A Novel Epstein-Barr Virus-Like Virus, HV\textsubscript{MNE}, in a Macaca Nemestrina With Mycosis Fungoides


Epstein-Barr virus (EBV) infection of humans has been associated with the development of lymphoid malignancies mainly of B-cell lineage, although occasionally T-cell lymphomas have been reported. We describe here the characterization of a novel EBV-like virus (HV\textsubscript{MNE}) isolated from a simian T-cell lymphotropic virus type I/II (STLV-I/II) seronegative pigtailed macaque (Macaca nemestrina) with a cutaneous T-cell lymphoma. Immunohistochemistry studies on the skin lesions demonstrated that the infiltrating cells were of the CD3\textsuperscript{+}/CD8\textsuperscript{+} phenotype. Two primary transformed CD8\textsuperscript{+} T-cell lines were obtained from cultures of peripheral blood mononuclear cells (PBMC) and skin, and, with time, both cell lines became interleukin-2-independent and acquired the constitutive activation of STAT proteins. Polymerase chain reaction analysis of the DNA from the cell lines and tissues from the lymphomatous animal demonstrated the presence of a 536-bp DNA fragment that was 90% identical to EBV polymerase gene sequences, whereas the same DNA was consistently negative for STLV-I/II sequences. Electron microscopy performed on both cell lines, after sodium butyrate treatment, showed the presence of a herpes-like virus that was designated HV\textsubscript{MNE} according to the existing nomenclature. In situ hybridization studies using EBV Epstein-Barr viral-encoded RNA probes showed viral RNA expression in both CD8\textsuperscript{+} T-cell lines as well as in the infiltrating CD8\textsuperscript{+} T cells of skin-tissue biopsies. Phylogenetic analysis of a 465-bp fragment from the polymerase gene of HV\textsubscript{MNE} placed this virus within the Lympocryptovirus genus and demonstrated that HV\textsubscript{MNE} is a distinct virus, clearly related to human EBV and other EBV-like herpesviruses found in nonhuman primates.

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Mycosis fungoides (MF) is a rare cutaneous T-cell lymphoma (CTCL) that may involve lymph nodes and viscera as well as skin.\textsuperscript{1,2} The Sézary syndrome (SS) is the erythrodermic variant of MF characterized by the presence of circulating tumor cells. The tumor cells in MF/SS are usually of the CD4\textsuperscript{+} mature-cell lineage, although CD8\textsuperscript{+} lineage has been described in a few cases.\textsuperscript{2} The diagnosis of MF is based on clinical features and histopathological findings that include infiltration of the dermis with lymphocytes with hyperconvoluted nuclei and Pautrier's microabcesses.\textsuperscript{1} MF and SS are the most frequent primary lymphomas involving the skin. Genetic predisposition, alteration in cytokine profile, and viruses such as herpesviruses I and II (HSV-I and HSV-II), Epstein-Barr virus (EBV), human herpesvirus 6 (HHV-6), and human T-cell lymphotropic virus type I (HTLV-I) have been suggested as possible causative agents.\textsuperscript{3}

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HSV-I/II–specific antigens and DNA have been found in lesions of CTCL.\textsuperscript{3,4} One report describes the finding of HHV-6 in 1 of 30 patients with CTCL.\textsuperscript{5} EBV DNA has been found in patients with cutaneous lymphomas\textsuperscript{6-12} and, in several cases, viral RNA expression has been demonstrated in the neoplastic tissue. Higher incidence of EBV seropositivity in CTCL patients with the consistent emergence of EBV in MF/SS-cultured lymphocytes has also been reported.\textsuperscript{13} However, a direct causative role of EBV in CTCL has been difficult to prove.\textsuperscript{13} In contrast, the importance of EBV in the development of B-cell malignancies in human immunodeficiency virus-infected individuals or iatrogenically immune-suppressed patients is more broadly accepted.\textsuperscript{14}

