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Basophil activation by mosquito extracts in patients with hypersensitivity to mosquito bites

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Key words

Basophils, Epstein–Barr virus, hypersensitivity to mosquito bites, IgE, natural killer cells

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Hypersensitivity to mosquito bites (HMB) is a cutaneous disorder belonging to the group of Epstein–Barr virus (EBV)-associated T/natural killer (NK)-cell lymphoproliferative diseases, and is primarily mediated by EBV-infected NK cells. It is characterized by intense local skin reactions accompanied by general symptoms after mosquito bites, and infiltration of EBV-infected NK cells into the bite sites. However, the mechanisms underlying these reactions have not been fully examined. We recently described the activation of circulating basophils by mosquito extracts *in vitro* in a patient with HMB. To further investigate this finding, we studied four additional patients with HMB. All patients showed typical clinical features of HMB after mosquito bites and they had NK lymphocytosis and high peripheral blood EBV DNA loads. We found evidence of EBV infection in NK cells through *in situ* hybridization that detected EBV-encoded small RNA-1, and flow cytometry showed HLA-DR expression on almost all NK cells. Basophil activation tests with the extracts of epidemic mosquitoes *Culex pipiens pallens* and *Aedes albopictus* showed positive responses to one or both extracts in all samples from patients with HMB, suggesting the presence of mosquito antigen-specific IgE and its binding to basophils. In particular, the extract of *Aedes albopictus* was able to activate basophils in all available patient samples. These results indicate that basophils and/or mast cells activated by mosquito bites may be involved in initiation and development of severe skin reactions to mosquito bites in HMB.

More than 90% of humans are infected with the Epstein–Barr virus (EBV) and the infection persists for life.[1] EBV mostly infects B cells in healthy persons who are often asymptomatic or may occasionally develop infectious mononucleosis (IM).[2] It rarely infects T or natural killer (NK) cells in healthy children and young adults, and the chronic proliferation of those EBV-infected cells causes EBV-associated T/NK-cell lymphoproliferative diseases.[3] EBV-associated T/NK-cell lymphoproliferative diseases present with diverse clinical and pathological findings, and were classified into the following disorders at the 2012 lymphoma workshop of the European Association for Haematopathology meeting: (i) chronic active EBV infection (CAEBV); (ii) systemic, malignant EBV-positive lymphoproliferative diseases; (iii) extranodal NK-cell/T-cell lymphoma, nasal type; and (iv) nodal T-cell/NK-cell lymphoma.[4] CAEBV include the systemic type, hydroa vacciniforme and hypersensitivity to mosquito bites (HMB).

Hypersensitivity to mosquito bites is a cutaneous disorder characterized by intense local skin reactions manifesting as erythema, bullae, ulcers and scar formation accompanied by systemic symptoms, including high fever, lymphadenopathy, liver dysfunction and the hemophagocytic syndrome after mosquito bites.[5] HMB has also been regarded as severe mosquito allergy, but such severe skin lesions and clonal proliferation of EBV-infected cells do not occur in simple allergy. The major cellular targets of latent EBV infection in HMB are NK cells, and NK cell lymphocytosis and high IgE levels are characteristic laboratory findings.[6] The pathogenesis of HMB has not been well defined. The mosquito bite site in patients with HMB showed perivascular mononuclear cell infiltration with an increased number of EBV-infected NK cells and $CD4^+$ T cells.[7] In addition, it has been reported that $CD4^+$ T cells from patients responded markedly to mosquito salivary gland extracts *in vitro* and that these $CD4^+$ T cells stimulated by the mosquito antigen were able to induce reactivation of latent EBV infection and the proliferation of NK cells *in vitro*.[5,8] Thus, $CD4^+$ T cells and EBV-infected NK cells are considered to be involved in the pathogenesis of HMB. Nevertheless, our previous study demonstrated that there were not only T and NK cells but also many $CD203c^+$ cells, representing basophils and/or mast cells, in the blister fluids from a patient with HMB.[9] In addition, we found basophil activation with exposure to the extract of *Aedes albopictus* (*Ae. albopictus*) in the basophil activation test (BAT). These findings suggest the possible involvement of mosquito-specific IgE and $CD203c^+$ cells in the severe skin reactions to mosquito bites in HMB.

