



Contents lists available at ScienceDirect

Allergology International

journal homepage: <http://www.elsevier.com/locate/alit>

Invited Review Article

Hypersensitivity to mosquito bites: A versatile Epstein–Barr virus disease with allergy, inflammation, and malignancy

Masaki Yamada¹, Yuriko Ishikawa¹, Ken-Ichi Imadome^{*}

Department for Advanced Medicine for Viral Infections, National Center for Child Health and Development, Tokyo, Japan

ARTICLE INFO

Article history:

Received 1 June 2021

Available online xxx

Keywords:

Chronic active Epstein–Barr virus disease (CAEBV)

Epstein–Barr virus (EBV)

Hypersensitivity to mosquito bites (HMB)

Lymphoproliferative disorders (LPD)

Severe mosquito bite allergy (SMBA)

ABSTRACT

Hypersensitivity to mosquito bites (HMB) is a rare disease characterized by transient intense skin reaction and systemic inflammation. Clinical presentation of HMB resembles other mosquito allergic responses, and it can also be difficult to clinically distinguish HMB from other severe allergic reactions. However, a distinctive pathophysiology underlies HMB.

HMB belongs to a category of Epstein–Barr virus (EBV)-associated natural killer (NK) cell lymphoproliferative disorders (LPD). Hence, HMB may progress to systemic diseases, such as hemophagocytic lymphohistiocytosis, chronic active EBV disease, and EBV-associated malignancies.

A triad of elevated serum IgE, NK lymphocytosis, and detection of EBV DNA in peripheral blood is commonly observed, and identification of EBV-infected NK cells usually facilitates the diagnosis. However, the effective treatment is limited, and its precise etiology remains unknown.

Local CD4⁺ T cell proliferation triggered by mosquito bites appears to help induce EBV reactivation and EBV-infected NK-cell proliferation. These immunological interactions may explain the transient HMB signs and symptoms and the disease progression toward malignant LPD.

Further research to elucidate the mechanism of HMB is warranted for better diagnosis and treatment of HMB and other forms of EBV-associated LPD.

Copyright © 2021, Japanese Society of Allergology. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

Mosquito bites usually induce local skin reactions via classic pruritic and inflammatory pathways triggered by a direct effect of salivary components, IgE-mediated hypersensitivity, and modulations of host immune response. On rare occasions, mosquito bite reactions may present as severe allergic reactions with intense local skin signs and symptoms and systemic constitutional signs/symptoms, attributable to an atypical form of Epstein–Barr virus (EBV) infection. This condition is recognized as hypersensitivity to mosquito bites (HMB) or severe mosquito bite allergy (SMBA), a cutaneous form of chronic active Epstein–Barr virus disease (CAEBV).

In this review, we summarize currently available knowledge of epidemiology, clinical features, and etiology of HMB and introduce future research directions to elucidate the pathophysiology of HMB.

Variety of mosquito bite reactions

Mosquito bite reactions refer to a broad range of cutaneous and systemic manifestations occurring after a mosquito bite. Typically, a skin reaction develops during two chronological phases after the mosquito bite, that is, first an immediate wheal and flare reaction develops within 15–30 min, and then a delayed pruritic induration arises within a day or two; the majority of these reactions are transient and self-limited.¹ Although it is rare, mosquito bites may induce large local reactions with systemic signs and symptoms, such as fever, urticaria, and angioedema (i.e., skeeter syndrome, also known as papular urticaria), and immediate hypersensitivity including anaphylaxis.² Only these severe conditions are referred to as “mosquito allergy”, as per the American Academy of Allergy, Asthma, and Immunology.^{3,4}

The etiologies of each reaction type are not fully understood; however, the components of mosquito saliva appear to be the major trigger of mosquito bite reactions. Mosquito saliva contains a large number of biological substances, including lysozymes, antibacterial glucosidases, anticoagulants, antiplatelet aggregating factors, vasodilators, immunomodulators, and others.²

^{*} Corresponding author. Department for Advanced Medicine for Viral Infections, National Center for Child Health and Development, 2-10-1 Okura, Setagaya-ku, Tokyo 157-8535, Japan.

E-mail address: imadome-k@ncchd.go.jp (K.-I. Imadome).

Peer review under responsibility of Japanese Society of Allergology.

¹ These authors contributed equally to this manuscript.

<https://doi.org/10.1016/j.alit.2021.07.002>

1323–8930/Copyright © 2021, Japanese Society of Allergology. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

The IgE-mediated response is considered the major cause of the immediate reaction triggered by mosquito salivary proteins. *In vitro*, some salivary proteins trigger histamine release from basophils,⁵ and IgE and IgG concentrations correlate with the intensity of immediate local reactions.⁶ On the other hand, lymphocyte proliferation in a sequestered lymph node appears to be the key player for delayed local reaction.⁷ This is supported by a few reports showing the presence of proliferating antigen-specific lymphocytes and the reproducibility of skin reaction by mosquito extract.^{2,8}

The spectrum of manifestations also differs depending on the host factors. For example, large local reactions, such as an erythematous induration and even a bullous reaction, may develop in some susceptible cases. The individual susceptibility differs depending on the frequency of mosquito bites and the species or populations of mosquito to which one has been sensitized with previous exposures.⁹

EBV and skin reactions

EBV is a ubiquitous human herpesvirus that infects more than 90% of the adult population worldwide. EBV primarily infects epithelial cells in Waldeyer's ring in the oropharynx and B cells, most frequently via saliva from EBV-infected individuals. Whereas the primary EBV infection occurring in infancy is usually asymptomatic, it can cause a febrile syndrome characterized by tonsillitis, lymphadenopathy, and hepatosplenomegaly, known as infectious mononucleosis (IM), especially when it occurs in adolescents and young adults. Following the primary infection, EBV establishes a life-long latency akin to other herpesviruses. A unique aspect of EBV is its carcinogenesis, only shared with human herpesvirus 8, another human gammaherpesvirus. Several diseases, such as Burkitt lymphoma, Hodgkin lymphoma, nasopharyngeal carcinoma, gastric carcinoma, and other hematological malignancies, have been recognized as EBV-associated malignancies. In EBV-infected cells, latency gene-coded proteins, such as latent membrane protein (LMP) and Epstein–Barr virus nuclear antigen (EBNA), manipulate the process of inhibition of apoptosis, cell proliferation, and escape from immune responses. This results in abnormal proliferation of the EBV-infected cells.¹⁰

EBV-lymphoproliferative disorders (LPD) refer to the proliferation of lymphocytes, including (but not limited to) B cells. When EBV infection occurs in T cells or natural killer (NK) cells, this condition is named separately as EBV-T-/NK-LPD because of its rarity, skewed age distribution, uneven geographic distribution,

and distinctive pathophysiology. A category of “EBV-positive T-cell and NK-cell lymphoproliferative diseases of childhood” is defined in the WHO classification of tumors of hematopoietic and lymphoid tissues (Table 1).¹¹ Here, the term CAEBV refers to EBV-positive LPD of T cells or NK cells. Whereas the persistence of IM-like signs/symptoms and high EBV DNA load in peripheral blood define systemic CAEBV, there are two cutaneous forms of CAEBV, namely, hydroa vacciniforme-like lymphoproliferative disorder (HV-LPD) and SMBA, the latter term being synonymous with the term “HMB”.