Although humans are the only known natural host for EBV, EBV-like agents have been described in Old World nonhuman primates, including chimpanzee,\textsuperscript{15-17} baboon,\textsuperscript{18-21} African green monkey,\textsuperscript{22} gorilla,\textsuperscript{23} and macaque species.\textsuperscript{24-29} Although the relative prevalence of these viruses in animals in captivity or in the wild is unknown,\textsuperscript{30} several studies suggest that it may be as high as that in EBV in humans. In fact, the inability to generate models of EBV-associated lymphomas in Old World monkeys with the human EBV has been ascribed to the presence of cross-reactive immunity against EBV in these species. In contrast, at least 2 New World monkey species, including the cotton-top tamarin (Sanguinus oedipus oedipus)\textsuperscript{31} and the owl monkey (Aotus trivirgatus),\textsuperscript{32} develop B-cell lymphoma upon human EBV exposure. We report here the occurrence of a rare case of CD8\textsuperscript{+} T-cell MF in a pigtailed macaque and the isolation of a novel EBV-like virus from 2 transformed CD8\textsuperscript{+} T-cell lines obtained from the blood and the skin of the lymphomatous macaque. These findings may help in the development of an animal model for EBV-like virus-induced malignant proliferation of T cells.

Materials and Methods

Establishment of macaque blood and skin T-cell lines. Peripheral blood mononuclear cells (PBMC) were isolated by density-gradient
centrifugation on lymphocyte separation medium (LSM; Organon Teknika Corp, Durham, NC) from anticoagulated blood obtained from pigtailed macaques J94356 before death. The cell layers were washed twice in Dulbecco’s phosphate-buffered saline (DPBS; GIBCO BRL), Gathersburg, MD) and were suspended in RPMI (GIBCO BRL) with 10% heat-inactivated (HI) fetal bovine serum (FBS; GIBCO BRL) with penicillin/streptomycin (500 U/mL and 500 g/mL, respectively; GIBCO BRL) and L-glutamine (2 mmol/L; GIBCO BRL) and stimulated with phytohemagglutinin (PHA: 5 g/mL; Murex Diagnostics, Norcross, GA). At 72 hours, the cells were washed twice in DPBS and resuspended in fresh RPMI with 10% HI FBS, penicillin/streptomycin, L-glutamine, and recombinant interleukin-2 (IL-2; 20 U/mL; Boehringer Mannheim, Indianapolis, IN). Fresh skin biopsy tissues from diseased areas were minced to release single cells. These were banded in Hank’s balanced salt solution (HBSS; Life Science (Cleveland, OH) according to the manufacturer’s instructions. After an overnight incubation at 37°C, DNA was extracted from the minced tissue was resuspended in ice-cold DPBS and until processing, were thawed on ice and finely minced with sterile blades. The minced tissue was resuspended in ice-cold DPBS and washed twice before lysis and extraction as detailed above. DNAs were initially amplified with the primer pools DFASA/GDTD1B and VYGA/EDR8/97 sense 5'-ATCTCTGTATTTTCACT-3', 8.5) containing 300 mmol/L Tris-HCl, 2.0 mmol/L MgCl₂, 75 mmol/L (NH₄)₂SO₄, 10 mmol/L dNTP, and 2.5 U Taq polymerase. The templates were subjected to 45 cycles of amplification as described above. The nested PCR products were analyzed by gel electrophoresis and subsequently cloned into Inv α cells, and sequence analysis was performed.

Other regions of the viral genome were amplified using the primer pairs described by Ino et al.α and included the 2a/2as pair covering IR1 region and the 11s/11as pair covering part of the EBV BRRF-1 region.α PCR products were analyzed by gel electrophoresis and cloned into Inv α cells, and the DNA sequence was obtained as described above.

Southern blot analysis. DNA (10 µg) of each sample was digested with Sau3AI or Pac I, electrophoresed in 0.8% agarose gels, and blotted onto Nylon membranes (Nyttran plus; Schleicher & Schuell, Keene, NH). The membranes were hybridized overnight at 42°C with the PCR-amplified EBV-like probe labeled using random-primer reaction (Boehringer Mannheim). Hybridization, washing, and detection were performed according to the manufacturer’s instructions.