Basophils and mast cells are similar in terms of expression of the high-affinity IgE receptor and the secretion of cytokines and inflammatory mediators upon stimulation.[10] Therefore, in human studies, basophils have often been utilized as surrogates of tissue-resident mast cells *in vitro* because basophils are easily accessible in the peripheral blood, in contrast to mast cells.[11,12] BAT is a flow cytometry-based test that measures basophil activation markers like CD63 and CD203c on the surface of basophils after *in vitro* stimulation with allergens.

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Because BAT has high sensitivity and specificity, it has been recently applied as an *in vitro* diagnostic method for various IgE-mediated allergic diseases.[13]

There are no studies apart from our previous study that examine basophils in patients with HMB. The present study aims to clarify the existence of mosquito antigen-specific IgE in HMB by using BAT and shows that in addition to mosquito antigen-specific $CD4^+$ T cells and EBV-infected NK cells, basophils and/or mast cells activated by mosquito bites may be involved in the pathogenesis of HMB.

Materials and Methods

Patients. We evaluated five Japanese patients with HMB. All patients were previously healthy, and their family histories were unremarkable. Table 1 presents the clinical and laboratory data of the patients. The data for P1 have been reported elsewhere.[9] All but P5 were diagnosed and analyzed within 1 year after onset. P5 was referred to our hospital when he was 19 years old because of a change in his address, and he was analyzed at that time. All patients showed typical clinical features of HMB, such as intense local skin reactions accompanied by general symptoms including fever and abnormal liver function after mosquito bites. Serological tests for EBV showed modestly elevated titers of antiviral capsid antigen immunoglobulin G, positive anti-EBV nuclear antigen titers and the absence of antiviral capsid antigen immunoglobulin M, which indicated past infection in the studied patients. EBV DNA loads in the peripheral blood were markedly increased in all patients. None of the patients had an increase in basophil counts. Consistent with a previous report,[6] all patients had elevated levels of serum IgE. Thus, all five patients were diagnosed with HMB according to the diagnostic guidelines.[14] Serum mosquito-specific IgE measured by commercial fluorenzymeimmunoassay was negative in P1 and P3 (<0.34 UA/mL), and weakly positive in P2 (0.80 UA/mL). The remaining patients were not tested because no appropriate sample was available. Approval for the study was obtained from the Human Research Committee of Kanazawa University Graduate School of Medical Science, and written informed consent was obtained in accordance with the Declaration of Helsinki.

Cell preparation and *in situ* hybridization for Epstein–Barr virus-encoded small RNA-1. Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll–Hypaque gradient centrifugation. Peripheral blood lymphocytes were prepared from PBMC by depletion of monocytes using anti-CD14 monoclonal antibody (mAb)-coated magnetic beads (Becton Dickinson San Diego, CA, USA). $CD4^+$ T, $CD8^+$ T, $CD19^+$ B and $CD56^+$ NK cells were then purified by positive selection from peripheral blood lymphocytes using the respective mAb-coated magnetic beads (Becton Dickinson). With P5, $CD16^+$ NK cells were purified by positive selection using phycoerythrin (PE)-conjugated anti-CD16 mAb and anti-PE-conjugated magnetic beads (Becton Dickinson). B cells were prepared by removal of $CD4^+$, $CD8^+$ and $CD16^+$ cells. The purity of cells from P1 has been reported elsewhere.[9] The purity of the isolated $CD4^+$ T, $CD8^+$ T, $CD19^+$ B and $CD56^+$ NK cells from P2 was 95.8%, 93.6%, 99.6% and 96.3%, respectively, as determined by flow cytometric analysis. Separation of lymphocyte subsets from P3 could not be done because of limited sample availability. The purity of the isolated $CD4^+$ T, $CD8^+$ T, $CD19^+$ B and $CD56^+$ NK cells from P4 was 98.4%, 97.9%, 80.7% and 93.2%, respectively. The purity of the isolated $CD4^+$ T, $CD8^+$ T, $CD20^+$ B and $CD16^+$ NK cells from P5 was 98.9%, 86.0%, 58.5% and 76.9%, respectively. Because of the negative selection, isolated B cells exhibited low purity and comprised 28.6% of the contaminating $CD56^+$ NK cells from P5. *In situ* hybridization for EBV-encoded small RNA-1 (EBER-1) was performed as described previously.[15]

