

# *Acta Medica Okayama*

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Volume 58, Issue 4

2004

Article 1

AUGUST 2004

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## A spectrum of clinical manifestations caused by host immune responses against Epstein-Barr virus infections.

Keiji Iwatsuki\*

Takenobu Yamamoto†

Kazuhide Tsuji‡

Daisuke Suzuki\*\*

Kazuyasu Fujii††

Hironori Matsuura‡‡

Takashi Oono§

\*Okayama University,

†Okayama University,

‡Okayama University,

\*\*Okayama University,

††Okayama University,

‡‡Okayama University,

§Okayama University,

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## Abstract

Epstein-Barr virus (EBV), or human herpesvirus 4 (HHV-4), infects the vast majority of adults worldwide, and establishes both nonproductive (latent) and productive (lytic) infections. Host immune responses directed against both the lytic and latent cycle-associated EBV antigens induce a diversity of clinical symptoms in patients with chronic active EBV infections who usually contain an oligoclonal pool of EBV-infected lymphocyte subsets in their blood. Episomal EBV genes in the latent infection utilize an array of evasion strategies from host immune responses: the minimized expression of EBV antigens targeted by host cytotoxic T lymphocytes (CTLs), the down-regulation of cell adhesion molecule expression, and the release of virokines to inhibit the host CTLs. The oncogenic role of latent EBV infection is not yet fully understood, but latent membrane proteins (LMPs) expressed during the latency cycle have essential biological properties leading to cellular gene expression and immortalization, and EBV-encoded gene products such as viral interleukin-10 (vIL-10) and bcl-2 homologue function to survive the EBV-infected cells. The subsequent oncogenic DNA damage may lead to the development of neoplasms. EBV-associated NK/T cell lymphoproliferative disorders are prevalent in Asia, but quite rare in Western countries. The genetic immunological background, therefore, is closely linked to the development of EBV-associated neoplasms.

**KEYWORDS:** latent infection, hydroa vacciniforme, mosquito allergy, chronic active EB virus infection, hemophagocytic syndrome

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\*PMID: 15551754 [PubMed - indexed for MEDLINE]

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*Review*

## A Spectrum of Clinical Manifestations Caused by Host Immune Responses against Epstein-Barr Virus Infections

Keiji Iwatsuki\*, Takenobu Yamamoto, Kazuhide Tsuji, Daisuke Suzuki,  
Kazuyasu Fujii, Hironori Matsuura, and Takashi Oono

*Department of Dermatology, Okayama University Graduate School of  
Medicine and Dentistry, Okayama 700-8558, Japan*

Epstein-Barr virus (EBV), or human herpesvirus 4 (HHV-4), infects the vast majority of adults worldwide, and establishes both nonproductive (latent) and productive (lytic) infections. Host immune responses directed against both the lytic and latent cycle-associated EBV antigens induce a diversity of clinical symptoms in patients with chronic active EBV infections who usually contain an oligoclonal pool of EBV-infected lymphocyte subsets in their blood. Episomal EBV genes in the latent infection utilize an array of evasion strategies from host immune responses: the minimized expression of EBV antigens targeted by host cytotoxic T lymphocytes (CTLs), the down-regulation of cell adhesion molecule expression, and the release of virokinines to inhibit the host CTLs. The oncogenic role of latent EBV infection is not yet fully understood, but latent membrane proteins (LMPs) expressed during the latency cycle have essential biological properties leading to cellular gene expression and immortalization, and EBV-encoded gene products such as viral interleukin-10 (vIL-10) and bcl-2 homologue function to survive the EBV-infected cells. The subsequent oncogenic DNA damage may lead to the development of neoplasms. EBV-associated NK/T cell lymphoproliferative disorders are prevalent in Asia, but quite rare in Western countries. The genetic immunological background, therefore, is closely linked to the development of EBV-associated neoplasms.

**Key words:** latent infection, hydroa vacciniforme, mosquito allergy, chronic active EB virus infection, hemophagocytic syndrome

**E**pstein-Barr virus (EBV) preferentially infects human B cells, epithelial cells, T cells, natural killer (NK) cells, and smooth muscle cells [1, 2]. 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 [3, 4], and these viral antigens are targeted by EBV-specific cytotoxic T lymphocytes (CTLs) [5]. The CTL responses to EBV infections induce a variety of inflammatory systemic and cutaneous symptoms, while the lack of CTLs allows EBV-infected cells to survive and proliferate. The primary infection may cause infectious mononucleosis, and, less frequently, induce Gianotti-Crosti syndrome and virus-associated hemophagocytic syndrome [6, 7] (Table 1). Latent EBV infection is associated with various types of neoplasms and hematological disorders including X-linked

Received April 30, 2004; accepted June 17, 2004.