Phylogenetic analysis. Phylogenetic analysis of the novel HVMNE pol-gene fragment (465 bp) was performed using sequences from an analogous strain from Macaca arctoidesα (HVMA) and the following related strains published previously: human EBV: V01556; retroperitoneal fibromatosis herpesvirus (RFHV)—Macaca mulatta: AF054579; herpesvirus Saimiri (HVS): M31122; Kaposi sarcoma herpesvirus (KSHV): AF005477; equine herpesvirus 2 (EHV-2): and U20824; herpesvirus Papio (HPV).α Sequences were aligned using ClustalX version 1.63B.α Genetic distance estimates among all pairs of pol sequences were estimated using the Tajima-Nei model of substitution.α Phylogenetic associations, using the computer program Mega Version 1.01,α were ascertained by minimum evolution estimated by neighbor-joining (NJ) in conjunction with the Tajima-Nei model of substitution. Bootstrap analyses, consisting of 100 iterations, were performed to determine the consistency of the data in recapitulating the same tree for NJ analysis. Values of 70% or more were considered strong support for the adjacent node.α

Electrophoretic mobility shift assay (EMSA). PHA-stimulated human PBMC were cultured in the presence of IL-2 (20 U/mL) for 8 days and used as a control for this assay. In starvation experiments, 1 × 10⁶ stimulated PBMC and IL-2–dependent and –independent J94356 cells were resuspended in 20 mL of RPMI 1640 with 1% FBS after washing with 1× PBS twice and incubated for 21 hours at 37°C in 5% CO₂. Protein lysates were prepared in 20 mmol/L HEPES (pH 7.9), 450 mmol/L NaCl, 0.4 mmol/L EDTA, 0.5 mmol/L dithiothreitol (DTT), 25% glycerol, 1 mmol/L NaVO₃, 1 mmol/L AEBSF, 20 µg/mL aprotinin, and 20 µg/mL leupeptin. The binding reaction was performed by preincubating 5 µg of nuclear extract with 1 µg of poly(dI-dC) in a buffer containing 5.9 mmol/L HEPES (pH 7.9), 30 mmol/L KCl, 5.9 mmol/L Tris (pH 7.4), 0.7 mmol/L DTT, 0.6 mmol/L EDTA, 8.9% glycerol, 0.1 mmol/L NaVO₃, 1 mmol/L AEBSF, 20 µg/mL aprotinin, and 20 µg/mL leupeptin in ice for 20 minutes. A 21P-labeled probe (20,000 cpm) corresponding to the MGF binding site in the β-casein gene promoter (5'-TAGATTCTGAGAATTGG-3') was added to the reaction mixture and incubated on ice for 30 minutes. For the supershift assay, STAT5 antibody (N-20; Santa Cruz Biotechnology, Santa Cruz, CA) was incubated with cell extracts on ice for 20 minutes after the addition of radiolabeled probe. Complexes were resolved on 4.5% polycrylamide gels.

Immunohistochemistry and EBV Epstein-Barr viral-encoded RNA (EBER) in situ hybridization. CD3 immunostaining was performed on formalin-fixed, paraffin-embedded tissue using a rabbit polyclonal anti-CD3 serum (A0452; DAKO, Carpinteria, CA) at 1:50 dilution followed by biotinylated antirabbit secondary antibodies and ABC reagent (Vector Labs, Burlingame, CA) and DAB substrate (Scytek Laboratories, Logan, UT). CD8 immunostaining was performed on tissue sections frozen in OCT using a mouse anti-CD8 monoclonal antibodies (Clone 1.3, Dako).

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lesions recurred in the perioral area and new lesions involving the face, trunk, forearms, and legs appeared. At this time, the serology was negative for herpes B and STLV; however, EBV serology was positive and the lesions improved on a 2-week course of acyclovir. Biopsy results of skin lesions examined early in October 1996 were consistent with a multifocal cutaneous lymphoma. The animal was continued on antibiotic therapy with mild improvement of the lesions.