Flow cytometric analysis. For the analysis, whole blood samples were used within 24 h after collection. None of the patients were receiving any treatment at the time of sample

Table 1. Patient characteristics

	P1	P2	P3	P4	P5	Normal range
Age at onset (years)	7	7	8	9	1	
Age at diagnosis	7	8	9	11	4	
Sex	M	M	M	F	M	
Skin findings	Swelling, blistering, necrosis	Swelling, blistering, necrosis	Swelling, blistering, necrosis, hemorrhage, scarring	Swelling, necrosis	Swelling, ulcer	
Systemic symptoms	Fever, liver damage	Fever	Fever, liver damage	Fever, liver damage	Fever, liver damage	
EBV VCA IgG (fold)	80	80	40	640	NA	<10
EBV VCA IgM (fold)	<10	<10	NA	<10	NA	<10
EBNA (fold)	40	20	80	10	NA	<10
EBV DNA loads (copies/10^6 WBC)	1 300 000	66 000	28 000	21 000	37 300†	<20
Lymphocyte subsets (%)						
$CD3^+$ T cells	26.2	47.5	15.2	42.7	44.8	69.5 ± 4.6
$CD4^+$ T cells	17.4	25.9	8.5	29.4	34.8	43.1 ± 6.0
$CD8^+$ T cells	7.4	19.2	6.6	11.4	9.8	22.0 ± 5.4
$CD20^+$ B cells	5.6	17.9	14.1	7.1	12.4	11.2 ± 3.5
$CD56^+$ NK cells	55.6	33.8	59.7	33.5	28.7	12.8 ± 5.3
$CD203c^+$ basophils	0.28	0.68	0.16	1.10	NA	0.53 ± 0.28
Total IgE (IU/mL)	1338	3480	10 020	3177	8910	<696

†It was expressed as copies/μg DNA in peripheral blood mononuclear cells. EBNA, Epstein–Barr virus nuclear antigen; EBV, Epstein–Barr virus; NA, not applicable; VCA, viral capsid antigen; WBC, white blood cells.

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collection. The distribution of lymphocyte subsets was analyzed using FACSCalibur flow cytometer using CellQuest software (BD Bioscience, Tokyo, Japan). HLA-DR was the activation marker that was evaluated on $CD3^+$ T cells and $CD16^+$ or $CD56^+$ NK cells. A total of 12 healthy subjects and 4 volunteers with simple mosquito allergies were also studied as controls. Simple mosquito allergy was defined as large local reactions but not systemic reactions after mosquito bites.[16] Those with simple mosquito allergies were excluded from the group of healthy controls. The following mAb were used: FITC-conjugated anti-HLA-DR, and PE-conjugated anti-CD3 or anti-CD56 (Becton Dickinson PharMingen, San Diego, CA, USA); PE-conjugated anti-CD4, anti-CD8 or anti-CD20 (DAKO, Glostrup, Denmark); and PE-conjugated anti-CD16 (Immunotech, Marseille, France). Analysis of differences among the data groups was performed using Student's *t*-test for unpaired samples. *P*-values < 0.05 were considered to indicate statistical significance.