Definition of HMB

HMB is an EBV-positive LPD characterized by episodic intense local skin signs and symptoms and high fever following mosquito

Table 2
Characteristics of HMB and CAEBV.^{11,13,19}

Clinical and laboratory findings	Hypersensitivity to mosquito bites	Chronic active EBV disease
General features		
Geographic distribution	East Asia, Latin America	East Asia, Latin America
Triggers	Mosquito bite, vaccination	Not specified
Anatomical site of disease	Dermis to subcutaneous tissue	Liver, lymphoid organ, bone marrow, and others
Systemic signs and symptoms [†]	Present only after the mosquito bite Not present between episodes	Present, often persistent, and progressive
Cutaneous findings	Gangrenous erythema, ulcerative lesions	Rash, unspecified May include the findings of HMB
Laboratory findings		
Granular lymphocytosis [‡]	Present with skin signs and symptoms	Present particularly in CAEBV NK type
Anti-EBV antibodies	Very high titers of anti-VCA IgG Anti-EBNA Ab can be low or negative	Very high titers of anti-VCA IgG Anti-EBNA Ab can be low or negative
Ferritin	Elevated when symptomatic	Elevated
Hypergammaglobulinemia	Occasionally	Occasionally
EBV-infected cells	NK cells with very few exceptions	T cells (60%) and NK cells (40%)
EBV-DNA load (copies/μg DNA)	>1000 when symptomatic Can be undetectable between episodes	>1000 persistently
Disease progression		
Hemophagocytic lymphohistiocytosis	26%	24%
Lymphoma and leukemia	22% to systemic CAEBV/lymphoma/leukemia mainly NK type	16% to lymphoma/leukemia
Mortality	53%	50%

HMB, hypersensitivity to mosquito bites; CAEBV, chronic active EBV disease; VCA, viral capsid antigen; EBNA, Epstein–Barr virus nuclear antigen; NK cells, natural killer cells.

[†] Systemic signs and symptoms include fever, lymphadenopathy, hepatosplenomegaly, and liver dysfunction.

[‡] CD56+ CD16+ HLA-DR+ NK cells with cytotoxic granules.

Table 1
Classification of EBV-positive T-cell and NK-cell proliferation.¹¹

Diagnosis	Usual patient age groups
EBV-positive hemophagocytic lymphohistiocytosis	Pediatric, adolescent
Systemic CAEBV	Pediatric, adolescent
Cutaneous CAEBV, hydroa vacciniforme-like lymphoproliferative disorder	Pediatric, adolescent
Cutaneous CAEBV, severe mosquito bite allergy	Pediatric, adolescent
Systemic EBV-positive T-cell lymphoma	Pediatric, adolescent
Aggressive NK-cell leukemia	Adult
Extranodal NK/T-cell lymphoma, nasal type	Adult
Nodal peripheral T-cell lymphoma, EBV-positive [†]	Adult

EBV, Epstein–Barr virus; NK cells, natural killer cells; CAEBV, chronic active EBV disease.

[†] Included within the category of peripheral T-cell lymphoma, NOS (not otherwise specified).

bites. When disease occurs, patients have NK-cell (occasionally T-cell) lymphocytosis and high EBV DNA load (typically above 1000 copies/ μ g DNA) in the peripheral blood (Table 2). The signs/symptoms may resolve spontaneously with or without topical therapy at the beginning of the disease course. However, the patients with HMB are at an increased risk of developing systemic diseases, such as hemophagocytic lymphohistiocytosis (HLH), systemic CAEBV, and even overt NK-cell/T-cell lymphoma or aggressive NK-cell leukemia in the longstanding clinical course.¹¹ Interestingly, 30%–70% of patients with systemic CAEBV experience the symptoms of HMB,^{12–14} indicating the overlapping disease spectrum between HMB and systemic CAEBV, especially of the NK type. Therefore, when systemic signs/symptoms persist, the diagnosis of CAEBV may be more appropriate rather than HMB. Considering the EBV-NK-LPD spectrum, the Japanese Society of Pediatric Infectious Diseases proposes an algorithm to establish the diagnosis of HMB. When a patient presents with signs/symptoms consistent with mosquito bite allergy, the presence of EBV is assessed either in the skin, blood, or both. If EBV is detected, HMB should be suspected. At this point, if severe multi-organ damage is present, an assessment for systemic CAEBV is recommended. Even after the initial diagnosis of HMB and its recovery, repeated assessment for systemic CAEBV should be considered if systemic signs/symptoms and/or multi-organ damage develop during the follow-up course of HMB (Fig. 1).¹⁵

Epidemiology of HMB

Most HMB cases have been reported in East Asian countries, such as Japan, Korea, China, Taiwan, and a few in Latin America (Table 2).^{16–23}

As its name implies, a mosquito bite is usually a trigger of HMB symptoms. *Aedes albopictus*, *Culex pipiens*, and *Culex tritaeniorhynchus* appear to be the most associated species, though this may be biased by the geographic distribution of those mosquitoes and frequency of exposure in the area where most HMB cases have been reported (e.g., Japan). Although the precise etiology is unknown, immunization with diphtheria–tetanus–pertussis vaccine might have also triggered the HMB-like skin reaction in a few patients.¹⁹

The overall incidence of HMB is extremely low, and there have been only a few hundred patients reported in the literature. HMB occurs typically in children, and there is no sex preponderance. Tokura *et al.* reported that the demographic characteristics of the largest HMB cohort consist of 58 Japanese patients with a primary diagnosis of HMB. The age at the diagnosis was 6.7 ± 5.0 (mean $\pm$ SD) years, and the age at death was 16.3 ± 5.7 (mean $\pm$ SD) years of age, indicating the 10-year survival rate was approximately 50%.

The poor outcomes are usually accompanied by the development of systemic forms of EBV-NK-LPD. At least 15/58 (26%) patients developed HLH, 13/58 (22%) developed systemic CAEBV of NK type, NK lymphoma, or NK leukemia, and 31/58 (53%) died in their longitudinal follow-up (Table 2).^{11,19} Although this prognosis was reported in the era before the standardized management for HLH and CAEBV was established, the unfavorable prognosis of HMB has been similarly reported by others. Kimura *et al.* reported a 10-year survival rate of 12 patients with CAEBV-NK type, including HMB, as 50% (6/12).¹³

Although the decisive factors to predict the prognosis of HMB have not been identified, Miyake *et al.* reported that BZLF1 (EBV-encoded lytic gene) mRNA in the skin tissue was correlated with poor survival, indicating that lytic induction of EBV is a potential risk factor.²⁴