However, in mid-January 1997, the lesions dramatically worsened and, after a course of steady deterioration, the animal was killed in January 1997. At the time of death, animal J94356 displayed an erythematous, hyperkeratotic rash, with alopecia and excoriated lesions with prominent red borders and numerous plaque-like lesions on extensive areas of the skin (Fig 1A and B). Hematoxylin Eosin staining of a skin-lesion sample showed profusely infiltrating mononuclear cells in the dermis and epidermis with prominence at the dermal-epidermal junction in periadnexal tissue and in perivascular tissue (Fig 1C). In the epithelium, the cells were arranged in small solid clusters at the dermal-epidermal interface, resembling Pautrier’s microabscesses. The cells had moderately sized nuclei containing 1 to 2 small nucleoli and up to 2 mitotic figures were present per 400× field (Fig 1D). A few histiocytes and eosinophils were admixed with the neoplastic cells. There were multifocal erosions in the epithelium, which was acanthotic and hyperkeratotic (Fig 1C). Immunohistochemical analysis of the skin lesion showed CD3+ infiltrating T cells both in the dermis (top left panel of Fig 2) and a small portion of the epidermis. Coalescing clusters of CD3+ cells were present along the dermal-epidermal junction and within the epidermis. The lineage of the infiltrating cell population was further characterized and found to be strongly CD8+ (right top and bottom panels of Fig 2) and CD4− (not shown). No staining was observed with an irrelevant antibody (left bottom panel of Fig 2). Thus, clinical features of the skin lesions and the histopathology were indicative of a CTCL with a CD8+ T-cell phenotype.

Infiltrates of mononuclear cells undergoing mitosis with a few eosinophils and neutrophils were also observed in several organs, including the lungs and lymph nodes (data not shown). Occasional mitoses were present in the enlarged lymphoid follicles and germinal centers. The spleen contained prominent lymphoid nodules and hyaline material in the germinal centers, and the liver had a mild multifocal lymphocytic infiltrate in portal areas and subcapsular tissue. Although staining with CD8 antibodies was not performed in the organs, it is highly likely that these infiltrates were composed of the same CD8+ T cells found in the skin.

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**RESULTS**

**MF in animal J94356: Clinical presentation and histopathological findings.** In May 1996, an 18-month-old pigtailed macaque (animal J94356), housed at the Washington Regional Primate Research Center, developed a unilateral eye infection with marked inflammation of the conjunctiva and eyelids, which eventually spread to the opposite eye. Improvement in the clinical manifestations was observed after treatment with antihistamine and antibiotics, but inflammation of both conjunctivae and eyelids recurred 8 days after treatment was discontinued. A small unilateral corneal ulceration was found on examination and antibiotic and antihistamine therapy was reinstituted. At this time, an extensive work-up to test for viral infections was performed. A serologic panel of tests was run at a commercial laboratory. Tests were negative for herpes B, HSV-1, measles, and simian immunodeficiency virus and were positive for cytomegalovirus. In August 1996, the ulcerative lesions recurred in the perioral area and new lesions involving the face, trunk, forearms, and legs appeared. At this time, the serology was negative for herpes B and STLV; however, EBV serology was positive and the lesions improved on a 2-week course of acyclovir. Biopsy results of skin lesions examined early in October 1996 were consistent with a multifocal cutaneous lymphoma. The animal was continued on antibiotic therapy with mild improvement of the lesions.