Mosquito extracts and basophil activation test. We prepared mosquito extracts and performed BAT using blood samples from five patients with HMB, 12 healthy controls and five volunteers with simple mosquito allergy as described previously.[9] Two mosquito species, *Culex pipiens pallens* (*C. p. pallens*) and *Ae. Albopictus*, which are endemic in Japan, were used (Sumika Technoservice, Takarazuka, Hyogo, Japan). Mosquito extracts were composed of whole bodies. A total of 100 mosquitoes were homogenized in 2 mL of phosphate-buffered saline, and the samples were centrifuged at 3000 *g* for 10 min. The supernatants were then centrifuged at 14 000 *g* for 10 min. The mosquito extracts were stored at −80°C until use. The total protein concentrations of the extracts of *C. p. pallens* and *Ae. albopictus* were 7.0 and 6.6 mg/mL, respectively, as assessed by BCA protein assay (Pierce, Rockford, IL, USA).[17] For BAT,[18] peripheral whole blood was obtained, washed twice in PBS and suspended in RPMI1640 medium (Gibco, Grand Island, NY, USA) containing 10% FBS and antibiotics. Aliquots (200 μL) were incubated with or without 10^{-1} or 10^{-2} dilutions of the mosquito extracts at 37°C for 40 min. Cells were then washed and incubated with FITC-conjugated anti-CD63 and PE-conjugated anti-CD203c mAbs (Immunotech) on ice in the dark for 20 min. Stained cells were analyzed in a FACSCalibur flow cytometer and basophils were detected on the basis of forward side scatter characteristics and expression of CD203c. Results of activated basophils were given as the percentage of basophils expressing CD63 among the total number of identified basophils and CD203c expression was evaluated by the mean fluorescence intensity. Analysis of differences among the data groups was performed using Student's *t*-test for unpaired samples. *P*-values < 0.05 were considered to indicate statistical significance.

Results

Cellular target of Epstein–Barr virus in hypersensitivity to mosquito bites. To determine which populations of lymphocytes were infected with EBV, we performed *in situ* hybridization to identify EBER-1 in each lymphocyte subpopulation that had been isolated from patients with HMB. Figure 1(a) shows representative pictures of *in situ* hybridization for EBER-1 where P1 had a number of EBER-1-positive cells among $CD56^+$ NK cells, in contrast to EBER-1 positivity in $CD4^+$, $CD8^+$ T and $CD19^+$ B cells. Figure 1(b) shows the percentages of EBER-1-positive cells in the population of PBMC and isolated lymphocyte subpopulations. The average (± standard deviation) percentage of EBER-1-positive cells among PBMC was 25.0 ± 11.4%. In the isolated cell subpopulations of four patients, the dominant population of EBER-1-positive cells was $CD56^+$ NK cells (61.8 ± 14.1%). Very few T and B cells were positive for EBER-1 in these patients. In spite of the low purity of B cells in P5, the percentage of EBER-1-positive cells was 1.7%.

Increased subpopulation of activated NK cells. Consistent with the findings of a previous report,[19] the number of $CD56^+$ NK cells was increased in all patients (Table 1) but not for those with simple mosquito allergy (11.1 ± 2.7%). As shown in Figure 2, the majority of these NK cells expressed HLA-DR (92.6 ± 4.2%), whereas a small number of NK cells in controls and simple mosquito allergy patients expressed HLA-DR (4.0 ± 2.5%, 5.7 ± 2.6%, respectively). The NK lymphocytosis likely caused a relative decrease in the percentage of T cells. Although some T cells expressed HLA-DR (23.8 ± 10.6%), the CD4/8 ratios were normal in patients with HMB. Our data showed that NK cells had significantly more HLA-DR expression than T cells in the group with HMB ($P < 0.001$).

Basophil activation test experiments. As shown in Figure 3(a), basophil activation occurred with exposure to the extracts of epidemic mosquitoes in all samples from patients with HMB, but no activation occurred in the absence of stimulation. Basophils from normal controls were not activated by mosquito antigens. When we examined in detail basophil activation on exposure to the respective antigens in samples from patients with HMB, we detected basophil activation on exposure to the extract of *Ae. Albopictus*, except in P4. Basophils from P2, P3 and P4 were activated by the extract of *C. p. pallens.* Both the extracts activated basophils in P2 and P3.