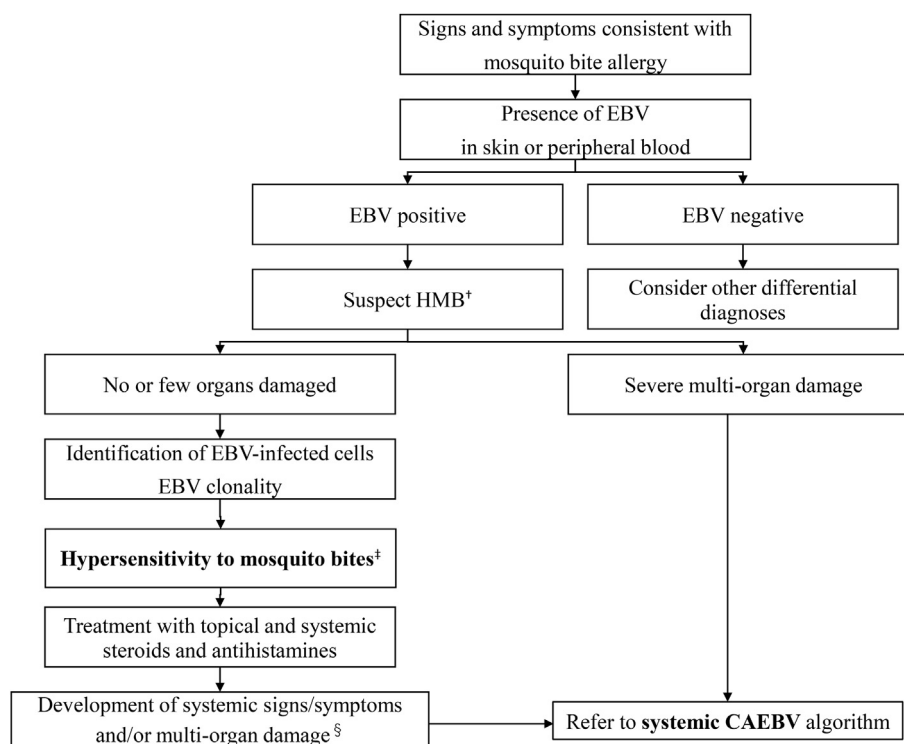


Fig. 1. Diagnostic algorithm for hypersensitivity to mosquito bites.¹⁵ When a patient presents with signs and symptoms consistent with mosquito bite allergy, the presence of EBV is assessed either in the skin, blood, or both. If EBV is positive, HMB should be suspected. ¹Signs, symptoms, and laboratory data should be reviewed. If severe multi-organ damage is present, an assessment for systemic CAEBV is recommended. ²Once the diagnosis of HMB is made, topical and systemic treatment with steroids and antihistamines may be considered. ³Even after the initial diagnosis of HMB and its recovery, repeated assessment for systemic CAEBV should be considered if systemic signs/symptoms and/or multi-organ damage develop during the follow-up course of HMB. EBV, Epstein–Barr virus; HMB, hypersensitivity to mosquito bites; CAEBV, chronic active EBV disease.

Clinical features of HMB

Signs and symptoms

The characteristic local skin signs of HMB include erythema, bullae, ulcers, necrosis, and scarring following the mosquito bite (Fig. 2).²⁵ High fever and general malaise are common symptoms along with cutaneous manifestations.¹⁹ Although similar signs and symptoms may be seen in a patient with non-HMB mosquito allergy or cellulitis, HMB patients can be distinguished by the presence of high EBV DNA load in the peripheral blood and NK cell lymphocytosis.²⁶ Lymphadenopathy, hepatosplenomegaly, hepatic dysfunction, hematuria, and proteinuria are occasionally noted during the symptomatic period, resembling IM and the systemic form CAEBV. However, signs/symptoms of HMB usually resolve within two to three weeks. After the resolution of symptoms, HMB patients remain asymptomatic until the next mosquito bite.

Laboratory findings

The typical laboratory findings have been reported in HMB patients. Elevation of EBV DNA load (>1000 copies/ μ g DNA), marked elevation of EBV VCA-IgG titer (960, [320–2560]) (median, [IQR]),

and EBV infection in NK cells are characteristic.^{19,27} Immunologically, a remarkable increase in CD16⁺ CD56⁺ HLA-DR⁺ NK cells, which have been morphologically recognized as large granular lymphocytes, in the peripheral blood up to 90% of lymphocytes (Fig. 3),^{28,29} and elevation of IgE level (4990, [1900–11,000] IU/mL) (median, [IQR]), have been frequently observed. Occasionally, the elevation of ferritin and gamma globulin levels are observed while systemic signs of inflammation exist.^{19,27,30}

Pathology

Pathology examination at the site of the skin lesion usually shows intraepidermal bullae in the acute phase and epidermal necrosis and ulceration in the recovery phase. Edematous changes in the dermis are usually accompanied by dense perivascular infiltration of mononuclear cells, and various degrees of vascular injury (Fig. 4).³¹ This angioinvasion may result in ischemic and necrotic changes in the affected tissue. Although polymorphic small lymphocytes, large atypical cells, histiocytes, basophils, and eosinophils exist, the major culprit of those changes is believed to be EBV-infected NK cells.²⁷ A considerable component of NK cells are EBV-encoded small RNAs (EBERs) and TIA-1 (a granule-associated protein) positive,¹¹ indicating these EBV infected-NK cells are cytotoxic and share the features with circulating NK cells.



Fig. 2. Skin lesions in a patient with hypersensitivity to mosquito bites.²⁵ Ulcerative, tender lesions surrounded by soft tissue swelling (A). Ulcerative lesions developed after rupture of blood-filled bullae on the face (B) and right lower leg (C). In the recovery phase, ulcerative lesions dried out with scabbing (D: An expanded image of C). A permission has been obtained from the patient's parents for use of this image.

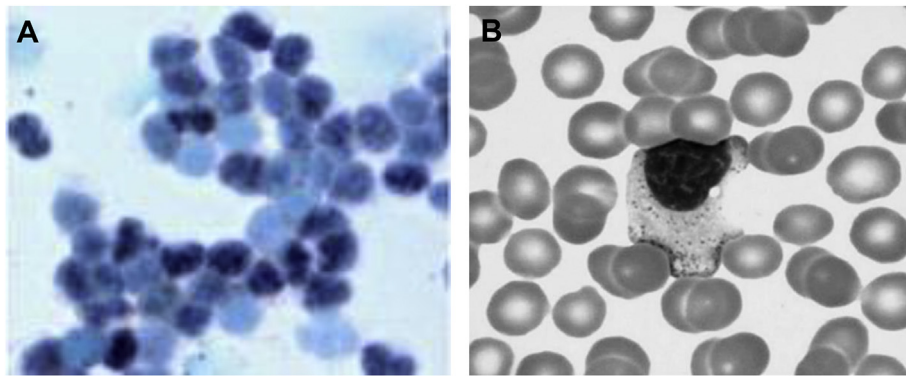


Fig. 3. EBV-infected NK cells in peripheral blood^{27,29} A. In-situ hybridization with EBER1 probe demonstrated EBER-positive EBV-infected NK cells in the cell block of purified NK cells from an HMB patient. B. Large granular lymphocyte in peripheral blood (Wright stain). HMB is associated with NK cell-derived large granular lymphocytes. EBERs, Epstein–Barr virus-encoded small RNAs; EBV, Epstein–Barr virus; NK cells, natural killer cells; HMB, hypersensitivity to mosquito bites.