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The transition of the J94356PBMC expressing HVMNE cell lines to IL-2 independence is associated with STAT5 activation and HVMNE expression. J94356PBMC cells hybridized with EBER RNA (A) or incubated with hybridization buffer in the absence of probe (B). (C) EMSA on control PBMC (lanes 1 through 4) and the J94356PBMC cell line in the IL-2-dependent (lanes 5 through 8) and IL-2-independent (lanes 9 through 12) status.
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Detection of an EBV-like virus in the transformed CD8+ T-cell lines from animal J94356. Peripheral mononuclear cells obtained from whole blood at the time of necropsy were cultured with PHA and recombinant IL-2. Skin-derived cells were also placed in culture under similar conditions and, like the PBMC culture, proliferated continuously in response to IL-2. Both cell lines have been in culture for approximately 2 years. Giemsa staining of cytospin preparations of these cells at 2.5 months after initiation of culture of the cells indicated their pleomorphism with myeloid-like features and multiple mitotic figures at higher magnification (lower panels of Fig 3). Cytogenetic analyses of the chromosomes confirmed the simian origin of both cell lines (2n = 42; data not shown).

Electron-microscopic analysis of the cell lines under standard culture conditions was negative even after an extensive search for any viral particles, including retroviruses. In addition, the supernatants of these cell lines were consistently negative for reverse transcriptase activity. However, electron microscopic analysis performed 48 hours after treatment with Na-butyrate allowed the detection of large enveloped virions in the cytoplasm of cells derived from the PBMC of animal J94356, consistent with the presence of a herpesvirus (Fig 4A). To assess the genetic composition of this herpesvirus, we used generic DNA primers previously demonstrated to amplify efficiently genomes from several members of the herpesvirus family,33 which amplified a DNA fragment of 536 bp from both CD8+ T-cell lines. This DNA fragment (p536) was molecularly cloned and used as a probe to hybridize the DNA from primary J94356 PBMC cultured for 46 days as well as from the J94356 skin culture at days 46, 226, and thereafter. After PCR amplification, the expected DNA fragment of 536 bp was found in the macaque cell lines but not in human PBMC (Fig 4B), suggesting that this putative herpesvirus persisted in both cell lines originated from animal J94356.

Genetic and phylogenetic characterization of the herpesvirus from animal J94356. To establish the genetic relationship of the putative viral fragment found in cells from animal J94356 to other known nonhuman primate herpesviruses, we obtained the DNA sequence of plasmid p536 and aligned it to the equivalent polymerase region of the human KSHV and EBV as well as EBV-like viruses or rhadinoviruses from various animal species (Fig 5).29,33,34,39 Both DNA sequence alignment and the phylogenetic analysis by the NJ method (Fig 6) indicated that the herpesvirus in J94356 cells clustered with the human EBV and the nonhuman primate herpesviruses, HVMA and HVPA,34 and was distantly related to the known rhadinoviruses.33,39,40

Two other EBV-like viruses, SiIIA and HVMF-1, have been isolated from *Macaca fascicularis*.26,41 To assess the genetic relationship of HV MNE to SiIIA, we analyzed the HV MNE DNA with primer sets for the BRRF-1 EBV-equivalent region. As demonstrated in Fig 7, HV MNE appears to be distinct from SiIIA, because the DNA sequence of this region was 85% identical to EBV and 90% identical to the SiIIA strains. In the case of HVMF-1, the DNA sequence from this region is not available; therefore, HVMF-1 and HV MNE DNA were compared by restriction enzyme and Southern blot analyses, which demonstrated differences in both restriction sites used and suggested genetic diversity between HV MNE and HVMF-1 (Fig 8). We therefore designated the virus from animal J94356 as HV MNE consistent with the common name used for other EBV-like viruses found in nonhuman primates.

**JAK/STAT constitutive activation in CD8+ T cells infected with HV MNE correlates with the acquisition of IL-2 independence.** Transforming viruses usually induce growth-factor independence by interfering with intracellular signaling pathways,42-45 and this event occurs also in EBV-transformed B-cell lines.46 In addition, in human hematopoietic malignancies,
including Sézary syndrome, constitutive activation of JAK/STAT protein has also been described.47-50

