Table 3. Comparison of genomic sequence of MHV variants and their pathogenesis in mice

Variants of MHV	Tumori-genicity	Spleno-megaly	t-RNA-like sequences	Genes		
				M1 through *M3*	*M4*	*M5* through *M12*
MHV-68	+	+	+	+	+	+
MHV-72	+	-	-	-	+	+
MHV-76	-	-	-	-	-	+

MHV, murine gammaherpesvirus.

no integration of HTLV-II genome (Hayashi et al., 1994). Later a type of oncogenic virus, EBV-related herpesvirus in Si-IIA cells (Si-IIA-EBV) was identified, and malignant lymphomas induced by Si-IIA in Japanese White rabbits as well as New Zealand White rabbits contained EBV-related DNA (Hayashi et al., 1995). I also established a rabbit lymphoma model via the natural oropharyngeal route with Si-IIA-EBV (Koirala et al., 1997) as well as rabbit lymphoma induction via transfusion with blood from Si-IIA-EBV-infected rabbits (Koirala et al., 2004). In addition I confirmed the lymphomagenesis of rabbits by another EBV-like herpesvirus variant from cynomolgus (Cyno-EBV) (Hayashi et al., 1995; Chen et al., 1997). However, intravenous inoculation of an HVMF1-infected cell line producing few virus particles (C54) made seroconversion in one of 10 rabbits and did not induce rabbit lymphomas. Based on the sequence analysis of these three viruses, these can be considered variant virus each other (Hayashi et al., 1999, 2002; Hayashi and Akagi, 2000). Therefore, I designate these three viruses of Si-IIA-EBV, Cyno-EBV and HVMF1 as Cynomolgus-EBV in this paper.

Intravenous or peroral inoculation of Cyno-EBV in rabbits induced a high rate of lymphomagenesis (77–90%). All of the sera from rabbits inoculated intravenously or perorally with Cynomolgus-EBV-producing cells or EBV-producing B95-8 cells showed increased anti-VCA IgG antibody titers (× 10–10,240). The autopsy of tumor-bearing rabbits revealed marked splenomegaly, lymph node swelling and/or hepatomegaly with multiple white nodules. White tumor nodules were less frequently found in the kidneys and heart. Rarely, multiple peritoneal and skin metastatic tumors were observed.

A histological examination of rabbit tissues revealed malignant lymphomas involving many organs. All of the involved tissues were classified as non-Hodgkin's lymphoma, diffuse, large-cell or diffuse mixed type (Figs. 3a, b, e and f). Bizarre giant cells were seen occasionally, admixed with lymphoma cells. Less often, bizarre giant cells were identified in a non-neoplastic background, mimicking the morphology of Hodgkin's lymphoma (Figs. 3c and d).

The *in situ* hybridization studies revealed that EBER-1 expression was detected in most Cynomolgus-EBV-producing cells and in about 90% cases of Cynomolgus-EBV-induced rabbit lymphoma. Most of the multinucleated bizarre giant cells among both neoplastic and non-neoplastic cells were positive (Figs. 3b and d).

Eleven T-cell lines harboring EBV-related DNA and EBER-1 expression were established from Cynomolgus-EBV-induced tumor-bearing rabbits (Fig. 3g). Type I/II latency of EBV infection was observed in Cynomolgus-EBV-induced lymphomas and their cell lines. Interestingly, six of them showed a deletion or translocation of 12q [12q–, 5 cases; t (7p+: 12q–), 1 case]. All cell lines except one (B6-J130LN) showed tumorigenicity in nude mice (Fig. 3h).

Direct sequencing of the three PCR products revealed that Si-IIA-EBV had about 82 % nucleotide similarity to the human EBV DNA in three regions (BRRF1 and IR1 regions) (Baer et al., 1984). Si-IIA-EBV had about 92.4% nucleotide similarity to HVMF1 (Ino et al., 1997). Cyno-EBV DNA has 77% base pair homology to EBV DNA from B95-8 cells and 91% base pair homology to HVMF1 DNA in the IR1 region (Hayashi et al., 1999). These sequence data indicate that Si-IIA-EBV has higher

