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

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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

Animal models for EBV infection and EBV-associated diseases

EBV-associated diseases and their animal models are tabulated in Table 2. In this review, I will focus only on *in vivo* animal models for EBV-related diseases.

Animal models using human EBV

Monkey models using human EBV

The host range of EBV is limited to humans and some new world monkeys (the cotton-top tamarin (*Sanguinus oedipus oedipus*), the common marmoset (*Callithrix jacchus*) and the owl monkey (*Aotus trivirgatus*). The cotton-top tamarin provides an *in vivo* model for EBV-persistent infection and EBV-related lymphomagenesis (Niedobitek et al., 1994). The EBV-infected cotton-top tamarins show a spectrum of responses that varies from unapparent infection to frank malignant lymphoma (Miller et al., 1977; Rickinson and Kieff, 1996). The cotton-top tamarin with inoculation of a high transforming dose of EBV developed multiple tumor foci composed of immunoblasts and plasmacytoid cells with the full spectrum of virus latent genes characteristic of latency type III infection (Cleary et al., 1985). These experimentally induced lymphomas are very good for the B-cell

LPD seen in immunocompromised humans. The common marmoset also provides an *in vivo* model for primary and persistent EBV infection, but not for EBV-related lymphoma. However, the value of tamarin and marmoset model as a more general model for EBV infections in humans is limited by the inability to infect these animals via the natural oropharyngeal route and/or virus persistence in animals, which do not develop lymphoma (Rickinson and Kieff, 1996). New World primates are endangered species, rare and so expensive that they are difficult to use for experiment.

SCID mouse model using human EBV

Severe combined immunodeficiency disease (SCID) mice with xenografts of human EBV-infected lymphocytes frequently developed oligoclonal or polyclonal multiple foci of EBV-positive human B-cell LPD (Moiser et al., 1998). These SCID tumor cells had a normal karyotype and showed latency type III infection with a small minority of cells expressing lytic viral proteins. Analysis of EBV clonality revealed that these tumors contained multiple viral episomes and linear DNA indicating viral replication. The SCID mouse model therefore is a convenient *in vivo* system in which to assess novel therapeutic regimes directed against EBV-associated B cell LPD (Johannessen and Crawford, 1999).

The other models using human EBV

Some of rabbits orally inoculated with EBV (B95-8) showed EBV infection with continuous detection of EBV-DNA from peripheral blood by PCR for 18 weeks and transient rise of antibodies to EBV but developed no tumors (Koirala et al., 1997; Chen et al., 1997).

Studies of tumorigenic mechanisms have been promoted by the application of transgenic mouse technology (Wilson., 1997). Candidate oncogenes can be definitively tested and their role in tumor formation dissected *in vivo*. In developing transgenic mouse models of EBV-associated diseases, the mechanism of action of the viral proteins, gleaned from molecular and biochemical analyses, can be visualized as phenotypic consequence in the whole organism. For examples, expression of the EBNA-1 or LMP-1 induces B cell lymphoma in transgenic mice (Wilson et al., 1996; Kulwichit et al., 1998).

Animal models using EBV-like herpesvirus (lymphocryptovirus)

Simian models using naturally infected proper herpesvirus (Rhesus and Cynomolgus models)

Lymphocryptoviruses (LCVs) are endemic in primate species and resemble each other in genomic structure and gene organization. Their structural and nonstructural proteins are frequently antigenically reactive across species (Kieff and Rickinson, 2001). Not only various Old World monkey species but New World monkeys carry their own EBV-related LCV (Dillneer et al., 1987; Kieff and Rickinson, 2001; Wang et al., 2001). And this is probably the reason why they were refractory to human EBV infection trials. These viruses show extensive colinear genome homology with EBV, encode many antigenically related proteins including EBNA-1 and EBNA-2 homologues, and can immortalize their natural target B cells *in vitro* but are not usually associated with any known disease of natural host monkeys. Their common evolutionary origin with EBV strongly suggests that the essential features of the virus-host interaction have been conserved (Rickinson and Kieff, 1996).