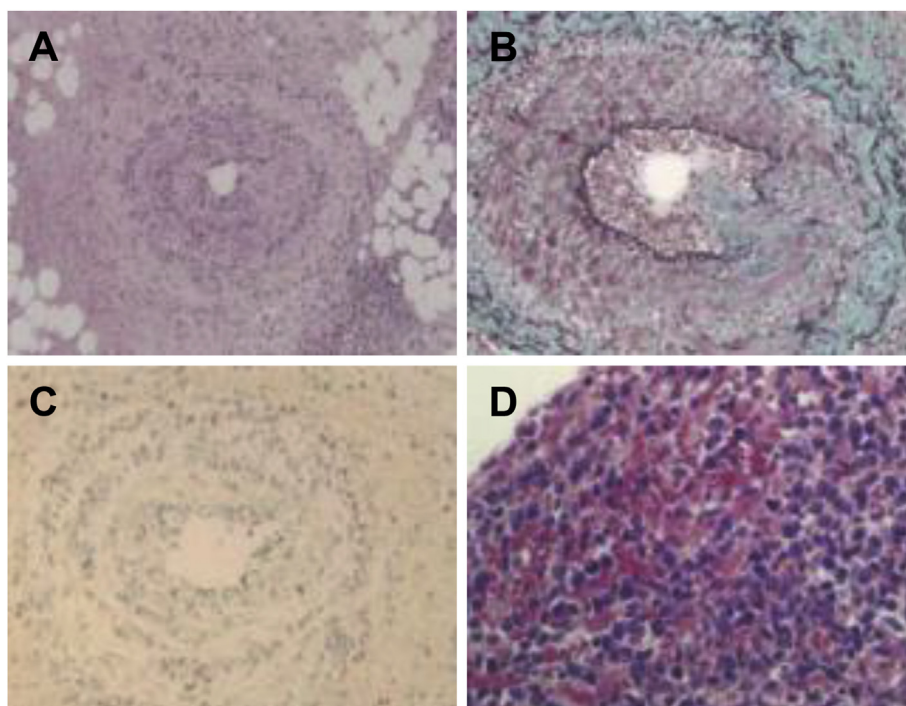


Fig. 4. Pathology examination of skin biopsy in a patient with HMB³¹ A. Intimal thickening of the arterial wall and the infiltration of mononuclear cells throughout the vessel wall (hematoxylin–eosin stain) in the primary mosquito bite lesion. B. Destruction of intimal lamina observed in the same area of image A (Elastica-Goldner stain). C. In-situ hybridization of the biopsy specimen of the primary mosquito bite lesion, using EBER1 probe. Positive staining is visible in many infiltrating cells. D. Biopsy sample from the skin necrosis lesion. The mononuclear cells showing nuclear atypia infiltrate into the vessel wall. HMB, hypersensitivity to mosquito bites; EBERs, Epstein–Barr virus-encoded small RNAs.

Mosquito bite triggering HMB disease cascade

Given the evidence of prior EBV infection, transient elevation of EBV DNA load in the peripheral blood, when the cutaneous signs/symptoms develop triggered by mosquito bite, the etiology of HMB flare appears to be associated with both EBV reactivation and proliferation of EBV-infected NK cells with some immunological triggers.

Asada *et al.* reported several key mechanisms of HMB. Salivary gland extract (SGE) was prepared as stimulants for peripheral blood mononuclear cells (PBMCs) obtained from HMB patients and for *in vitro* as well as *in vivo* scratch patch tests on an HMB patient. They

demonstrated two possible major cascades in the pathogenesis of HMB explained by lytic infection and latent proliferation of EBV-infected NK cells.

Viral reactivation upon stimulation by mosquito bite

Regarding viral reactivation, they demonstrated the following: 1) only CD4⁺ T cells, but not NK cells, sorted from PBMCs of an HMB patient, proliferated in response to SGE stimulation, indicating the presence of SGE-specific CD4⁺ T cells, 2) co-culture of SGE-stimulated CD4⁺ T cells and NK cells from the HMB patient resulted in EBV reactivation confirmed by expression of VCA and early

antigen (EA) in NK cells, 3) scratch patch test with SGE-triggered, site-limited, marked increase of BZLF1 mRNA expression, confirming SGE-induced viral reactivation in the dermis of the HMB patient.³²

These results indicated that the SGE-specific CD4⁺ T cells induce EBV reactivation in EBV-infected NK cells. IM-like symptoms probably develop due to immune responses against EBV lytic antigens with potent antigenicity and other viral components produced upon EBV reactivation.

Proliferation of EBV-infected NK cells induced by mosquito bite

Regarding the proliferation of EBV-infected cells, they also demonstrated the following: 1) NK cells co-cultured with SGE-stimulated CD4⁺ T cells from the PBMCs of HMB patients increased the expression of LMP1, which is the key latent oncogene of EBV, 2) NK cells proliferate while maintaining type 2 latency (described in the next section) when the PBMCs were cultured with SGE, and 3) scratch patch test with SGE on an HMB patient triggered a marked increase in LMP1 mRNA expression only around the site of antigen exposure, confirming SGE-induced proliferation of latently infected NK cells in the dermis of the HMB patient.³³

These results indicated that the SGE-stimulated CD4⁺ T cells also induce the proliferation of NK cells with latent EBV infection in both dermis and peripheral blood, resulting in cutaneous tissue damage and NK lymphocytosis.

HMB in the spectrum of EBV-infected NK-LPD

EBV latency pattern in HMB

EBV latency pattern is defined based on the combination of latency gene expression (Table 3).¹⁰ Type 2 latency is defined by EBER⁺ EBNA-1⁺ EBNA-2/3/LP[−] LMP1/2⁺ and has been confirmed in EBV-T/NK-LPD, including systemic CAEBV, HV, HMB, nasopharyngeal carcinoma, and lymphoma.³⁴

Several known mechanisms of immune escape and carcinogenesis in type 2 latency have been elucidated; absence of EBNA expression helps immune evasion because the antigenicity of EBNA is relatively strong compared to other latent-gene coded proteins; LMP1/2 negatively regulates the cytotoxic T lymphocyte (CTL) responses against EBV-infected cells by inducing apoptosis and upregulating IL-10; and LMP1 mimics CD40, a receptor of CD40L, and upregulates A20 and bcl-2, which inhibit the apoptosis of EBV-infected cells utilizing signals from CD40L⁺ CD4⁺ T cells.^{10,35}

Table 3
EBV latency gene expression patterns.¹⁰

Latency	EBERs	EBNA-1	EBNA-2/3/LP	LMP 1/2	BART	Associated disease
0	+	−	−	−	Unknown	Blood in healthy individuals
1	+	+	−	−	+	Burkitt lymphoma, Gastric carcinoma
2	+	+	−	+	+	NPC, Hodgkin lymphoma, CAEBV
3	+	+	+	+	+	PTLD

BART, Bam-HI A rightward transcripts; EBERs, Epstein–Barr virus-encoded-small RNAs; EBNA, Epstein–Barr virus nuclear antigen; LMP, latent membrane protein; NPC, nasopharyngeal carcinoma; CAEBV, chronic active Epstein–Barr virus disease; PTLD, post-transplant lymphoproliferative disorder.