\*Corresponding author. Phone: +81-86-235-7282; Fax: +81-86-235-7283  
E-mail: keijiwa@cc.okayama-u.ac.jp (K. Iwatsuki)

lymphoproliferative disorder (Duncan disease), malignant lymphomas arising in patients with acquired immunodeficiency syndrome (AIDS) and the recipients of organ transplantations, gastric cancer, and pyothorax-associated lymphoma [8–11]. Recent studies have shown that cutaneous disorders such as hydroa vacciniforme and hypersensitivity to mosquito bites are mediated by EBV-infected T and NK cells, respectively [12, 13]. In certain geographic areas, characteristic EBV-associated hematological disorders or neoplasms may occur endemically despite the absence of immunodeficiency [14–20]. These conditions include African Burkitt's lymphoma, nasopharyngeal carcinoma in the southern part of China, and nasal-type NK/T cell lymphomas in Asian and Central and South American countries. Furthermore, most patients with chronic active EBV

infection and EBV-associated hemophagocytic lymphohistiocytosis (HLH) have been reported from Japan [7]. These observations suggest that an HLA-restricted immunological background and environmental co-factors are strongly associated with the development of EBV-associated neoplasms. In the process of disease progression, EBV evolves a number of skillful strategies to escape the host immune surveillance system.

## 1. Primary EBV infection and host immune responses

**Infectious mononucleosis.** Patients with acute infectious mononucleosis (IM) present with fever, lymphadenopathy, tonsillitis, pharyngolaryngitis, hepatosplenomegaly, puffy eyelids, and skin eruptions. Skin rashes are most frequently induced by the administration of ampicillin, amoxicillin, and  $\beta$ -lactam antibiotics [21]. In most patients with acute IM, the illness is caused by a primary EBV infection, but similar symptoms may occur in an acute cytomegalovirus infection, and in drug-induced hypersensitivity syndrome caused by sulfones, anticonvulsants, allopurinol, and other drugs [22, 23]. The complications of IM include neutropenia, thrombocytopenia, splenic rupture, airway obstruction by tonsillar hypertrophy, central nervous system involvements, and fulminant hepatitis [21]. Because primary EBV infections occur early in life in Asia and developing countries, asymptomatic primary infections are common in these areas, whereas acute IM is fairly common in the United States and Western Europe, where a primary infection often occurs during adolescence.

EBV virions bearing gp350/220 infect B cells via CD21 (CR2) or a receptor for C3d, and form an episomal EBV in the nucleus [24]. A complex of gp85(gH)/gp25(gL)/gp42 binds to HLA class II molecules to induce cell membrane fusion in B cells [25]. Binding of the gp42 molecule to HLA class II is essential for virus entry into B cells. 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. In the reactivation process, EBV virions originating in epithelial cells efficiently infect B cells, whereas B cell-derived virions better infect epithelial cells [26]. Following an incubation period of 2–7 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 natural killer (NK) cells,

**Table 1** Diseases associated with EBV infection

(primary infections)
Infectious mononucleosis
EB virus-associated hemophagocytic syndrome (EB-VAHS)
Gianotti-Crosti syndrome
(chronic infections/ lymphoproliferative disorders)
X-linked recessive lymphoproliferative disorder (Duncan disease)
Lymphoproliferative disorders in immunocompromised hosts
Hemophagocytic lymphohistiocytosis (HLH)
Chronic active EBV infection
Hypersensitivity to mosquito bites (HMB)
Hydroa vacciniforme
(lymphomas/leukemias)
Burkitt's lymphoma
Lymphomas in immunocompromised hosts
Pyothorax-associated lymphoma
Primary effusion lymphoma (co-infection with HHV-8)
Methotrexate-associated lymphoma
Lymphomatoid granulomatosis
Extranodal NK/T cell lymphoma, nasal type
Hydroa vacciniforme-like lymphoma
Aggressive NK/T cell lymphoma/leukemia
Chronic NK cell leukemia
Hodgkin lymphoma
Angioimmunoblastic T cell lymphoma (bystander EBV+ cells)
(carcinomas)
Nasopharyngeal carcinoma
Gastric cancer
Salivary gland cancer
Oral hairy leukoplakia
(sarcomas)
Leiomyosarcoma

activated T cells, and antibody-dependent cell-mediated cytotoxicity (ADCC) [27, 28]. CD8<sup>+</sup>, HLA-DR<sup>+</sup> activated T cells increase in peripheral blood, and are defined as “mononucleosis” by hemograms when systemic symptoms manifest. The sera of acute IM patients contains elevated levels of the interleukins (IL)-1 $\alpha$ , IL-2, IL-6, IL-12, IL-18 and interferon (IFN)- $\gamma$ , probably corresponding to the expansion of activated CD8<sup>+</sup> CTLs, although other effector cells besides EBV-infected cells also produce cytokines. Setsuda *et al.* reported that the expression of IL-18, IFN- $\gamma$ , Mig, and RANTES in the lymphoid tissues of patients with acute EBV-induced IM was greater than in the lymphoid tissues of patients with posttransplantation lymphoproliferative disorders (LPD), suggesting the possibility that these mediators participate in critical host responses to EBV infection [29]. A range of CTL-specific epitopes contains the EBV lytic-cycle proteins, including immediate early, early, and late proteins [30]. Therefore, EBV-replicative lesions are subjected to direct CTL control *in vivo*, which may induce intense systemic symptoms as observed in acute IM. In contrast, the  $\alpha$ - and  $\beta$ -herpesviruses interfere with antigen-processing pathways during lytic infection, which renders the lytic-cycle antigens much less immunogenic. Although selective expansion of the V $\beta$ 6.1-2<sup>+</sup> and V $\beta$ 7<sup>+</sup> T cell subsets has been reported in acute IM [31], this phenomenon does not always reflect the expansion of EBV-specific T cells, but may be induced by EBV-induced superantigens [32]. CTL precursors in the latent infection are directed against immunodominant EBV epitopes including EBNA3A, -3B and -3C, although LMP1- and LMP2-specific CD8<sup>+</sup> T cells exist [33]. EBV-specific CD4<sup>+</sup> T cells are believed to maintain EBV-specific CD8<sup>+</sup> memory cells.