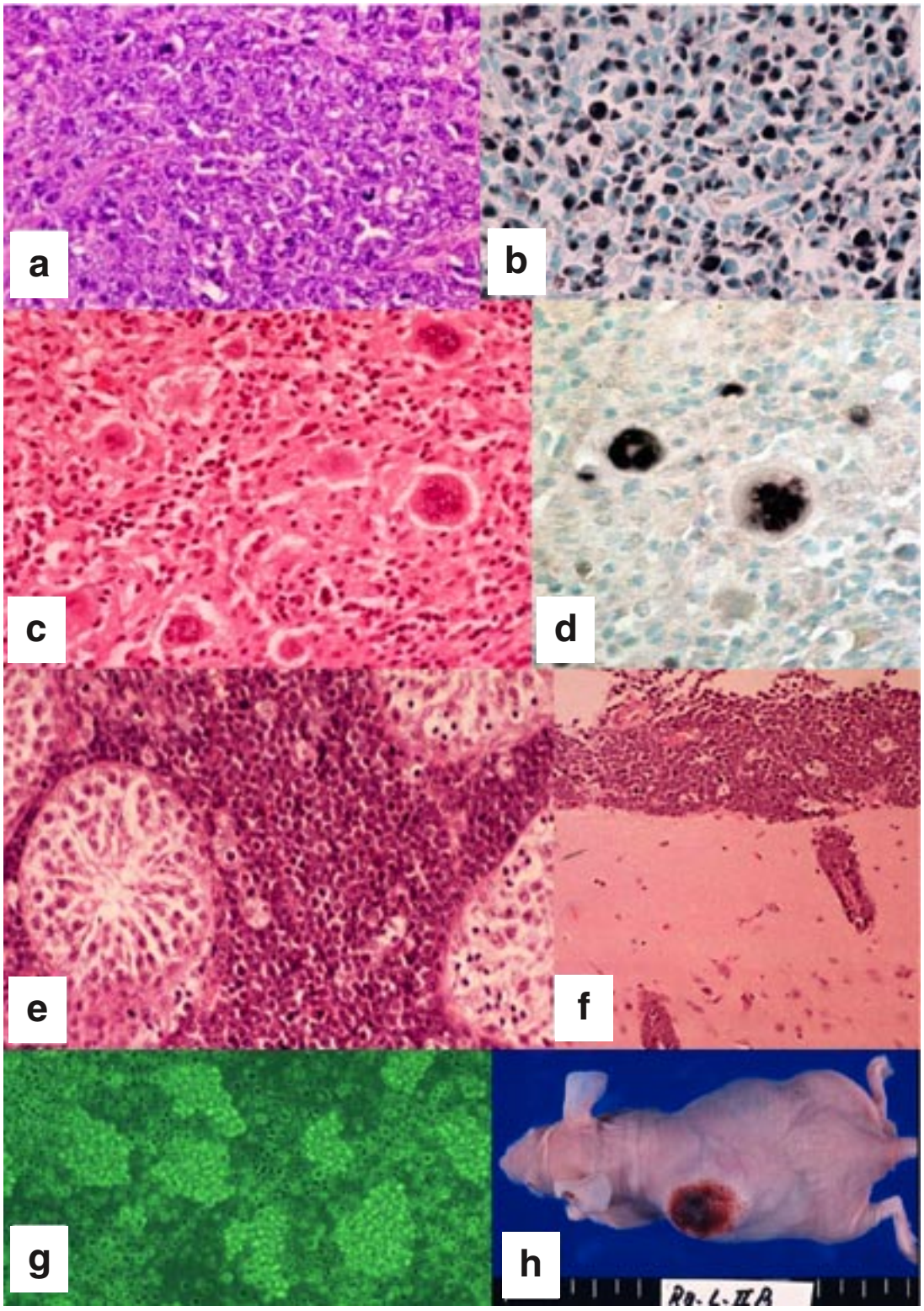

Fig. 3. Cynomolgus-EBV-induced lymphomas in rabbits. Diffuse large cell lymphoma of the spleen (hematoxylin and eosin stain) (**a**) and EBER-1 (*in situ* hybridization) (**b**). Hodgkin-like lymphomas with EBER-1 expression are rarely observed (hematoxylin and eosin stain) (**c**) and EBER-1 (*in situ* hybridization) (**d**). Lymphoma cells also infiltrate into the testis (**e**) and brain (**f**). Phase-contrast microphotograph of the rabbit lymphoma cell line (**g**) and its transplanted tumor in a nude mouse (**h**).

sequence homology to HVMF1 and Cyno-EBV than human EBV from B95-8 suggesting that Cyno-EBV, Si-IIA-EBV and HVMF1 differ from B95-8-EBV and may be variants of each other (Hayashi and Akagi, 2000: Ohara et al., 2000).