Clonality

Most HMB and other EBV-NK-LPD cases have demonstrated monoclonal to oligoclonal expansion of EBV by Southern blotting analysis of EBV terminal repeat.³⁶ An assessment of clonality in EBV-infected NK cells is not required to establish the diagnosis of HMB. However, a monoclonal expansion is often observed when the HMB progresses towards a true malignancy, such as lymphoma. Hence, the clonality can be used to assess the degree of malignancy when a high EBV DNA load and an increase in NK cells are present.³⁰

Gene expression profiles of HMB and systemic CAEBV

Although there are many common biological features among HMB and other EBV-NK-LPDs, the presence of unique gene expression patterns in HMB is suspected due to the distinctively different clinical features of HMB.

Washio *et al.* reported the gene expression profiles amongst EBV-infected cell lines with type 2 latency.³⁷ Cell lines derived from an HMB patient (named as KAI3), CAEBV patients, and EBV-infected lymphoma were used for the analysis. They identified over-expression of genes responsible for 1) promoting cell-cycle progression, 2) both induction and evasion of apoptosis, and 3) activation of JAK/STAT pathways amongst all EBV-infected cell lines, but only KAI3 (HMB cell line) showed no expression of Src homology 2 domain-containing protein tyrosine phosphatase 1 (SHP-1). Notably, SHP-1 is essential for NK cell activation.³⁸ Whereas all EBV-infected cell lines shared the overall features of dysregulated cell proliferation, the absence of SHP-1 gene expression implied that KAI3 is incapable of activation, whereas others can be activated upon stimulation. The different degrees of inflammation in HMB patients compared to systemic CAEBV may be explained by the EBV-infected NK cells being in a different state regulating activation.

Cytokine profiles of HMB and systemic CAEBV

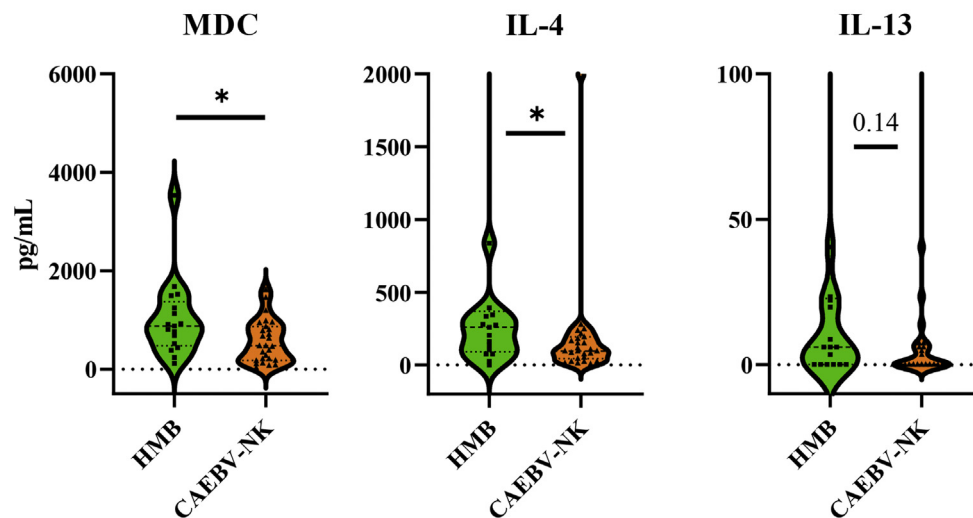
Serum cytokine profiling can be a useful tool to estimate the degree of inflammation and types of immune response in each subject. Given the clinical and transcriptomic differences between HMB and systemic CAEBV, we hypothesized that there were different cytokine profiles between HMB patients (n = 17) and CAEBV-NK type patients (n = 27) and performed a multiplex cytokine array (Milliplex®) to investigate them (unpublished data).

Notably, some Th2 cytokines such as macrophage-derived chemokine (MDC) and IL-4 were higher in HMB patients than in CAEBV patients (*P* = 0.02 and 0.04, respectively). In contrast, Th1 cytokines, such as interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α), were significantly elevated in patients with CAEBV-NK type (*P* = 0.04) (Fig. 5).

CCR4, also known as CCL22, is a ligand for chemokine receptor 4 (CCR4). CCR4 is preferentially expressed in Th2 cells, and the frequency of CCR4⁺ cells correlates with serum IgE levels, reflecting Th2 polarization.³⁹ Notably, MDC is expressed in inflamed skin tissue, and elevation of serum MDC has been observed in patients with atopic dermatitis. MDC promotes chemotaxis of CCR4⁺ CD4⁺ T cells toward the inflamed skin.⁴⁰

As a remarkable increase of Th2 cytokines and a high level of IgE have been observed in HMB patients,⁴¹ an elevation of MDC and IL-4 may reflect Th2 polarization and their effect to promote migration of CCR4⁺ CD4⁺ T cells to the site of the mosquito bite in the HMB patients.

A. Th2 cytokines



B. Th1 cytokines

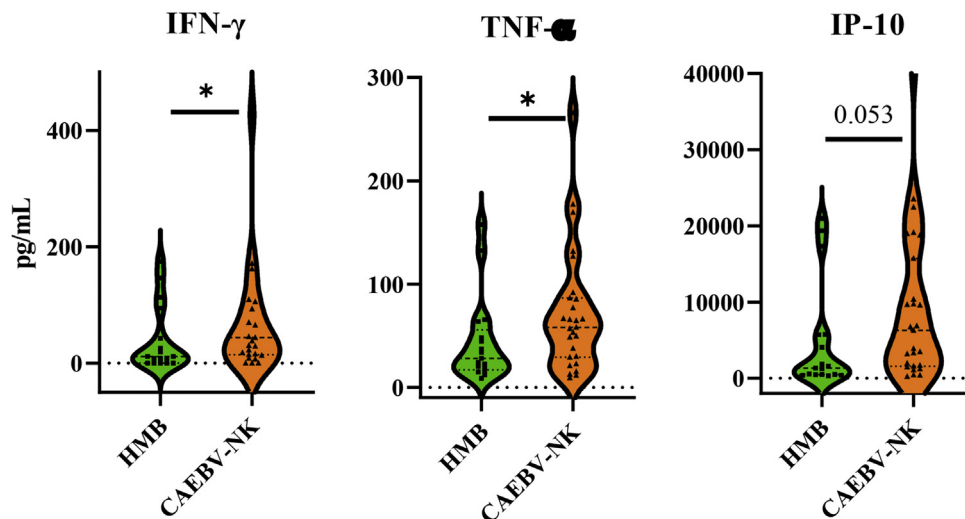


Fig. 5. Cytokine profiles of EBV-associated NK cell lymphoproliferative disease. Several cytokines were measured in plasma from the patients with HMB (n = 17) and CAEBV-NK type (n = 27). A. MDC and IL-4 showed significant elevation in the patients with HMB. B. IFN- γ and TNF- α showed significant elevation in the patients with CAEBV-NK type. EBV, Epstein–Barr virus; CAEBV, chronic active EBV disease; MDC, macrophage-derived chemokine; IFN- γ , interferon gamma; TNF- α , tumor necrosis factor alpha; IP-10, interferon-gamma-induced protein 10. * $P < 0.05$.