In the symptomatic phase, 10<sup>3-4</sup> copies of EBV-DNA are detected in peripheral mononuclear cells or plasma by quantitative real-time PCR amplification. Positive IgM and IgG antibodies to viral capsid antigens (VCA) and early antigens (EA) are usually detected in acute IM, whereas no IgG antibodies to EBV nuclear antigen-1 (EBNA-1) are found in the early stage of primary infections [34]. After the resolution of acute IM, both IgG anti-VCA and anti-EBNA-1 antibodies have a lifelong persistence in the body. During stable carrier stages, CTLs directed against EBV antigens suppress the proliferation of EBV-infected B cells. Although most EBV-infected cells are in the latent cycle *in vivo*, the reactiva-

tion of EBV may occur in various conditions associated with the elevated levels of antibodies to the lytic-cycle EBV antigens such as VCA and EA. Stimuli or reagents that can induce the reactivation of EBV *in vitro* include the cross-linking of surface immunoglobulins, 12-O-tetradecanophorbol-13-acetate (TPA), n-butyrate, 5-azacytidine, trichostatin A (histone acetylation), TGF- $\beta$ 1, high Ca<sup>2+</sup>, and the superinfection of cytomegalovirus or human herpesvirus (HHV)-6. It is intriguing to note that HHV-6, another lymphotropic human herpesvirus, can activate EBV replication and may thus contribute to the pathogenesis of EBV-associated diseases.

**Gianotti-Crosti syndrome.** Gianotti-Crosti syndrome, or papular acrodermatitis of childhood, is characterized by discrete papules on the cheeks, dorsal surfaces of the hands and buttocks, and the extensor aspects of the arms and thighs of infants (Fig. 1). Although hepatitis B infection was found in earlier reported cases, the primary infection of EBV and other viruses may cause essentially the same clinical manifestations. Therefore, a diagnosis of Gianotti-Crosti “disease” is used for hepatitis B-induced cases, excluding cases caused by other viruses. The association of primary EBV infection with Gianotti-Crosti syndrome has been diagnosed by serological studies [6]. In our preliminary studies, however, no EBER-positive cells were found in cutaneous lesions by *in situ* hybridization. No direct evidence for the implication of EBV-infected cells was found in skin lesions by immunohistochemistry [35].

## 2. Systemic disorders associated with latent EBV infection

**Hemophagocytic syndrome.** Hemophagocytic syndrome (HPS), or hemophagocytic lymphohistiocytosis (HLH), is an unusual syndrome character-



Fig. 1 Gianotti-Crosti syndrome.

ized by fever, splenomegaly, jaundice, pancytopenia, disseminated intravascular coagulation (DIC), and features of hemophagocytosis of erythrocytes, leukocytes, and platelets by macrophages in the bone marrow and other tissues [7]. HLH may be associated with various conditions including familial HLH with or without perforin gene defects [36], viral and bacterial infections, lymphomas, cancers, and autoimmune diseases, acute IM, and EBV-associated LPD. EBV-associated HLH commonly occurs in children and adolescents in Asia, but is rarely seen in Western countries [7]. Abnormal laboratory test results reveal pancytopenia, liver dysfunction, coagulopathy, elevated levels of lactate dehydrogenase, ferritin,  $\beta$ 2-microglobulin, and serum cytokines including IFN- $\gamma$ , IL-6, IL-10, sIL-2R, sFas, and Fas L [7]. Overexpression of these cytokines in the process of CTL responses directed against EBV-infected cells may induce hemophagocytosis by activated macrophages, thereby forming beanbag cells. It has been demonstrated that the EBV genome is found in abundance in CD8+ T cells from patients with EBV-associated HLH [37, 38], although latent EBV infection is also detected in other lymphocyte subsets of CD4+ T cells, CD16+ NK cells, and CD20+ B cells.

**Chronic active EBV infection.** Chronic active EBV infection (CAEBV) is a disease of LPD characterized by abnormally high titers of anti-EBV antibodies and increased levels of EBV-DNA in the peripheral blood mononuclear cells and plasma [39]. This disease entity is distinct from chronic fatigue syndrome, which has been misidentified as an EBV-mediated disorder. Japanese children and adults comprise most reported cases of CAEBV. Approximately half of such patients may die of complications, including hepatic failure, gastrointestinal bleeding, HLH and malignant lymphomas several years after disease onset [39, 40, 41]. During the clinical course, some patients may have vesiculopapular eruptions suggestive of hydroa vacciniforme and hypersensitivity to mosquito bites [12, 13, 41]. 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. The presence of small deletions and intragenic mutations that specifically disrupt a gene named DSHP/SH2DIA/SLAM-associated protein (SAP) was detected in unrelated patients with X-linked lymphoproliferative syndrome (Duncan disease), an inherited immunodeficiency characterized by increased susceptibility

to EBV [42]. This gene encodes a predicted protein of 128 amino acids composing a single SH2 domain with extensive homology to the SH2 domain of SHIP, an inositol polyphosphate 5-phosphatase that functions as a negative regulator of lymphocyte activation.

