

A Quantitative Study of Specific Immunoglobulins to Mosquito Salivary Gland Antigen in Hypersensitive and Common Types of Mosquito Bite Reaction

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Abstract

This study was designed with two purposes: first, to elucidate immunologic mechanisms in different cutaneous reactions, particularly in hypersensitivity to mosquito bites, and, second, to develop a more reliable and safer method of identifying the causative species of mosquito in severe cases.

The amounts of IgG, IgG₄ and IgE specific to the mosquito salivary gland extract of *Aedes albopictus* were determined in the sera of 116 volunteers with normal reactions, either immediate or delayed, and 4 patients with severe systemic symptoms caused by mosquito bites. Titers of IgG and IgE in the severe cases were considerably higher than in volunteers with normal reactions, but there were no differences in IgG₄ titers between the two groups. These results indicate that high titers of IgG and IgE may be involved in development of systemic symptoms in severe cases and verify the possibility of *in vitro* tests to identify causative species of the mosquito.

Key words: mosquito hypersensitivity; IgG; IgE; IgG₄; *Aedes albopictus*

Introduction

When bitten by a venomous insect, the human skin may develop a wide variety of symptoms. Cutaneous reactions to mosquito bites consist of two types; immediate and delayed. The former is characterized by a small wheal and erythema, which appear soon after a bite and then disappear gradually within one hour. In the latter, erythematous swelling appears several hours after the bite and lasts 3 days or more (1, 2). The development of an immediate reaction is considered to depend

on the occurrence and increase of immunoglobulins against mosquito saliva (3, 4). The cell mediated immune systems may be involved in the delayed type of reaction (3).

In addition to these common reactions, single mosquito bites have produced, in some cases, very severe reactions with systemic symptoms such as high fever, lymphadenopathy, and hepatosplenomegaly (5-10).

In Japan, it has previously been reported that 15 patients with mosquito allergy have died from malignant histiocytosis, lymphoma or DIC with glomerulonephritis (10-13). Although it is not clear why a single mosquito bite induces such severe systemic symptoms, it can be assumed that these patients may have had some immunologic abnormalities which finally resulted in cells, such as histiocytes and lymphocytes, becoming malignant. Therefore, it seemed important to determine the comparative amounts of specific immunoglobulins to mosquito saliva in the sera of normal persons with different cutaneous reactions and that of severe cases.

In severe cases, identification of the causa-

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tive species of mosquito is necessary to prevent repeated bites which worsen the disease, because hypersensitivity to mosquito bites is species specific (5). Although skin tests are usually administered for this purpose, their specificity is not sufficient to identify the causative species (5, 7).

In this paper, we report the relative amounts of IgG, IgG₄, and IgE specific to salivary gland extract of *Aedes albopictus* in the sera of 4 patients who exhibited severe systemic reactions to bites of the *Aedes* species and in that of 116 persons with common reactions. These studies should form the basis essential for further elucidation of the immunologic mechanisms that induce the cutaneous and systemic symptoms caused by insect bites in severe cases. Another purpose of this study was the development of a quick, safe, and reliable method for identification of the causative species of mosquito by immunologic techniques, so patients may avoid repeated bites which aggravate symptoms.

Materials and Methods

Four patients with severe symptoms caused by mosquito bite

Case 1. A 20-year-old female. From the age of 5, she has experienced high fevers and red swelling following mosquito bites. Anti Epstein-Barr virus IgG was high. Hypersensitivities to *Aedes* sp. and *Armigeres* sp. were proven by experimental biting test. Other diagnostic data have been published in a previous report (5).

Case 2. A 5-year-old male with high fever, lymphadenopathy, leucocytosis, and high IgE. The causative species of mosquito was caught and identified as *A. albopictus*. Details have been previously published (14).

Case 3. A 52-year-old male with high fever and hemorrhagic bulla. A bloody bulla was produced by skin test using *Aedes* salivary gland extract (15).

Case 4. A 17-year-old female with high fever and large erythema. Hypersensitivity was proven by an experimental biting test using *Aedes* sp. (16).

Dissection of salivary glands and preparation of SGE

Aedes albopictus were routinely cultured in the laboratory of Tokyo Medical and Dental University. Newly emerged females were segregated from the

cultures, and, after light anesthesia by cooling, their salivary glands were removed under a dissecting microscope. After washing with insect Ringer (NaCl 0.75 g, KCl 35 mg, CaCl₂ 21 mg in 100 ml DW) twice, some 300 salivary glands were pooled and stored in the insect Ringer at -80°C until use. The salivary glands were homogenized by ultrasonication for 15 minutes. The homogenate was centrifuged at 1,000×g for 40 minutes. The precipitate was washed with insect Ringer and centrifuged twice. The combined supernatant was filtrated through a millipore filter (pore size: 0.22 µm, Millipore Co., Mass.). The obtained filtrate, named "salivary gland extract" (SGE), was used as an antigen. Other details have been mentioned in previous papers (3, 17).

Determination of specific IgG, IgG₄, IgE to SGE

Titer of SGE specific IgG and IgG₄ in the sera obtained from 116 volunteers with normal skin reactions to mosquito bites and the 4 severe cases were determined by a modification of ELISA assay.