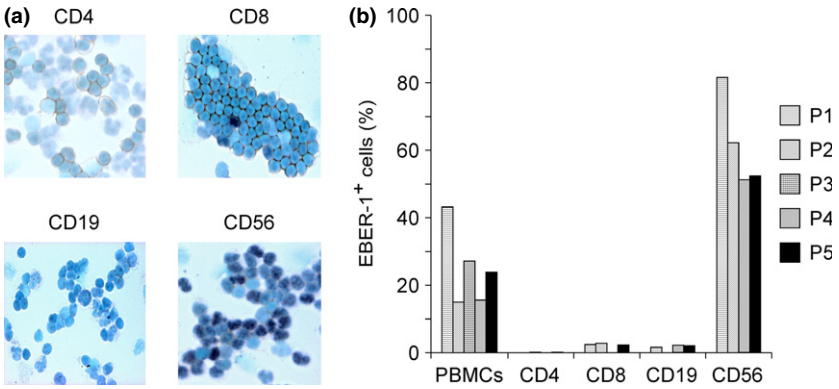


Fig. 1. Cellular target of Epstein–Barr virus (EBV) infection. (a) EBV-encoded small RNA-1 (EBER-1) *in situ* hybridization of the lymphocyte subpopulations in a representative patient with hypersensitivity to mosquito bites. (b) The percentages of EBER-1-positive cells in peripheral blood mononuclear cells (PBMC) and the lymphocyte subpopulations of patients with hypersensitivity to mosquito bites are shown.

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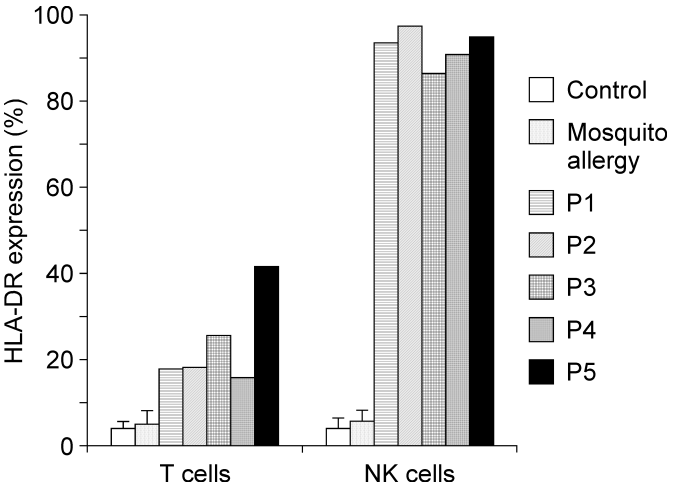


Fig. 2. Expression of HLA-DR on T and natural killer (NK) cells. The percentages of HLA-DR$^+$ cells among CD3$^+$ T and CD56$^+$ NK cells in the peripheral blood of healthy controls (n = 12), simple mosquito allergy patients (n = 4) and patients with hypersensitivity to mosquito bites are shown. Error bars indicate standard deviation.

When we compared the extent of CD63 and CD203c expression between the HMB group and the healthy control group, the HMB group showed a significantly higher percentage of CD63$^+$ basophils that were stimulated by the extracts of *C. p. pallens* or *Ae. Albopictus* ($P < 0.05$ and $P < 0.001$, respectively) (Fig. 3b). Furthermore, both *C. p. pallens* and *Ae. Albopictus* significantly upregulated CD203c expression in the HMB group more than the control group ($P < 0.05$ and $P < 0.001$, respectively) (Fig. 3c). In the HMB group, there were significantly more basophils that upregulated CD63 and CD203c expression after exposure to *Ae. Albopictus* compared with *C. p. pallens* ($P < 0.05$ and $P < 0.05$, respectively) (Fig. 3b,c). Similar to HMB, basophil activation by mosquito extracts was detected in all samples from simple mosquito allergy patients.