Recently, Kimura *et al.* have analyzed 30 Japanese patients with apparent CAEBV manifestations, and demonstrated that the major cell types carrying latent EBV infection were T cells in 16 patients, and NK cells in 12 patients [43]. The authors reported that the T cell type of CAEBV tends to show high titers of anti-EBV-related antibodies, whereas the NK cell type exhibits the symptoms of hypersensitivity to mosquito bites and high serum concentrations of serum IgE. The real-time PCR method demonstrated that both the T cell and NK cell types contained high levels of EBV load in the circulation, but early mortality is observed in the T cell type of CAEBV [43]. Previous reports demonstrated that the main EBV-infected T cell subset is CD4+ cells in the T cell type of CAEBV, whereas CD8+ T cells are a major target in patients with EBV-associated HLH [38].

### 3. Cutaneous manifestations associated with latent EBV infection and host immune responses

**Hydroa vacciniforme.** In 1999, we reported for the first time that hydroa vacciniforme (HV), a photosensitivity dermatosis of childhood, is mediated by the infiltration of EBV-infected T cells and reactive CTLs [12] (Fig. 2). Skin biopsy specimens from typical HV contain EBV-infected T cells in 3-20% of dermal infiltrates, and PCR amplification demonstrates the presence of EBV DNA sequences in biopsy specimens.

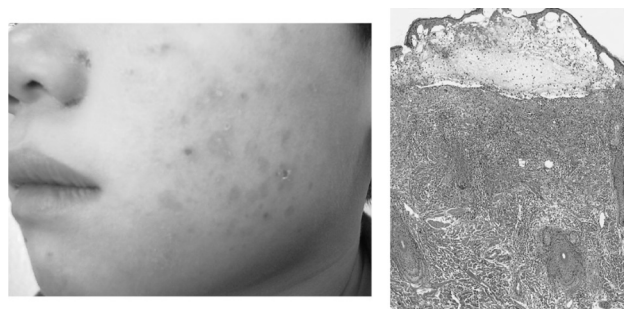


Fig. 2 Hydroa vacciniforme: clinical features and histopathologic findings.

Many reactive T cells containing cytotoxic molecules such as TIA-1 and granzyme B are present in skin lesions. Although no hematological abnormalities are found in typical HV patients, real-time PCR amplifications demonstrate elevated levels of EBV-DNA in peripheral blood mononuclear cells compared with the levels of healthy volunteers. The amounts of EBV-DNA, however, are lower than those of CAEBV (unpublished data). These data indicate that a small number of EBV-infected cells circulate in the blood without detectable hematological abnormalities, and that circulating EBV-infected T cells migrate to sun-exposed skin, together with the infiltration of CTLs.

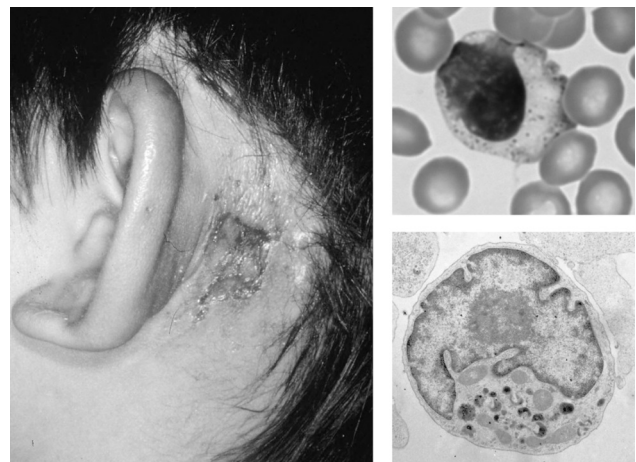
HV is believed to resolve spontaneously with age in most patients. However, our retrospective study disclosed that although a few patients had typical eruptions of HV at disease onset, they progressed to EBV-associated lymphomas 2 to 14 years later [44]. The alarming clinical and laboratory findings which predict progression include 1) no spontaneous resolution by age, 2) the aggravation of eruptions associated with facial swelling, 3) systemic complications such as a high-grade fever and liver damage, 4) dense and deep lymphocytic infiltration containing atypical cells, 5) an increased number of EBER + cells, 6) an episode of hypersensitivity to mosquito bites, 7) abnormal antibody titers to EBV, and 8) an increased level of EBV-DNA in peripheral blood.