Both T-cell lines from animal J94356 acquired the ability to grow independently from exogenous IL-2, and, to assess whether constitutive activation of STAT5 protein also correlated with the acquisition of IL-2 independence, cell extracts from normal human PHA-stimulated PBMC and the J94356PBMC cell line, at 3 months and 17 months from the start of culture, were analyzed after IL-2 withdrawal. At 3 months from initiation of culture, some degree of constitutive STAT5 binding to the MGF probe could be already observed in the absence of IL-2 and serum (Fig 9C, lanes 7 and 8), and, at 17 months, the cells in culture in absence of exogenous IL-2 displayed constitutive binding of activated STAT5 to the MGF probe (Fig 9C, lanes 9 through 12). Constitutive STAT1 and STAT3 binding was also observed when the specific SIE probe was used (data not shown). Thus, as in the case of HTLV-I–infected cells, the transition to IL-2 independence correlates with the acquisition
of constitutive activation of STAT proteins.\(^{43,44}\) To assess whether the resident herpesvirus expression was contained in the IL-2–independent cells, viral expression was analyzed using the human EBV EBER probe. As demonstrated in Fig 9A and B, a clear nuclear signal was evident in most HVM\(_\text{MNE}\)-infected cells, indicating also a good conservation of this sequence in the HVM\(_\text{MNE}\).

Detection of EBV-like DNA in the tissues of animal J94356. To investigate whether HVM\(_\text{MNE}\) found in the CD8\(^+\) T-cell lines from animal J94356 was also present in the infiltrating CD8\(^+\) neoplastic T cells from the skin lesion as well as other organs, the DNA from various tissues was analyzed using the same primer set used to amplify the HVM\(_\text{MNE}\) polymerase gene from the DNA of the J94356 cell lines.

As demonstrated in Fig 10A, viral DNA sequences were found in the skin but not the PBMC obtained from animal J94356 at the time of CTCL diagnosis (1996). However, at time of death (1997), viral DNA was also found in the uncultured PBMC as well as in multiple skin specimens, lymph nodes, and muscles. The finding of viral DNA in PBMC at time of death is consistent with the clinical finding of an abnormal T-cell count (18,000/mL) in the blood of animal J94356.

To assess whether the HVM\(_\text{MNE}\) sequences found in the tissues were also detectable in the CD8\(^+\) neoplastic T cells in vivo, the postmortem tissue specimens of animal J94356 were stained with the EBER RNA probe. As demonstrated in the left panels of Fig 10B and 10C, several cells in the skin expressed EBER RNA and the distribution pattern closely mirrored the pattern observed with immunohistochemical analyses using CD8-specific antibody (Fig 2); this suggests that HVM\(_\text{MNE}\) was present in the CD8\(^+\) T cells that infiltrated the dermis and epidermis of the lymphomatous animal.

DISCUSSION

Herpesviruses have been found in most animal species, and the family Herpesviridae includes the 3 subfamilies Alphaherpesvirinae, Betaherpesvirinae, and Gammaherpesvirinae. Within the Gammaherpesvirinae subfamily, 2 genuses have been distinguished, the lymphocryptoviruses (Epstein-Barr–like viruses) and rhadinoviruses (Saimiri-ateles–like herpesviruses).\(^{51}\) Only members of the Gammaherpesvirinae subfamily have been associated with human malignancies. EBV causes B-cell lymphoma in immunodeficient individuals and is epidemiologically associated with Burkitt’s lymphoma, Hodgkin’s disease,
Fig 7. Alignment of BRRF-1 DNA sequences of HV MNE, SiIIA, and EBV.