Rhesus monkey provides a new model for primary and persistent EBV infection. Experimen-

Abbreviations: AIDS, acquired imunodeficiency syndrome; CTL, cytotoxic T lymphocyte; EBER, EBV-encoded small RNA; EBNA, EBV-determined nuclear antigen; EBV, Epstein-Barr virus; EBV-AHS, EBV-associated hemophagocytic syndrome; HHV, human herpesvirus; HPS, hemophagocytic syndrome; HTLV, human T lymphotropic virus; HVMA, Herpesvirus *Macaca arctoides*; HVMF1, Herpesvirus *Macaca fascicularis*-1; HVMNE, Herpesvirus *Macaca nemestrina*; HVP, Herpesvirus *papio*; IM, infectious mononucleosis; LCV, lymphocryptvirus; LMP, latent membrane protein; LPD, lymphoproliferative disease; MHV, murine gammaherpesvirus; SCID, severe combined immunodeficiency; SIV, simian immunodeficiency virus; VAHS, virus-associated hemophagocytic syndrome; VCA, viral capsid antigen

tal oral inoculation of rhesus LCV in LCV-naïve rhesus monkeys resulted in acute and persistent LCV infection mimicking EBV infection in humans. Acute responses resemble to those seen in humans infectious mononucleosis with atypical lymphocytosis and activated CD23-positive B cells

Table 2. Animal models of EBV-associated diseases

Animal models Animals used	Viruses	Diseases and pathology	Similar EBV-associated diseases of humans
Animal models using human EBV			
Monkey models			
Cotton-top marmoset	EBV	EBV-associated LPD	PTLD-like lymphoma (B-LPD)
Calithrix marmoset	EBV	EBV-associated LPD, mild	PTLD-like lymphoma (B-LPD)
Owl monkey	EBV	EBV-associated LPD, disseminated	PTLD-like lymphoma (B-LPD)
Mouse model			
SCID mouse	EBV-infected lymphocyte	Transplanted human LPD	PTLD-like lymphoma (B-LPD)
The other animal models			
Hamster (newborn)	EBV-infected lymphocyte	Transplanted human LPD	PTLD-like lymphoma (B-LPD)
Rabbit	EBV	Acute infection (mild IM)	Mild IM
Animal models using EBV-like herpesvirus (lymphocryptovirus)			
Monkey models using naturally infected proper herpesvirus			
Rhesus monkey	Rhesus-EBV (lymphocryptovirus)	Acute Rhesus-EBV infection or IM; oral hairy leukoplakia	Acute EBV infection or IM; oral hairy leukoplakia
Cynomolgus with SIV infection	Cynomolgus-EBV (herpesvirus from *Macaca fascicularis*)	Cynomolgus-EBV-associated LPD	PTLD-like lymphoma (B-LPD)
Mouse models using MHV			
Mouse (Balb/C)	MHV-68	LPD	PTLD-like lymphoma (B-LPD)
Mouse (Balb/C)	**MHV-72**	**LPD**	**PTLD-like lymphoma (B-LPD)**
Mouse (Balb/C)	MHV-76	Acute infection	Acute EBV infection or IM
Rabbit models using simian EBV-like herpesvirus			
Rabbit	**Cyno-EBV, Si-IIA-EBV (HVMF1)**	**Cyno-EBV-associated ML (T-cell)**	**EBV-associated ML (T-cell)**
Rabbit	Herpesvirus from *Macaca arctoides* (HVMA)	HVMA-associated ML	EBV-associated ML (?)
Rabbit	Herpesvirus from *Macaca nemestrina* (HVMNE)	HVMNE-associated ML (T-cell)	EBV-associated ML (T-cell)
Rabbit	**Boboon-EBV [Herpesvirus *papio* (HVP)]**	**HVP-associatd fatal LPD with VAHS (T-cell)**	**EBV-associated fatal LPD with VAHS (T-LPD)**

B-LPD, B-cell LPD; LPD, lymphoproliferative disease; PTLD, post-transplant LPD; T-LPD, T-cell LPD; VAHS, virus-associated hemophagocytic syndrome.

in peripheral blood with cross-reacting antibodies to EBNA2 and EBV-VCA. Acute infection was followed by a persistent infection with shedding virus in saliva and harboring asymptomatic LCV in the peripheral blood. However, without overt immunosuppression, LCV-related tumors have not developed in this model (Wang, 2001; Wang et al., 2001). Rhesus LCV can infect epithelial cells in immunosuppressed rhesus macaques and can induce epithelial cell lesions resembling oral hairy leukoplakia in AIDS patients. Electron microscopy, immunohistochemistry and DNA-RNA *in situ* hybridization were used to identify the presence of a lytic rhesus LCV infection in these proliferative, hyperkeratotic or parakeratotic epithelial cell lesions (Kutok et al., 2004).