Additionally, Kumai *et al.* reported that EBV-infected NK cell lines, such as SNK-6 and SNK-1, but not EBV-negative NK cell lines, secreted MDC and expressed CCR4, and the moderate component of EBV-infected NK cells expresses CCR4 in nasal NK lymphoma.⁴² Also, Nakayama *et al.* revealed that LMP1 induces MDC in EBV-infected B cell lines.⁴³

Taken together, our results imply a positive feedback loop promoting the migration of mosquito salivary antigen-specific CD4⁺ T cells and EBV-infected NK cells into the affected skin lesion via the MDC-CCR4 axis. The accumulation of CD4⁺ T cells and EBV-infected NK cells promotes interaction between those cells and further enhances the Th2 immune response, IgE production, lytic induction,

and proliferation of EBV-infected NK cells, which are believed to be the key aspects of pathogenesis seen in symptomatic HMB patients.

On the other hand, when a patient develops systemic CAEBV, the Th1 immune response appears to take over. Both IFN- γ and TNF- α are Th1-inflammatory cytokines that activate NK cells. This inflammatory process is usually effective in destroying viral-infected cells. However, EBV-infected NK cells utilize these signals for their activation and proliferation by enhancing anti-apoptotic signals. One patient demonstrated a remarkable decrease in IgE levels while developing lymphoma, suggesting the switch from Th2 to Th1 polarization as EBV-LPD progresses.⁴¹ Accordingly, dysregulated production of inflammatory/anti-apoptotic cytokines is

even more exaggerated by activated NK cells, which are potent sources of IFN- γ , and may lead to the development of the systemic form of EBV-NK-LPD (Fig. 6).^{44,45}

It is not known what stimulates the change in mosquito bites with Th2 polarization from indolent EBV-infected NK cells with transient proliferation to the aggressive proliferation of activated EBV-infected NK cells in Th1-polarization. However, our observations in conjunction with other reports may provide new insight to elucidate the mechanistic etiology of HMB.

Future research directions

Mechanistic elucidation of HMB is extremely challenging due to the rarity of the disease, lack of practical animal models, and the transient immune response occurring only after the mosquito bites. However, there are newer experimental approaches that are potentially useful to investigate the pathogenesis of HMB and other EBV-T/NK-LPD.

Phenotypic and functional assays of EBV-infected NK cells

Identification of EBV-infected cells is crucial to make a diagnosis of HMB and other EBV-NK-LPD. There are several ways to identify the type of EBV-infected cells.

EBERs are the most widely used target by in-situ hybridization to identify EBV-infected cells in pathological examinations, as EBERs exist ubiquitously in the cytoplasm of EBV-infected cells. However, extensive phenotypic assays and functional analysis cannot be performed. Detection of EBV-DNA in sorted peripheral

lymphocytes has also been used, particularly when tissue examination is not practically possible. However, detection of EBV DNA and phenotypic and functional analysis on limited patient samples are usually incompatible.

Fournier *et al.* reported that a new fluorescent in-situ hybridization flow cytometry technique enables the detection of EBERs and other EBV-encoded RNAs, as well as surface and intracellular molecules.^{46,47} This can be applied to investigate the detailed immunophenotype, function, and latency patterns of EBV-infected cells at a single-cell level. By using this technique, they demonstrated the increased de-granulating and proliferating ability in EBV-infected CD8⁺ T cells, compared to the bulk CD8⁺ T cells.

Genetic and epigenetic factors for the development of HMB and its disease progression

How and why B-lymphotropic EBV infects NK cells remains an unsolved research question.⁴⁸ Because of the restricted geographic distribution of HMB, the presence of a genetic predisposition to allow EBV to infect NK cells is strongly suspected. Although the diagnosis of HMB (and other forms of CAEBV) requires the exclusion of known immunodeficiency, malignancy, and autoimmune diseases, several gene mutations have been confirmed by next-generation sequencing methods in CAEBV patients.⁴⁹ These mutations do not cause apparent immunodeficiency according to the current knowledge but may predispose the risk of developing HMB and other forms of CAEBV.

Newer techniques of single-cell DNA and RNA sequencing are expected to answer these research questions. It may help discover

Disease progression of HMB in the spectrum of EBV-NK-LPD

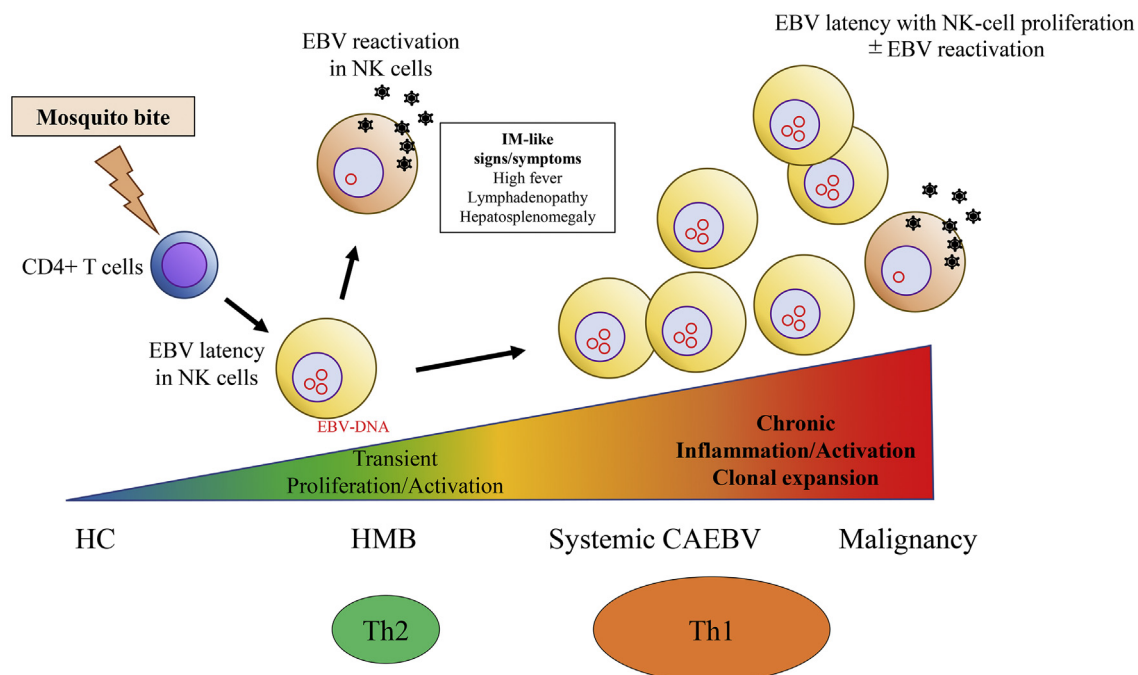


Fig. 6. Disease progression of HMB in the spectrum of EBV-NK-LPD.¹⁴ Mosquito bite stimulates antigen-specific CD4⁺ T cells, which subsequently help EBV-infected NK-cell proliferation in dermis and EBV reactivation in a Th2 environment. When EBV DNA is detected in the peripheral blood, IM-like signs/symptoms, such as high fever, lymphadenopathy, and hepatosplenomegaly may develop. This condition is initially transient and usually resolves until the next episode of mosquito bite occurs. The indolent HMB may progress to systemic CAEBV and even EBV-related malignancies. When the disease progresses, EBV-infected NK cells often clonally proliferate and lyse, producing inflammatory granules and other cytokines. EBV, Epstein–Barr virus; NK cells, natural killer cells; HC, healthy control; HMB, hypersensitivity to mosquito bites; CAEBV, chronic active EBV disease.

unreported genetic backgrounds, epigenetics, and gene expression patterns towards the malignant proliferation of EBV-infected cells and surrounding immune cells in the patients with HMB and other EBV-T-/NK-LPD.