Discussion

We demonstrated basophil activation by mosquito antigens in HMB. In particular, the extract of *Ae. albopictus* was able to activate basophils in all available patient samples. *Ae. albopictus* has been known to have a larger variety and amount of allergens in the saliva compared with other species of mosquitoes.[20] Furthermore, *Ae. albopictus* is the most common species associated with systemic allergic reactions to mosquito bites.[21] In fact, the antigen of *Ae. albopictus* tended to induce more basophil activation than *C. p. pallens* in our patients with HMB. However, it remains unclear whether the antigen of *Ae. albopictus* causes more intense clinical symptoms in patients with HMB. In contrast, the extract of *C. p. pallens* activated basophils from three patients with HMB, two of whom showed basophil activation after exposure to either extract. These different profiles of response to the mosquito antigens may simply reflect the frequency of exposure to bites of each species. Because the saliva of *Ae. Albopictus* contains allergens that are common among different species, it is possible that basophil activation by *C. p. pallens* is the consequence of the cross-reactivity of such allergens.[21]

Studies of T cells from patients with HMB show that mosquito antigens activate and induce proliferation of CD4$^+$ T cells and that these mosquito antigen-specific T cells induce EBV reactivation in NK cells.[5,8] It is well known that significant numbers of EBV-infected NK cells and reactive T cells infiltrate into the bite sites in HMB.[7] Therefore, mosquito antigen-specific CD4$^+$ T and EBV-infected NK cells have been considered to be responsible for intense skin lesions as well as systemic symptoms in HMB. In the present study, we demonstrated the presence of mosquito-specific IgE and basophil activation by mosquito antigens *in vitro*. The discrepancy regarding mosquito-specific IgE between the BAT data and the commercial fluorenzymeimmunoassay may be due to the different sensitivity of each method used for assessing mosquito-specific IgE. It seems reasonable that mosquito antigens can bind to mosquito-specific IgE attached to the high-affinity IgE receptor of basophils and/or mast cells at the bite sites, resulting in activation of such cells in HMB. In two patients whose basophils were evaluated after successful hematopoietic stem cell transplantation, basophil activation by mosquito antigens was no longer detected *in vitro* (data not shown). Indeed, these post-stem cell transplantation patients have had no symptoms after mosquito bites.

Immediate allergic skin reactions via IgE-dependent mechanisms soon after mosquito bites are also seen in simple mosquito allergy.[22] In fact, we demonstrated basophil activation by mosquito antigens in all samples from simple mosquito allergy patients. Subsequent bite-induced indurations in simple mosquito allergy correlate with T-cell proliferation responses to mosquito antigens. However, unlike simple mosquito allergy, patients with HMB exhibit severe skin reactions and systemic symptoms that might be the result of activation of mosquito-specific CD4$^+$ T cells and EBV-infected NK cells. Basophils and/or mast cells activated by the mosquito antigens may produce humoral factors such as cytokines that potentially activate CD4$^+$ T cells and NK cells at the reaction sites. In fact, activated basophils have been reported to produce various cytokines, including IL-4, IL-9 and IL-13.[23,24] IL-4 is known to induce proliferation of T cells, differentiation of naïve CD4$^+$ T cells into Th2 cells and a class switch to IgE in B cells.[25–27] IL-13 can also promote a class switch to IgE in B cells *in vitro*.[28] IL-9 has been showed to increases the number of EBV-infected NK cell lines of nasal NK/T lymphoma *in vitro*.[29] An analysis of serum cytokines from patients with HMB showed high levels of IL-13, which might explain the high serum IgE levels.[30] Further analysis of these cytokines in serum or local skin lesions is needed to reveal the inflammatory processes in HMB. Taken together, these findings suggest that antigen-induced activation of basophils and/or mast cells that bind to mosquito-specific IgE could be the initial event and an additional mechanism leading to severe local skin reactions and systemic symptoms in HMB.