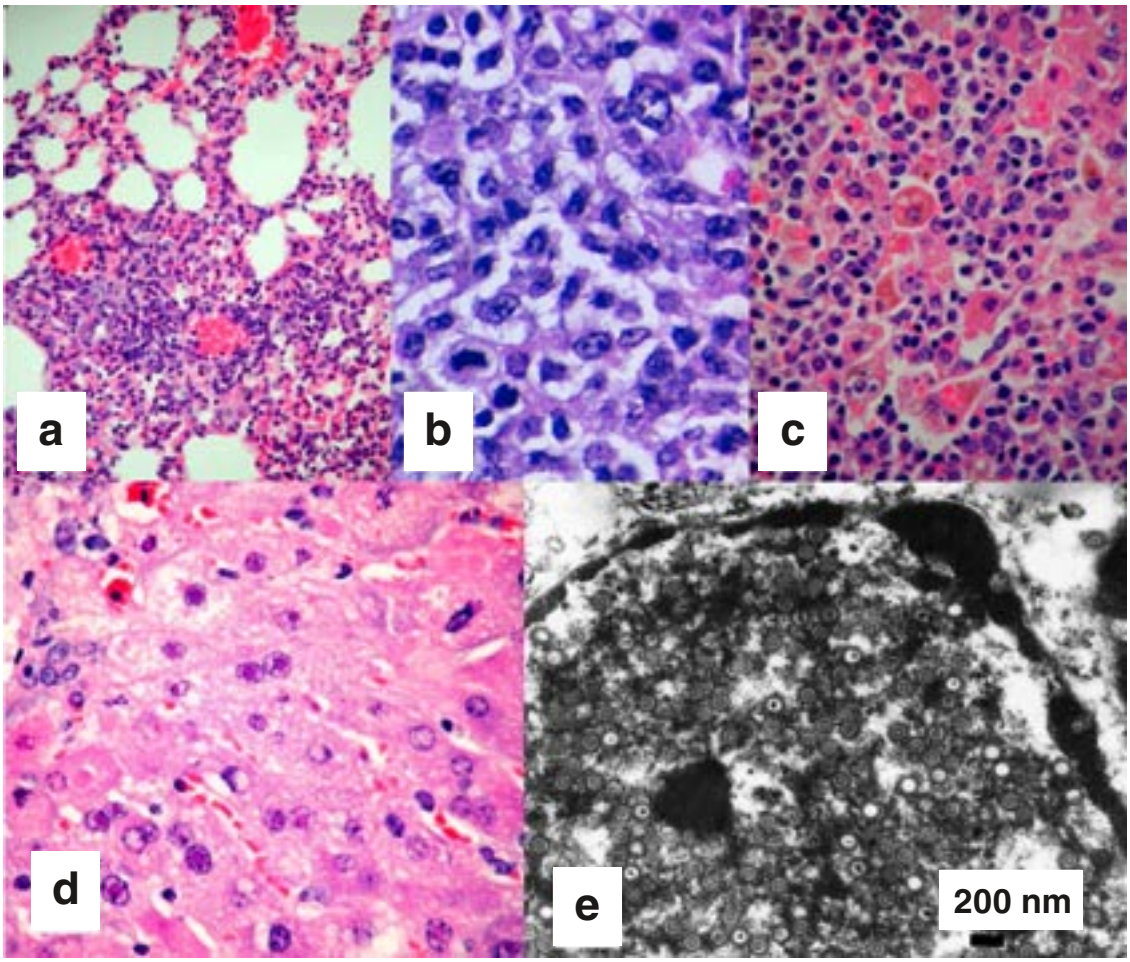
An EBV-like herpesvirus (Herpesvirus *Macaca fascicularis*-1, HVMF1) isolated from lymphomas of simian immunodeficiency virus (SIV)-infected Cynomolgus monkeys (*Macaca fascicularis*) has been identified as a causative agent for a monkey model of EBV-associated lymphomagenesis in human AIDS (Feichtinger et al., 1992; Rezikyan et al., 1995). Rhesus monkeys (*Macaca mulatta*) and Cynomolgus monkeys infected with a SIV developed B cell lymphomagenesis at 4% and 31% incidence, respectively, associated with an EBV-related simian herpesvirus (Rhesus LCV and HVMF1, respectively), providing a monkey model for EBV-associated lymphomagenesis at 3 to 6% incidence in human AIDS (Habis et al., 1999). Of 160 consecutive renal transplants of cynomolgus, 5.6% developed B-cell LPD with EBER expression 28 to 103 days after transplantation (Schmidtko et al., 2002).