Acknowledgments

This work was supported by research grants (30-E3) from the National Center for Child Health and Development (NCCHD). The authors thank the senior medical editor from the Division of Postgraduate Education and Training at the NCCHD, laboratory technical staff, Naomi Wada and Miyuki Ogura at the NCCHD.

Conflict of interest

The authors have no conflict of interest to declare.

References

1. Fostini AC, Golpanian RS, Rosen JD, Xue R-D, Yosipovitch G. Beat the bite: pathophysiology and management of itch in mosquito bites. *Itch* 2019;**4**:e19.
2. Engler RJM, Crisp HC. *Mosquito Hypersensitivity: Clinical Updates*. Springer International Publishing; 2017. p. 203–30.
3. Peng Z, Simons FE. Mosquito allergy: immune mechanisms and recombinant salivary allergens. *Int Arch Allergy Immunol* 2004;**133**:198–209.
4. Golden DBK, Moffitt J, Nicklas RA, Freeman T, Graft DF, Reisman RE, et al. Stinging insect hypersensitivity: a practice parameter update 2011. *J Allergy Clin Immunol* 2011;**127**: 852.4.e23.
5. Reunala T, Brummer-Korvenkontio H, Rasanen L, Francois G, Palosuo T. Passive transfer of cutaneous mosquito-bite hypersensitivity by IgE anti-saliva antibodies. *J Allergy Clin Immunol* 1994;**94**:902–6.
6. Peng Z, Yang M, Simons FE. Immunologic mechanisms in mosquito allergy: correlation of skin reactions with specific IgE and IgG antibodies and lymphocyte proliferation response to mosquito antigens. *Ann Allergy Asthma Immunol* 1996;**77**:238–44.
7. Peng Z, Simons FE. Advances in mosquito allergy. *Curr Opin Allergy Clin Immunol* 2007;**7**:350–4.
8. Karppinen A, Rantala I, Vaalasti A, Palosuo T, Reunala T. Effect of cetirizine on the inflammatory cells in mosquito bites. *Clin Exp Allergy* 1996;**26**:703–9.
9. Peng Z, Simons FE. A prospective study of naturally acquired sensitization and subsequent desensitization to mosquito bites and concurrent antibody responses. *J Allergy Clin Immunol* 1998;**101**:284–6.
10. Fields BN, Knipe DM, Howley PM. *Fields Virology*. 6th ed. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2013.
11. Swerdlow SHCE, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. 69372 Lyon Cedex 08, France: International Agency for Research on Cancer (IARC); 2017.
12. Kimura H, Hoshino Y, Hara S, Sugaya N, Kawada J-i, Shibata Y, et al. Differences between T Cell-type and natural killer cell-type chronic active Epstein-Barr virus infection. *J Infect Dis* 2005;**191**:531–9.
13. Kimura H, Hoshino Y, Kanegane H, Tsuge I, Okamura T, Kawa K, et al. Clinical and virologic characteristics of chronic active Epstein-Barr virus infection. *Blood* 2001;**98**:280–6.
14. Asada H. Hypersensitivity to mosquito bites: a unique pathogenic mechanism linking Epstein-Barr virus infection, allergy and oncogenesis. *J Dermatol Sci* 2007;**45**:153–60.
15. Japanese Society of Pediatric Infectious Diseases. [Practice Guideline for Chronic Active Epstein-Barr Virus Infection, EBV-associated Hemophagocytic Lymphohistiocytosis, Hydroa Vacciniiforme, Severe Mosquito Bite Allergy]. Tokyo: Shindan-to-chiryo-sha; 2016 (in Japanese).
16. Shigekiyo T, Ohmori H, Chohraku M, Ohtsuka S, Yamabe K, Takishita Y, et al. Unusual skin reactions after mosquito bites and Epstein-Barr virus reactivation in a patient with mantle cell lymphoma. *Intern Med* 2004;**43**:986–9.
17. Zhang Z, Shi Q, An X, Ma H, Zhou H, Ma J, et al. NK/T-cell lymphoma in a child with hypersensitivity to mosquito bites. *J Pediatr Hematol Oncol* 2009;**31**: 855–7.
18. Seon HS, Roh JH, Lee SH, Kang EK. A case of hypersensitivity to mosquito bites without peripheral natural killer cell lymphocytosis in a 6-year-old Korean boy. *J Korean Med Sci* 2013;**28**:164–6.
19. Tokura Y, Ishihara S, Tagawa S, Seo N, Ohshima K, Takigawa M. Hypersensitivity to mosquito bites as the primary clinical manifestation of a juvenile type of Epstein-Barr virus-associated natural killer cell leukemia/lymphoma. *J Am Acad Dermatol* 2001;**45**:569–78.
20. Pacheco SE, Gottschalk SM, Gresik MV, Dishop MK, Okmaura T, McCormick TG. Chronic active Epstein-Barr virus infection of natural killer cells presenting as severe skin reaction to mosquito bites. *J Allergy Clin Immunol* 2005;**116**:470–2.
21. Kyriakidis I, Vasileiou E, Karastrati S, Tragiannidis A, Gompakis N, Hatzistilianou M. Primary EBV infection and hypersensitivity to mosquito bites: a case report. *Viol Sin* 2016;**31**:517–20.
22. Jiang S, Manandhar U, Zheng KP, Liu Q, Liang P, Song JQ. A case of nodal marginal zone lymphoma with hypersensitivity to mosquito bites as initial symptom. *J Cutan Pathol* 2019;**46**:769–74.
23. Roh EJ, Chung EH, Chang YP, Myoung NH, Jee YK, Seo M, et al. A case of hypersensitivity to mosquito bite associated with Epstein-barr viral infection and natural killer cell lymphocytosis. *J Korean Med Sci* 2010;**25**:321–3.
24. Miyake T, Yamamoto T, Hirai Y, Otsuka M, Hamada T, Tsuji K, et al. Survival rates and prognostic factors of Epstein-Barr virus-associated hydroa vaccini-forme and hypersensitivity to mosquito bites. *Br J Dermatol* 2015;**172**:56–63.
25. Shoji K, Imadome KI, Miyairi I, Yoshida K, Ishiguro A. Multiple round ulcers after mosquito bites in a pediatric patient with chronic active EBV infection. *Pediatr Int* 2019;**61**:109–10.
26. Wada T, Yokoyama T, Nakagawa H, Asai E, Toga A, Sakakibara Y, et al. Flow cytometric analysis of skin blister fluid induced by mosquito bites in a patient with chronic active Epstein-Barr virus infection. *Int J Hematol* 2009;**90**:611–5.
27. Sakakibara Y, Wada T, Muraoka M, Matsuda Y, Toma T, Yachie A. Basophil activation by mosquito extracts in patients with hypersensitivity to mosquito bites. *Cancer Sci* 2015;**106**:965–71.
28. Tokura Y, Tamura Y, Takigawa M, Koide M, Satoh T, Sakamoto T, et al. Severe hypersensitivity to mosquito bites associated with natural killer cell lymphocytosis. *Arch Dermatol* 1990;**126**:362–8.
29. Chung JS, Shin HJ, Lee EY, Cho GJ. Hypersensitivity to mosquito bites associated with natural killer cell-derived large granular lymphocyte lymphocytosis: a case report in Korea. *Korean J Intern Med* 2003;**18**:50–2.
30. Ishihara S, Ohshima K, Tokura Y, Yabuta R, Imaishi H, Wakiguchi H, et al. Hypersensitivity to mosquito bites conceals clonal lymphoproliferation of Epstein-Barr viral DNA-positive natural killer cells. *Jpn J Cancer Res* 1997;**88**: 82–7.
31. Kanno H, Onodera H, Endo M, Maeda F, Chida S, Akasaka T, et al. Vascular lesion in a patient of chronic active Epstein-Barr virus infection with hypersensitivity to mosquito bites: vasculitis induced by mosquito bite with the infiltration of nonneoplastic Epstein-Barr virus-positive cells and subsequent development of natural killer/T-cell lymphoma with angiodestruction. *Hum Pathol* 2005;**36**: 212–8.
32. Asada H, Miyagawa S, Sumikawa Y, Yamaguchi Y, Itami S, Suguri S, et al. CD4+ T-lymphocyte-induced Epstein-Barr virus reactivation in a patient with severe hypersensitivity to mosquito bites and Epstein-Barr virus-infected NK cell lymphocytosis. *Arch Dermatol* 2003;**139**:1601–7.
33. Asada H, Saito-Katsuragi M, Niizeki H, Yoshioka A, Suguri S, Isonokami M, et al. Mosquito salivary gland extracts induce EBV-infected NK cell oncogenesis via CD4 T cells in patients with hypersensitivity to mosquito bites. *J Invest Dermatol* 2005;**125**:956–61.
34. Tsuge I, Morishima T, Morita M, Kimura H, Kuzushima K, Matsuoka H. Characterization of Epstein-Barr virus (EBV)-infected natural killer (NK) cell proliferation in patients with severe mosquito allergy: establishment of an IL-2-dependent NK-like cell line. *Clin Exp Immunol* 1999;**115**:385–92.
35. Imadome K, Shimizu N, Arai A, Miura O, Watanabe K, Nakamura H, et al. Coexpression of CD40 and CD40 ligand in Epstein-Barr virus-infected T and NK cells and their role in cell survival. *J Infect Dis* 2005;**192**:1340–8.
36. Ohga S, Ishimura M, Yoshimoto G, Miyamoto T, Takada H, Tanaka T, et al. Clonal origin of Epstein-Barr virus (EBV)-infected T/NK-cell subpopulations in EBV-positive T/NK-cell lymphoproliferative disorders of childhood. *J Clin Virol* 2011;**51**:31–7.
37. Washio K, Oka T, Abdalkader L, Muraoka M, Shimada A, Oda M, et al. Gene expression analysis of hypersensitivity to mosquito bite, chronic active EBV infection and NK/T-lymphoma/leukemia. *Leuk Lymphoma* 2017;**58**:2683–94.
38. Viant C, Fenis A, Chicanne G, Payrastre B, Ugolini S, Vivier E. SHP-1-mediated inhibitory signals promote responsiveness and anti-tumour functions of natural killer cells. *Nat Commun* 2014;**5**:5108.
39. Nakatani T, Kaburagi Y, Shimada Y, Inaoki M, Takehara K, Mukaida N, et al. CCR4+ memory CD4+ T lymphocytes are increased in peripheral blood and lesional skin from patients with atopic dermatitis. *J Allergy Clin Immunol* 2001;**107**:353–8.
40. Yoshie O, Matsushima K. CCR4 and its ligands: from bench to bedside. *Int Immunol* 2015;**27**:11–20.
41. Kunitomi A, Konaka Y, Yagita M. Hypersensitivity to mosquito bites as a potential sign of mantle cell lymphoma. *Intern Med* 2005;**44**:1097–9.
42. Kumai T, Nagato T, Kobayashi H, Komabayashi Y, Ueda S, Kishibe K, et al. CCL17 and CCL22/CCR4 signaling is a strong candidate for novel targeted therapy against nasal natural killer/T-cell lymphoma. *Cancer Immunol Immunother* 2015;**64**:697–705.
43. Nakayama T, Hieshima K, Nagakubo D, Sato E, Nakayama M, Kawa K, et al. Selective induction of Th2-Attracting chemokines CCL17 and CCL22 in human B cells by latent membrane protein 1 of Epstein-barr virus. *J Virol* 2004;**78**: 1665–74.
44. Mizuki M, Ueda S, Tagawa S, Shibayama H, Nishimori Y, Shibano M, et al. Natural killer cell-derived large granular lymphocyte lymphoma of lung developed in a patient with hypersensitivity to mosquito bites and reactivated Epstein-Barr virus infection. *Am J Hematol* 1998;**59**:309–15.