**Hypersensitivity to mosquito bites.** NK cell lymphocytosis with latent EBV infection is usually associated with patients with severe hypersensitivity to mosquito bites (HMB) which is characterized by intense local skin reactions and systemic symptoms such as high fever, lymphadenopathy, and hepatosplenomegaly [13] (Fig. 3). The levels of EBV-DNA in the plasma and peripheral blood mononuclear cells are usually elevated in these patients, compared to those of healthy volunteers. Furthermore, many patients with HMB have high antibody titers to lytic-cycle viral proteins such as viral capsid antigens (VCA) and early antigens (EA), suggesting a booster phenomenon to the repetitive reactivation of EBV. The dermal infiltrates in the mosquito-bitten sites are composed of dense infiltration of T and NK (CD56+) cells with cytotoxic molecules, and a small number of EBER-positive cells. Asada *et al.* have reported that patient CD4+ T cells, but not CD8+ T cells or NK cells, respond to mosquito salivary gland extracts [45]. Interestingly, co-culture of the NK cells and CD4+ T cells activated by mosquito extracts induced the expres-

sion of EBV lytic-cycle proteins in the NK cells. Therefore, CD4+ T cells are important for primary skin reactions to mosquito bites, and may play a key role in the reactivation of latent EBV infection in NK cells. Although a specific immune response mediated by CD4+ T cells seems to be important as a trigger, subsequent CTL responses against lytic-cycle viral proteins may be more responsible for the pathogenesis of the IM-like systemic symptoms observed in these patients.

**EBV-associated NK/T cell lymphomas.** EBV is involved in the majority of LPD arising in patients with congenital and acquired immunodeficiencies, and methotrexate-associated lymphoproliferative disorders [8, 9, 46]. Most EBV-associated LPDs are of B cell lineage, but T cell neoplasms and Hodgkin lymphoma may occur. Latent EBV infection plays a pivotal role in the occurrence of African Burkitt's lymphoma, pyothorax-associated lymphomas (PAL), Hodgkin lymphoma, primary effusion lymphoma (PEL) induced by HHV-8 co-infection, and various types of B cell lymphomas [8, 11, 46, 47]. The association of latent EBV infection with NK/T cell lymphomas is less common than B cell lymphomas, but their clinical and histological features are characteristic enough to predict the presence of EBV infection [16, 47, 48].

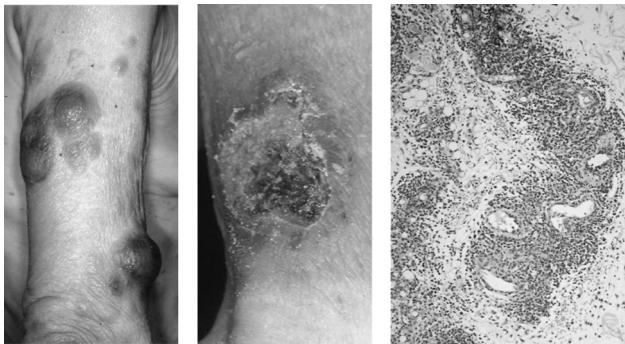
Extranodal NK/T cell lymphoma, nasal type (WHO classification), is a prototype of EBV-associated NK/T cell lymphomas preferentially arising in nasal cavities [16, 47], which often affects the nasopharynx, palates, skin,



**Fig. 3** Hypersensitivity to mosquito bites: clinical features, large granular lymphocytes in the blood (upper right), and an EBV-infected cell line cell (lower right).

soft tissues, gastrointestinal tract, and testis (Fig. 4). Neoplastic cells in most cases appear to be of NK cell lineage, but rare cases show a cytotoxic T cell phenotype [16]. It is, therefore, designated as NK/T (NK or T) cell lymphoma. Cases involving the nasal cavity are identical to the former categories, including nasal lymphoma, angiocentric T cell lymphoma (REAL classification), and lethal midline granuloma. EBV-associated NK/T cell lymphoma is more prevalent in Asia, Mexico, and Central and South America [47]. Angiocentric or angiodestructive infiltration is a hallmark of NK/T cell lymphoma, and prominent ulceration or tissue necrosis is often seen. The neoplastic cells of typical cases express CD2, cytoplasmic CD3 $\epsilon$ , CD 56 and cytotoxic molecules such as TIA-1 and granzyme B, without surface CD3. EBV is usually present in neoplastic cells in a clonal episomal form.

Apart from the prototypic nasal NK/T cell lymphoma, EBV-associated cutaneous lymphomas often show characteristic clinical features [48]: 1) HV-like lymphoma in children or young adults [17, 18] (Fig. 5), 2) puffy



**Fig. 4** Extranodal NK/T cell lymphoma, nasal type: cutaneous lesions and angiocentric infiltration of neoplastic cells.



**Fig. 5** Hydroa vacciniforme-like lymphoma.

eyelid swelling with intramuscular infiltration, masquerading dermatomyositis [19], and 3) panniculitis-like plaque associated with high fever, pancytopenia, and beanbag cells, mimicking cytophagic histiocytic panniculitis. Other clinical phenotypes associated with EBV infections include aggressive NK/T cell lymphoma, and chronic NK/T cell leukemia. NK/T cell lymphoma occurs most often in adults, but children who have suffered from CAEBV and HMB often progress to EBV-associated NK/T cell lymphomas. We believe that EBV-associated NK/T cell lymphomas can be separated into 2 groups: de novo, adult-onset of EBV-associated NK/T cell lymphomas, and those arising in children or young adults, preceded by various EBV-related complications.