EBV-MNE  CGAGGAGCCCTGCGCTG..GCAGTTCTTTAACCAGGGTT
EBV-SiIIA T--A--G------G------G--CC--
EBV  T------G------G------

CTTGGACAGTTGGCCCTCTTGCAAGTTCTGCTATCTACCACATCTCA--G
--C------G------G------A------T------

TCCATAGCCACCTCAACCCAGCTTTCTGACCACTCGAGATCAAGCTCTAGT
--------T------A------G------C------

GTCTCAGGAGGGGATTGGAACGACCTCTCTCCTTCAGGGC
A------GC------A------T------G------
A------C------A------A------A------T------

GAGCTDGCGACCGCCTCAGGCCACAGGACGGCTCTACTGCTACTGAAT
A-------AT------T------C------TGC---ATCT------

ATATTGGACCTCTGAAGCAGACCAACCTCAGATAGTG..GGCTATA
--------C------C------A------A------A------T------G------
--------NCA--T------C------

GCAATAAGCTCCTCCTACCC...CTCTGTTGTCTAGTCTGTTGTCCTTCGATAGGA
T------TC------A------G------CA------TGCTAT--A--A------G------

TCTGCTAGCAAAAATACGCGGCCTGCAATGGGCATTGGGC..TTTTATAGGCCATTTCCAT
--------N------N------T------A------GT------
--------AT------C------T------G------GG------

..GGCCGGACAATAGCAATATAAAAACCTCAGCTACTGCTAGGCTTAGCGAACCTCTG
TG------C------A------A------T------G------C------

CATCAGGTATCGGCTTTACTGATTGAGGGCCACAGGAAGCTCTGGTTATTTT
--------AC------C--G------G------
--------C------GT------G------AG------

Fig 8. Southern blot analysis of HV MNE and HVMF-1-infected cell DNAs. Human PBMC DNA was used as negative control. The molecular-weight complexes are indicated at the left of each panel and the restriction enzyme used is indicated at the bottom of each panel.
and nasopharyngeal carcinoma. Similarly, human herpesvirus 8 has been epidemiologically linked to Kaposi sarcoma and rare forms of lymphoma. EBV induces B-cell lymphoma in previously unexposed New World monkeys but not in Old World primates, presumably because of preexisting cross-immunity against EBV, as demonstrated by their frequent EBV seropositivity. However, after simian immunodeficiency virus infection, approximately one third of the infected macaques develop B-cell lymphoma, and this event has been associated with the presence of an EBV-like virus in *Macaca fascicularis*.

We describe here the identification and partial characterization of HV*<sub>MNE</sub>* from a pigtailed macaque with MF. HV*<sub>MNE</sub>* is distinct from the known nonhuman primate EBV-like herpesviruses as demonstrated by the phylogenetic analysis of the polymerase gene and the BRRF-1 region. The finding of HV*<sub>MNE</sub>* in the CD8<sup>+</sup> infiltrating T cells in vitro as well as in transformed CD8<sup>+</sup> T-cell lines in vitro supports the notion that HV*<sub>MNE</sub>* might target T cells and might have been involved in the development and/or progression of MF in animal J94356.

EBV has been reported to induce B-cell lymphoma in New World monkeys but not in rabbits, whereas other EBV-like strains from nonhuman primates, such as HVMA (*Macaca arctoides*) and EBV-related strains from cynomolgus monkeys, have been demonstrated to induce B-cell lymphoma in rabbits. Conversely, the pathogenicity of nonhuman-primate EBV-like viruses has not been assessed in New World monkeys. Interestingly, HSV strains A and B cause lymphoma in New World primates but not in New Zealand white rabbits, suggesting that pathogenicity of different viral strains varies in different species. Transfusion of blood from the lymphomatous animal discussed in this study to other pigtailed macaques did not result in lymphoma (data not shown), presumably because of a preexisting infection of EBV-related virus in this species of Old World monkey. It would be of interest to assess whether HV*<sub>MNE</sub>* induces lymphoma in the rabbit model. If so, HV*<sub>MNE</sub>* may provide a model to study viral and cellular determinants involved in oncogenesis. Lastly, the findings reported here may prompt a further investigation on the possible role of human EBV strains in T-cell lymphomas.

![Detection of herpesvirus DNA by PCR and of EBV-specific RNA by in situ hybridization in tissues of animal J94356.](image)
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A Novel Epstein-Barr Virus-Like Virus, HV\textsubscript{MNE}, in a \textit{Macaca Nemestrina} With Mycosis Fungoides