Mouse models using murine gamma-herpesvirus

Murine herpesvirus 68 (MHV-68), a murine gammaherpesvirus, was isolated from a murid rodent, the bank vole in Slovakia (Blaskovic et al., 1980). Seven more isolates similar to MHV-68 (MHV-60, MHV-72, MHV-76, MHV-78, MHV-Sumava, MHV-4556 and MHV-5682) were also obtained. At least three isolates, MHV-68, MHV-72 and MHV-Sumava seem to be involved in malignant neoplasm development in mice (Mistrikova et al., 2000). Especially MHV-68 has been intensively investigated to be used as a mouse model for LPD induced by EBV. Intranasal inoculation of MHV-68 in Balb/c mice induced viral infection and replication in the lung alveolar epithelia and mononuclear cells and subsequently followed by infectious mononucleosis and/or persistent infection in murine B cells. Twenty of 220 (9%) persistently infected mice developed MHV-68-associated LPD during 3 years observation and the LPD incidence of MHV-68 infected mice with Cyclosporin A treatment increased to 60% (Sunil-Chandra et al., 1994). *In situ* hybridization revealed the presence of viral DNA and the expression of viral RNA in the lymphoid cells of LPD lesions. An MHV-68-infected B cell line derived from an LPD lesion showed tumorigenicity in nude mice (Usherwood et al., 1996). Atypical lymphocytosis in acute phase of mouse MHV-72 infection, like infectious mononucleosis in humans and LPD development in MHV-72-infected Balb/c mice, were reported (Mistrikova and Mrmusova, 1998). Pathology of MHV-72-infection in CB17 +/+ and CB17 scid/scid mice was examined (Fig. 2; Oda et al., submitted). Comparative genomic sequence analysis of MHV variants and their different pathogenesis were shown in Table 3 (Macrae et al., 2001; Oda et al., submitted). Many aspects of MHV-68 or MHV-72 infection in mice are similar to those of human EBV infection and this is a useful model for the study of gammmaherpesvirus infection *in vivo.*

Rabbit models using simian EBV-like herpesviruses

Rabbit T-cell lymphoma model induced by Cynomolgus-EBV (herpesvirus from *Macaca fascularis*): I previously established a simian (Cynomolgus monkey) leukocyte cell line (Si-IIA) by cocultivation with an human T lymphotropic virus (HTLV)-II-producing human CD8+ T cell line (HTLV-IIA) (Miyamoto et al., 1990). Si-IIA cells immortalize human T cells (Hayashi et al., 1993; Ohara et al., 1993). During a study on the prevention of HTLV-II infection using Si-IIA, I found by chance that malignant lymphoma develops in Japanese White rabbits when they were inoculated intravenously and that these rabbit lymphomas had



f: Detection of MHV-DNA in MHV-72-infected mice

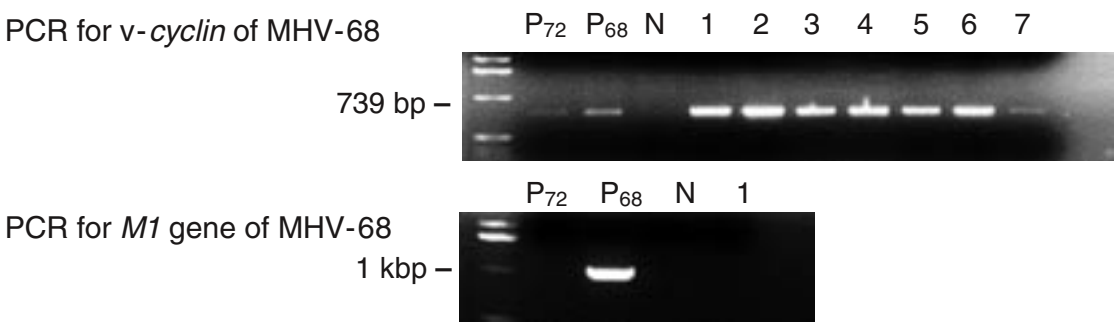


Fig. 2. Pathology of MHV-72-infected mice.
a: Acute bronchopneumonia induced by nasal inoculation of MHV-72 in CB17 +/+ mouse.
b: Malignant lymphoma occurred in the spleen of MHV-72-infected CB17 +/+ mouse.
c: Hemophagocytosis detected in the spleen of MHV-72-infected CB17+/+ mouse.
d & e: Intranuclear inclusions of herpesvirus in the many hepatocytes of MHV-72-infected CB17 scid/scid mouse.
f: Detection of MHV-DNA in MHV-72-infected mouse tissues by PCR using MHV-68 primers.
v-*cyclin* gene was detected in MHV-72-infected mouse tissues (lanes 1 through 7), MHV-68 (lane P68) and MHV-72 (lane P72). However, *M1* gene of MHV-68 was only amplified in the positive control (lane P68) and not in MHV-72 (lane P72) and MHV-72-infected tissues (lane 1). N, the negative control.