Overt EBV-associated lymphomas are usually resistant to conventional chemotherapy, including adriamycin, probably because of multidrug resistant (MDR) gene expression [49], and frequently complicate a fatal hemophagocytic syndrome. No treatment protocol has been established at the present time. Recent advances have made it possible to apply the adoptive immune transfer of EBV-specific cytotoxic T cells for EBV-associated lymphomas arising in patients with bone marrow transplantation or severe CAEBV [50]. This procedure might be applicable for patients with Latency II or III infection, in which some EBV antigens for CTLs are expressed by neoplastic cells. The infusion of donor leukocytes or gene-modified virus-specific T cells has been successfully used to control EBV-associated lymphoproliferative disorders after allogeneic bone marrow transplantation [51]. Because EBV-specific T cells are selectively proliferating in this system, GVHD caused by alloreactive CTL clones are absent or minimal.

#### 4. EBV-infected lymphocyte subsets and related disorders

In patients with CAEBV and HMB, a clonal expansion of EBV-infected NK cells or T cells is often detected by the southern blotting method using a cDNA probe directed against BamHI-digested EBV DNA fragments containing terminal repeat (TR) [52]. Although such patients often have a dominant clone in the blood, latent EBV infection is usually observed in various cell types, including NK, T, and B cells [38, 43]. The patients' blood cells, therefore, contain an oligoclonal pool of lymphocyte subsets infected with EBV, which might give rise to a diversity of clinical symptoms observed in



patients with CAEBV. Among EBV-associated symptoms, NK cell lymphocytosis is closely related to the occurrence of HMB [13], and EBV-infected CD8+ T cells are responsible for that of HLH [37, 38] (Fig. 6). Vesiculopapular skin eruptions mimicking HV are induced by EBV-infected T cells without the infiltration of NK (CD56+) cells [12]. Furthermore, either NK cell or T cell lymphoma may occur from the oligoclonal pool of EBV-infected lymphocyte subsets. These observations suggest that a variety of clinical manifestations result from the existence of various EBV-infected lymphocyte subsets in the blood, but are not induced by a dominant EBV-infected clone alone.

## 5. Molecular mechanisms leading to latent infection and tumor development

**Restricted expression of EBV gene products during latent infection.** During latent infection, EBV is capable of expressing selective viral antigens: 6 EBV-determined nuclear antigens (EBNAs) consisting of EBNA-1, 2, 3A, 3B, 3C, and leader protein (LP), and 3 latent membrane proteins (LMPs), including LMP-1, 2A and 2B [3, 4] (Fig. 7, Table 2). All EBNAs and LMPs, except for EBNA-1, are target

molecules for EBV-specific cytotoxic T cells. In addition to these proteins, 2 kinds of untranslated transcripts, BARF0 and EBV-encoded small nuclear RNA (EBER), are always present during latent infection. Based on the expression patterns of EBV antigens, there are at least 3 distinct forms of latent infection *in vivo*, *i.e.*, Latency I, II, and III [4]. Neoplastic cells from patients with Burkitt lymphoma express only EBNA-1, without any other virus-associated antigens (Latency I). Under this condition, EBV becomes invisible to the immune system because cell mediated immunity is directed against foreign proteins, and is not programmed to recognize foreign nucleic acids. In patients with nasopharyngeal carcinoma in China, Hodgkin lymphoma and T cell lymphomas, neoplastic cells express EBNA-1 and LMPs without any other EBNAs (Latency II). The restriction of EBV gene expression allows EBV-infected cells to evade immune surveillance, and persists throughout latent infection. In contrast, B cell lymphomas in patients with AIDS and immunocompromised hosts can express all EBV antigens (Latency III) because of the exhaustion of host CTL responses.

**Biological functions of EBV gene products expressed in Latency II.** EBNA-1 is essential for replicating EBV episomes during latency by binding to

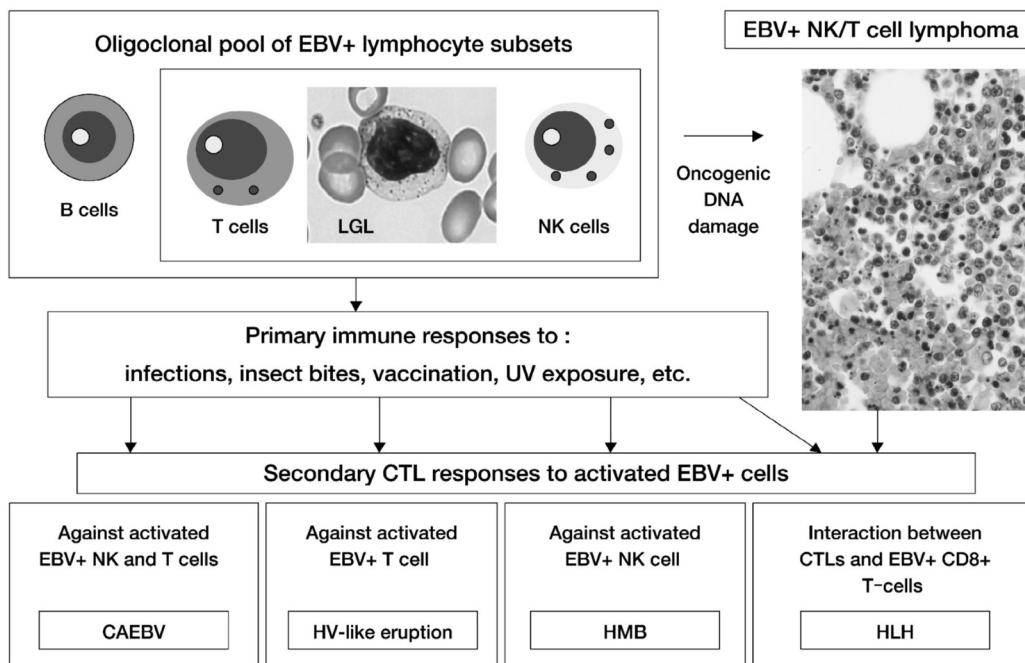


Fig. 6 An oligoclonal pool of EBV-infected lymphocyte subsets in the blood and related disorders.