Our immunological analysis clearly demonstrated prominent EBV infection in NK cells, the proliferation of NK cells and HLA-DR expression on almost all NK cells in HMB. Normal NK cells do not usually express HLA-DR.[31,32] Consistent with our previous study,[9] the percentage of activated NK cells expressing HLA-DR was much higher than that of EBV-infected NK cells in all patients with HMB. The reason why the majority of non-infected NK cells expressed HLA-DR is unclear. In contrast to NK cells, the minority of T cells expressed HLA-DR in our patients. These findings differ from those of IM, in which many T cells express HLA-DR.[33] The difference in HLA-DR expression on T cells between HMB and IM may be due to the types of antigens of EBV-infected cells. EBV-infected B cells in IM express all EBV latent

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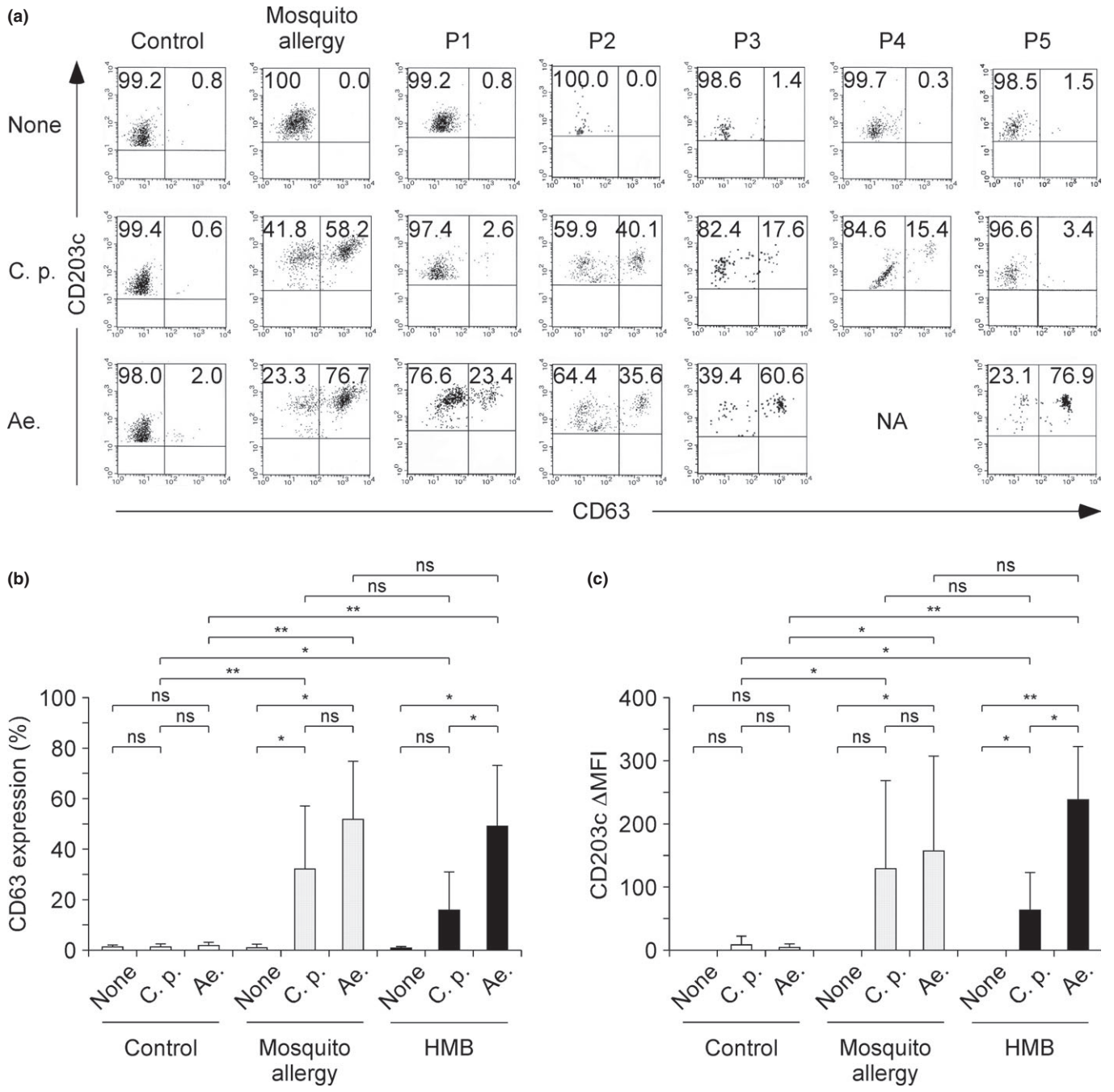