Rabbit lymphoma model induced by EBV-like herpesvirus from *Macaca arctoides*: Herpesvirus *Macaca arctoides* (HVMA) is an EBV-like herpesvirus isolated from lymphoid cell line of the rhesus monkey species *Macaca arctoides* (Lapin et al., 1985). Both simian T-cell leukemia virus (STLV-1) and HVMA are produced by MAL cell lines established from *Macaca arctoides.* Inoculation of MAL cells in rabbits induced malignant lymphomas and PCR analysis revealed the presence of T-cell leukemia virus-like sequence but not the presence of EBV-like sequence in rabbit lymphomas (Schatzl et al., 1993). However, Wutzler et al. (1995) demonstrated the association of HVMA with the etiology of malignant lymphoma of rabbits inoculated with HVMA by detecting EBER-like transcripts expression and HVMA-DNA with PCR in the lymphoma cells. Inoculation of HVMA into 32 rabbits resulted in the seroconversion to EBV-VCA and EBV-EA in all infected rabbits and showing symptoms in 16 cases (50%) between 21 and 143 days after inoculation, and the development of 17 LPD (13 high-grade non-Hodgkin's lymphomas and 4 lymphoid hyperplasia). The phenotype of LPD was not described and latency type of EBV infection in rabbit LPD could not be determined. However, these findings suggested that HVMA caused a symptomatic infection and subsequent LPD development in rabbits (Wutzler et al., 1995).

Rabbit T-cell lymphoma model induced by EBV-like-herpesvirus from *Macaca nemestrina*: Herpesvirus *Macaca nemestrina* (HVMNE) is a novel EBV-like virus isolated from a *Macaca nemestrina* with CD8+ T-cell mycosis fungoides-cutaneous T-cell lymphoma (Rivadeneira et al., 1999). A new rabbit T-cell lymphoma model by HVMNE has been reported (Ferrari et al., 2001). Intravenous inoculation of HVMNE-infected T-cells or cell-free HVMNE in New Zealand White rabbits resulted in seroconversion to EBV-VCA in 7 of 10 rabbits and 1 of 4 rabbits, respectively. And all 8 seroconverted rabbits developd T-cell lymphoma within 3 to 9 months after inoculation. Necropsy revealed splenomegaly or hepatomegaly or both in most tumor-bearing animals. White nodules were frequently found in kidneys, heart and lungs. Lymph node enlargement and skin involvement were rarely observed. Histologically, diffuse mixed lymphoma cells infiltrated involving many organs of rabbits. Viral sequences from tissue DNA were detected by PCR in all lymphomatous rabbits. Not all cells within the lymphoid infiltrates expressed EBER-specific viral RNA, and the intensity of the EBV EBER staining varied among cells of the same tissue. Possibly the low-level expression found using EBER was related to a suboptimal sensitivity of this technical approach owing to the incomplete homology of the probe used because the DNA sequence encoding EBER from HVMNE is unknown. HVMNE-DNA and EBV-like RNA expression was also detected in two transformed T-cell lines established from two lymphomatous rabbits. Analysis of one of these T-cell lines demonstrated the persistence of HVMNE-DNA, expression of an LMP1-like protein, acquisition of interleukin-2 independence, and constitutive activation of the Jak/STAT pathway. HVMNE infection of rabbits provides a valuable animal model for human EBV-associated T-cell lymphoma whereby genetic determinants for T-cell transformation by this EBV-like animal virus can be studied.

Baboon EBV (herpesvirus *papio*)-induced rabbit model for EBV-associated fatal LPD with virus-associated hemophagocytic syndrome (VAHS): Human EBV-associated hemophagocytic syndrome (EBV-AHS), has a poor prognosis and is often noted in patients with fatal IM (Mroczek et al., 1987; Okano and Gross, 1996), fatal childhood with T-cell LPD (Su et al., 1994, 1995; Kikuta, 1995), chronic active EBV infection (Yamashita et al., 1998), and malignant lymphomas (MLs), particularly EBV-infected T-cell lymphoma (Craig et al., 1992; Su et al., 1993). Patients with HPS exhibit common clinicopathologic features such as

fever, skin lesions, lung infiltrates, hepatosplenomegaly with jaundice and liver dysfunction, pancytopenia and coagulopathy. The liver, spleen, lymph nodes and bone marrow are usually infiltrated with proliferated florid histiocytes with hemophagocytosis as well as proliferated atypical lymphocytes. Increased serum levels of many cytokines including soluble IL-2, IL-1, IL-3 and IL-6, macrophage colony stimulating factor, interferon-γ, prostaglandins and tumor necrosis factor-alpha (TNF-α) have also been reported (Su et al., 1995; Okano and Gross, 1996).