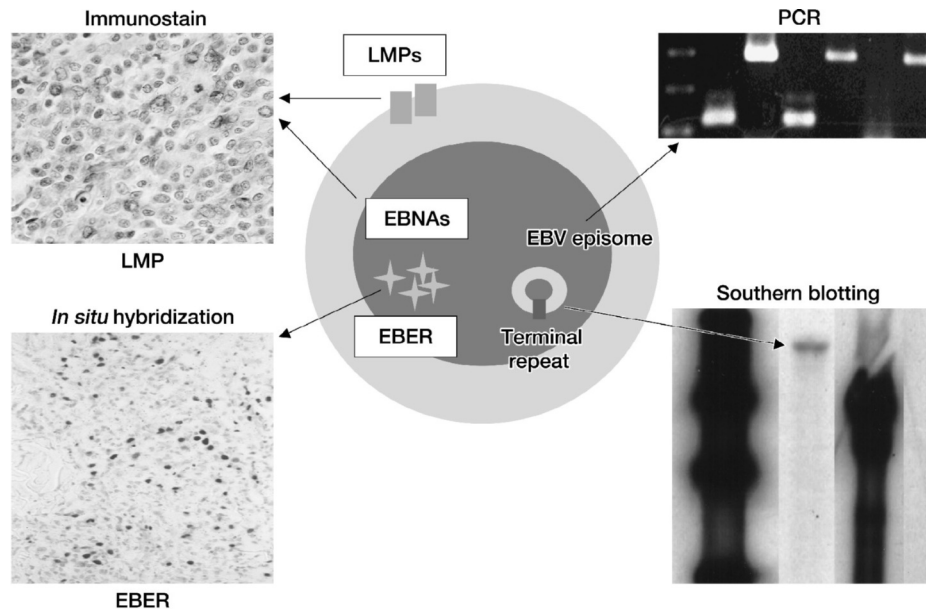


Fig. 7 Detection of latent EBV infection.

Table 2 Patterns of EBV latent infections

EBV gene products	Latency		
	I	II	III
EBNA 1	+	+	+
EBNA 2	-	-	+
EBNA 3A	-	-	+
EBNA 3B	-	-	+
EBNA 3C	-	-	+
EBNA-LP	-	-	+
LMP 1	-	+/-	+
LMP 2A	+/-	+/-	+
LMP 2B	-	+/-	+
EBERs	+	+	+
BARF 0	+	+	+
Diseases	Burkitt lymphoma gastric cancer	Nasopharyngeal carcinoma NK/T-cell lymphoma	Opportunistic lymphoma AIDS-related lymphoma

OriP, a cis-acting element of the EBV genome, and by promoting the replication of viral episomes by host cell DNA polymerase during the S phase of the host cell cycle [53, 54] (Table 3). Furthermore, EBNA-1 is known to upregulate the expression of the V(D)J recombination-activating genes (RAG) 1 and 2 [55]. Levitskaya *et al.* demonstrated that glycine-alanine repeats in EBNA-1 interfere with antigen processing by proteasome and MHC class I-restricted presentation [56]. Other groups,

however, have reported the presence of EBNA-1-specific T cells [57].

LMP-1 shows oncogene activity with the up-regulation of cellular gene expression in various cell types, and prevents apoptosis by the induction of bcl-2 [58]. Recently, it was found that LMP-1 is a viral analogue of the tumor necrosis factor (TNF) receptor family which binds to the TNF receptor-associated factor (TRAF), a signal transduction molecule to the nuclear factor- $\kappa$ B

(NF- $\kappa$ B) [59]. The carboxyl terminus of LMP-1, which contains TES (transformation effector site) 1 and 2, is important for primary B cell transformation, and contains sequences functionally related to the half-lives of LMP-1 proteins [60]. TES1 binds TRAF1, 2, 3, and 5, and TES2 binds TRADD and RIP. LMP-1, therefore, may activate B cells by mimicking the CD40 signaling pathway [61]. The presence of 30-bp deletion mutants within the carboxyl terminal end of LMP-1 has been reported in EBV-associated neoplasms. This deleted LMP-1 variant may have a higher tumorigenic potential, and be preferentially selected in lymphomatous progression.

LMP-2 is composed of 2 splicing variants, LMP-2A and LMP-2B, and contains a molecular ITAM motif. LMP-2 has no function in cell transformation, but blocks the tyrosine kinase phosphorylation of B cell antigen receptors, preventing reactivation of EBV from the latent-cycle infection [62]. In EBV-induced B cell transformation, LMP-1 and LMP-2 may mimic the function

of CD40 signaling, and that of B cell antigen receptors, respectively [61].