Fig. 3. Results of basophil activation tests. (a) Expression of CD63 and CD203c on basophils stimulated *in vitro* by the antigens of *Culex pipiens pallens* (*C. p.*) or *Aedes albopictus* (*Ae.*) from representative individuals of normal control or simple mosquito allergy and patients with hypersensitivity to mosquito bites (HMB). The percentage of cells gated in each region is shown. (b) The percentages of $CD63^+$ cells within the population of basophils stimulated by the antigens of *Culex pipiens pallens* or *Aedes albopictus* in the healthy control group ($n = 12$), simple mosquito allergy ($n = 5$) and HMB group ($n = 5$ or 4, respectively). (c) The difference in mean fluorescence intensity (ΔMFI) of CD203c on basophils. ΔMFI of CD203c was calculated by subtracting the MFI of unstimulated cells from those of stimulated cells. Error bars indicate standard deviation of mean. $*P < 0.05$; $**P < 0.001$ based on Student's *t*-test. NA, not applicable; ns, not significant.

proteins, whereas EBV-infected T/NK cells in CAEBV do not express EBNA-3 family proteins that are dominant in cytotoxic T lymphocytes (CTL) responses.[34,35] The absence of these antigens is thought to impair CTL recognition of infected cells in CAEBV. Thus, a low percentage of activated T cells in HMB may be associated with markedly reduced CTL activity.[36]

From the clinical perspective, our results suggest an auxiliary method for the diagnosis of HMB. Although the proposed guidelines have been shown to be helpful for the diagnosis of HMB, the analysis of EBV viral load, clonality and cellular target of EBV is only available in specialized laboratories. We demonstrated that the existence of mosquito antigen-specific IgE by BAT and selective activation and expansion of NK cells, both of which reflect the pathogenesis of HMB, were important features in HMB. These findings may help in the diagnosis of HMB, although each isolated finding might not be specific. Mosquito antigen-specific IgE has already been

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reported in patients with simple mosquito allergy.[22] Similarly, an increased subpopulation of HLA-DR$^+$ NK cells has been found in patients with infections caused by herpes simplex virus, dengue virus and *Mycobacterium tuberculosis*, as well as aggressive NK cell leukemia.[37–40] However, concurrent assessment of basophil activation by mosquito extracts and NK cells might provide clues that lead to the diagnosis of HMB. Further studies on whether this auxiliary method is useful as a screening test of HMB are needed.

Limitations of the present study include the small sample size, the ethnically homogeneous sample, and the absence of patients with other forms of EBV infection. It has been reported that the symptoms of HMB also occur in patients with T-cell type CAEBV or NK cell leukemia/lymphoma.[3,41] Further evaluation of patients with various types of EBV infection or patients with a different ethnic background is necessary. It is also necessary to assess whether mosquito-specific IgE is present in cases of EBV-associated T/NK-cell lymphoproliferative diseases without HMB. In addition, we used whole body extracts that were easy to prepare. However, this might not be ideal because they contained many extraneous proteins that were not present in mosquito saliva.[42] Although saliva from living mosquitoes would be the best source of antigens, it is technically difficult and time-consuming to obtain large enough quantities of saliva.[21,42] It has been reported that recombinant *Aedes aegypti* salivary antigens can be used in the *in vitro* and *in vivo* diagnosis of mosquito allergy and are more sensitive than whole body extracts.[43,44] These antigens may increase the sensitivity and specificity of BAT.

In conclusion, our study demonstrated the activation of circulating basophils by mosquito extracts *in vitro* and suggested the involvement of activated basophils and/or mast cells carrying mosquito-specific IgE on their cell membranes in the initiation and development of severe skin reactions to mosquito bites in HMB.

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Disclosure Statement

The authors have no conflict of interest to declare.

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