45. Ohshima S, Ishii M, Asada H, Tatekawa T, Yamaguchi N, Kobayashi H, et al. A possible mechanism of NK cell-lineage granular lymphocyte proliferative disorder (NK-GLPD) in a patient with chronic active Epstein-Barr virus infection (CAEBV) and severe hypersensitivity to mosquito bites (SHMB). *Intern Med* 2002;**41**:651–6.
46. Kimura H, Miyake K, Yamauchi Y, Nishiyama K, Iwata S, Iwatsuki K, et al. Identification of Epstein-Barr virus (EBV)-infected lymphocyte subtypes by flow cytometric in situ hybridization in EBV-associated lymphoproliferative diseases. *J Infect Dis* 2009;**200**:1078–87.
47. Fournier B, Boutboul D, Bruneau J, Miot C, Boulanger C, Malphettes M, et al. Rapid identification and characterization of infected cells in blood during chronic active Epstein-Barr virus infection. *J Exp Med* 2020;**217**:e30192262.
48. Fujiwara S, Nakamura H. Chronic active Epstein-Barr virus infection: is it immunodeficiency, malignancy, or both? *Cancers* 2020;**12**:3202.
49. Okuno Y, Murata T, Sato Y, Muramatsu H, Ito Y, Watanabe T, et al. Defective Epstein-Barr virus in chronic active infection and haematological malignancy. *Nat Microbiol* 2019;**4**:404–13.