***Genetic backgrounds of EBV-associated NK/T cell lymphoproliferative disorders.*** Patients with typical HV are observed worldwide and independent of race, but HV-like lymphomas occur preferentially in Asian countries and Central and South America, where other EBV-associated lymphoproliferative disorders, including HLH, CAEBV, hypersensitivity to mosquito bites, and NK/T cell lymphomas are common [12-20]. Since EBV gene expression is restricted in these disorders (Latency II), only LMPs expressed by the EBV-infected cells are targeted by host CTLs. Despite the absence of overt immunodeficiency, EBV-infected cells are insufficiently abrogated by CTLs in those disorders. Therefore, selective immunological defects to latent EBV infection should be considered in such patients, including an HLA-restricted low response of CTLs to LMPs, immunological tolerance, ignorance or anergy to LMPs, and selective deletion of LMP-

**Table 3** EBV gene products in latent EBV infections

Protein	Functions
EBNA1	Binds to OriP to replicate EBV genome Binds to metaphase chromosome
EBNA-2	Transactivation of LMPs, CD23, c-fgr, and c-myc Binds to EBNA2 responsive element by interacting with RBP-J $\kappa$ /CBF-1
EBNA-3A	Regulation of CD21 expression
EBNA-3B	Regulation of CD40, and CD77
EBNA-3 C	Regulation of LMP-1, CD21, and CD23
EBNA-LP	Interaction with the human metastatic suppressor Nm23-H1 Interaction with HSP 70 family, Rb and p53 Co-operation with EBNA-2
LMP-1	TNF receptor family Contains TES1(transformation effector site 1) which binds to TRAF 1, 2, 3 and 5 Contains TES2 which binds to TRADD and RIP Upregulation of cellular gene expression (CD23, CD39, CD40, CD44, vimentin, LFA-1, LFA-3, ICAM-1, transferrin receptor, bcl-2, cyclin D2, IL-6, and IL-10) Prevention of Ras-induced senescence Inhibition of apoptosis Induction of EGFR expression on epithelial cells Mimic CD40 signaling pathway
LMP-2A/2B	Prevention of virus reactivation by blocking B cell antigen receptor signaling Contains ITAM motif, and mimics B cell antigen receptors
EBER 1/2	Binds to La antigen, EBER-associated protein(EAP)/L22, and dsRNA-dependent protein kinase (PKR) Inactivation of interferon-induced protein kinase, PKR Induction of cellular IL-10
BARTs (BARF0)	Inhibition of virus reactivation? Interaction with Notch

(Adapted from Ref. 53-61)

specific CTLs. Recent studies have disclosed that patients with nasal type EBV-associated NK/T cell lymphomas have a low frequency of HLA-A\*0201 allele, suggesting the importance of this allele in CTL responses [63]. Candidate amino acid sequences for CTL epitopes recognized by the A\*0201 allele may include YLQQ-NWWTL and YLLEMLWRL of LMP-1, and LLWT-LVLL of LMP-2 [2].

***Virokines and EBV-encoded proteins that regulate immune responses and cell kinetics.*** EBV encodes a unique gene product, BCRF1, that has high amino acid identity with human IL-10 [64]. Like human IL-10, vIL-10 inhibits the synthesis of IFN- $\gamma$  by lymphocytes and NK cells and suppresses IFN- $\gamma$ -mediated cellular events such as the up-regulation of the MHC class I expression and CTL responses. The EBV-induced gene 3 (EBI3) heterodimerizes with IL-12 p35, and a complex of the EBI3 and IL-2 p35 subunit functions as a novel, heterodimeric hematopoietin to modulate IL-12-mediated cell immunity [65]. The BARF1 gene encodes a unique, soluble colony-stimulating factor (CSF)-1 receptor, which may act as a decoy receptor to block CSF1-induced IFN- $\alpha$  expression [66]. Low levels of ICAM-1 and LFA-3 expression are observed in B cell lines with EBV latency type I and II, which is associated with an impaired ability to interact with EBV-specific CTL in the antigen-independent phase of effector/target conjugation [67]. HLA class II-mediated antigen presentation to T helper cells is hampered in the presence of the lytic-phase protein, gp42, by interfering with T cell receptor-HLA-DR interaction [68]. The release of virokines and the down-regulation of cell adhesion molecules are additional strategies for EBV-infected cells to evade the host immune system. BHRF1 encodes a 17 kDa protein with both sequence and functional homology to bcl-2, which can protect epithelial cells from apoptosis induced by TNF- $\alpha$ , anti-Fas, and serum deprivation [69].

### Perspectives

Latent EBV infection is maintained by a balance between viral persistence and host CTL responses. Cells carrying episomal EBV in the latent-cycle evade the host immune system by down-regulation of the immunodominant viral antigens and direct modulation of the host CTLs by EBV-encoded proteins. Gaining an understanding of the precise mechanism of latent EBV infection

and host immune responses may contribute to promising therapeutic strategies for controlling EBV-associated disorders.

**Acknowledgments.** This work was supported in part by Grant-in-Aid for Scientific Research (B) (No.14370261) from the Japan Society for the Promotion of Science, and a Grant-in Aid for Exploratory Research (No. 14657200) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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