK/T cell lymphomas are common in Asia (Iwatsuki et al., 2004). Since EBV gene expression is restricted in these disorders (latency II), only LMPs expressed by

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Fig. 1. Representative EBV-associated tumors in humans.
a: Hodgkin's lymphoma, hematoxylin and eosin stain.
b: EBER-1+ Hodgkin and Reed-Sternberg cells observed in Hodgkin's lymphoma. EBV-EBER-1 ISH.
c: Gastric adenocarcinoma, hematoxylin and eosin stain.
d: EBER-1 expression in adenocarcinoma of stomach. EBV-EBER-1 ISH.

the EBV-infected cells are targeted by host CTLs. Despite the absence of overt immunodeficiency, EBV-infected cells are insufficiently abrogated by CTLs in these disorders. Therefore, selective immunological defects to LMPs as genetic predisposition can be considered in those patients, including an HLA-restricted low response of CTLs to LMPs, immunological tolerance to LMPs and selective deletion of LMP-specific CTLs.

The release of virokines such as vIL-10 and the down-regulation of cell adhesion molecules are additional strategies for EBV-infected cells with latency type I/II to evade the host immune system. vIL-10 inhibits the synthesis of IFN-γ from lymphocytes and NK cells, and suppresses IFN-γ-mediated cellular events such as the up-regulation of the MHC class I expression and CTL responses. Low levels of intercellular adhesion molecule 1 and leukocyte function-associated antigen 3 expression are associated with an impaired ability to interact with EBV-specific CTL (Iwatsuki et al., 2004).

Most EBV-associated tumors arise with a very long latency in long-term EBV carriers. This suggests the multistep oncogenesis through malignant transformation from a single cell within the EBV-infected pool (Rickinson and Kieff, 2001). EBV may require certain risk factors to induce malignancy in humans. These risk factors are immunologic risk factor, genetic risk factors such as racial predisposition and personal predisposition, and environmental risk factors like Euphorbia tirucalli and malarial infection for African Burkitt’s lymphoma (Osato, 1998). The translocation of c-myc proto-oncogene and mutation or deletion of genes
like p53 are needed for the development of the most EBV-associated tumors in addition to EBV infection.

Despite intensive investigations on the role of EBV infection in the pathogenesis of EBV-associated tumors, a causal relationship between EBV and these tumors has not been established except for LPD arising in immunosuppressed individuals. Therefore, animal models of human EBV-associated diseases are essential to elucidate the pathogenesis of EBV-infection and EBV-associated diseases.

References


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