Herpesvirus *papio* (HVP) infection-related rabbit fatal LPD with VAHS, which is described in detail as in the following, is the first animal model for human EBV-fatal LPD with VAHS (Hayashi et al., 2001, 2003a).

An HVP-producing baboon lymphoblastoid cell line (594S) or cell-free HVP virion pellets obtained from 594S culture were intravenously inoculated into female New Zealand White rabbits. Of the 13 rabbits inoculated intravenously with HVP-producing simian 594S cells, 11 (85%) died of LPD 22 to 105 days after inoculation. LPD was also accompanied by VAHS in 9 of these 11 rabbits. Peroral inoculation of cell-free HVP resulted in viral infection in 3 of 5 rabbits, with 2 of the 3 infected rabbits dying of LPD with VAHS (51–81 days). LPD with VAHS was also induced in 7 of 7 rabbits (100%) by intravenous injection of cell-free HVP 21 to 28 days after inoculation. In total, only 3 infected rabbits remained free of LPD. Two of the rabbits that showed no seroconversion after peroral inoculation exhibited no abnormalities.

Increased anti-EBV-VCA IgG antibody titers (× 40–2,560) were detected in all sera from rabbits inoculated intravenously with 594S (HVP). However, increased anti-VCA-IgG antibodies levels were found in only 3 of the 5 rabbits inoculated perorally with cell-free virion pellets. Peripheral blood (PB) examination of some rabbits with LPD and VAHS revealed elevated GOT (≤ 116 IU/L), GPT (≤ 109 IU/L) and LDH (≤ 1,557 IU/L), and leukocytosis (≤ 21,500/mm^3) with mildly increased levels of atypical lymphocytes (1–10 %). Transient mild leukopenia (3,700–5,400/mm^3) was also found in 4 of the 10 rabbits examined.

Except for anorexia and emaciation, most rabbits inoculated with HVP appeared physically normal, but showed severe bloody rhinorrhea (Fig. 4a) and dyspnea during the few days before death. Autopsy of the infected rabbits frequently revealed pulmonary congestion and edema, often accompanied with severe hemorrhage of the lungs. Mild or marked splenomegaly with congestion and hemorrhage (Fig. 4b) was often observed, as well as dark purple, swollen lymph nodes with hemorrhage (Fig. 4c) and/or hepatomegaly. White nodules were sometimes found in spleen, liver or heart cross-sections. Histological examination of rabbit tissues revealed mild to severe infiltration of atypical pleomorphic lymphoid cells involving many organs. Atypical large or medium-sized lymphoid cells without Hodgkin's cell-like morphology infiltrated around perivascular areas with a diffuse or nodular pattern. Apoptotic cells (individual cell necrosis) accompanied by histiocytes containing cellular debris were often observed in the atypical cell-infiltrated lesions. Lymph nodes, spleen, and liver were frequently and markedly involved. Most involved lymph nodes showed diffuse infiltrations of atypical lymphoid cells and marked hemophagocytosis in the sinus (Figs. 4d and e). Involved livers showed severe periportal and sinusoidal infiltration of atypical lymphoid cells (Figs. 4f and g), which was often accompanied by central necrosis of the hepatic lobules. Atypical lymphocytes were often found in the blood vessels. Hemophagocytosis was also found in the spleen, bone marrow and thymus.

Six rabbit T-cell lines with IL-2 dependency were established from 3 of the 5 HVP-infected Japanese White rabbits. Five of 6 cell lines had the normal rabbit female karyotype (44, XX), while one had an abnormal karyotype (Hayashi et al., 2003a). In 18 of 20 LPD cases (90%), EBER-1 expression was detected in virtually all atypical lymphoid cells (Figs. 4e and g). EBER-1-positive atypical lymphoid cells infiltrated not only the parenchyma and stroma of the various organs, but were also sometimes demonstrated in the vessels, lymph nodal sinus or hepatic sinusoid. The six rabbit lymphoid cell lines also expressed EBER-1.