First, the insides of 10 ml test tubes were coated with SGE; 100 µl of SGE solution at a concentration of 25 µg protein/ml was introduced into each tube and centrifuged for 10 min at 1,000×g. After standing at 37°C for 2 hrs, the SGE tubes were stored overnight at 4°C. The inside of each tube was then washed with phosphate buffered saline (pH 7.4). Next, 100 µl aliquots of 25-fold dilutions of sera obtained from a total of 120 persons were added to the SGE coated tubes. After standing at room temperature for 3 hrs, the inside of each tube was washed with phosphate buffered saline; then 100 µl of peroxidase conjugated antihuman IgG or IgG₄ antibodies was added, and the tubes were left overnight at room temperature.

The next day tubes were washed with phosphate buffered saline; 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium and H₂O₂ were added; and the color reaction was stopped by NaN₃ after standing for 40 minutes at room temperature. Then, each tube was read in a spectrophotometer to determine OD at 415 nm.

Determination of SGE specific IgE was performed by an ELISA assay using paper discs. Detailed procedures have been described in a previous paper (3).

The amounts of SGE specific IgG, IgG₄ and IgE in the sera were expressed as relative values.

Chemicals and reagents

Anti-human IgG, IgG₄, and IgE antibodies were

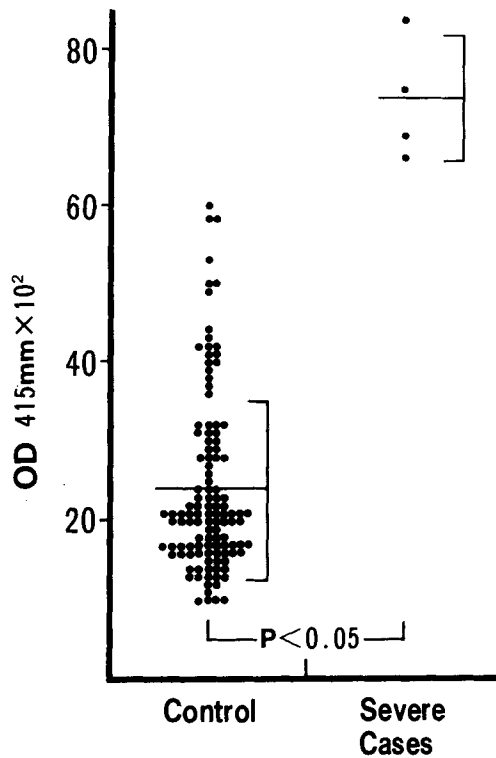


Fig. 1. Amounts of IgG specific to mosquito salivary gland extract (SGE) in the sera of 116 normal reaction volunteers and 4 severe cases. Each dot represents a relative amount of IgG in the serum of one subject. Vertical lines with side bars near the dots indicate mean values and standard deviations.

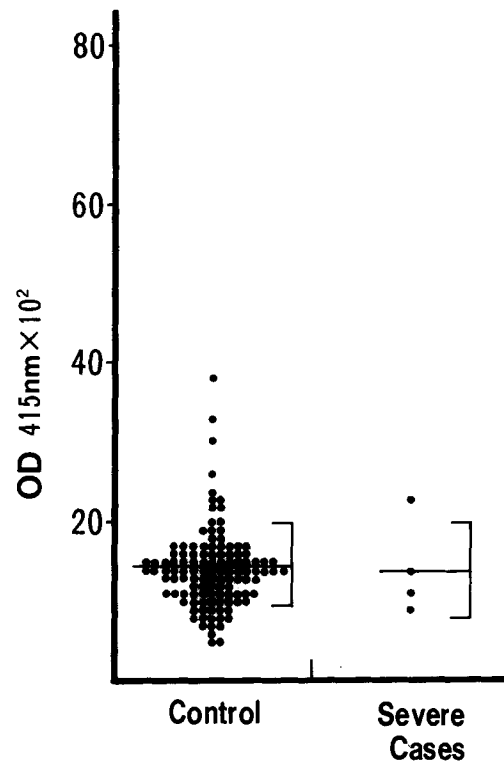


Fig. 2. Amounts of IgG₄ specific to SGE in the sera of 116 normal reaction volunteers and 4 severe cases. Explanations are the same for Fig. 1.

purchased from Shionogi Pharmaceutical Inc., Osaka, Japan. For the IgE ELISA assays, ELISA kit from Pharmacia Chem., Uppsala, Sweden was used. Other chemicals were purchased from Wako Pure Chem. Co., Osaka, Japan.

Results

As shown in Figure 1, the average value of SGE specific IgG in the sera of the 4 severe cases was more than 3 times that obtained from the 116 persons who exhibited common reactions to bites. The Student's t-test (Welch) clearly indicates that this difference is significant at the 5% level. Although the IgG titers of the common reaction group scattered in a wide range, all of them were lower than those of the 4 patients with severe reactions. On the other hand, the amount of IgG₄ specific to SGE did not show any conspicuous difference between

the two groups (Fig. 2). It is worthy of note, however, that the IgG₄ values of 4 volunteers with normal reactions were clearly higher than those of the 4 severe cases.

The titers of IgE specific to SGE among the common reaction volunteers ranged from an undetectable level to a high one. Notably, the IgE titers in the sera of 13 volunteers who did not show immediate reactions to *Aedes* mosquito bite were very low, but about 80% of the volunteers who exhibited immediate reactions by the test bites possessed considerable amounts of specific IgE.

The IgE value specific to *Aedes* SGE in one severe case (Case 1) was about five times higher than the average value of the immediate reaction positive group of normal volunteers (Fig. 3). Only two out of the 116 normal volunteers possessed higher titers of the specific IgE than did the severe case. Among the 116 volunteers 13 subjects did not show immediate reactions and the IgE levels in their sera were almost

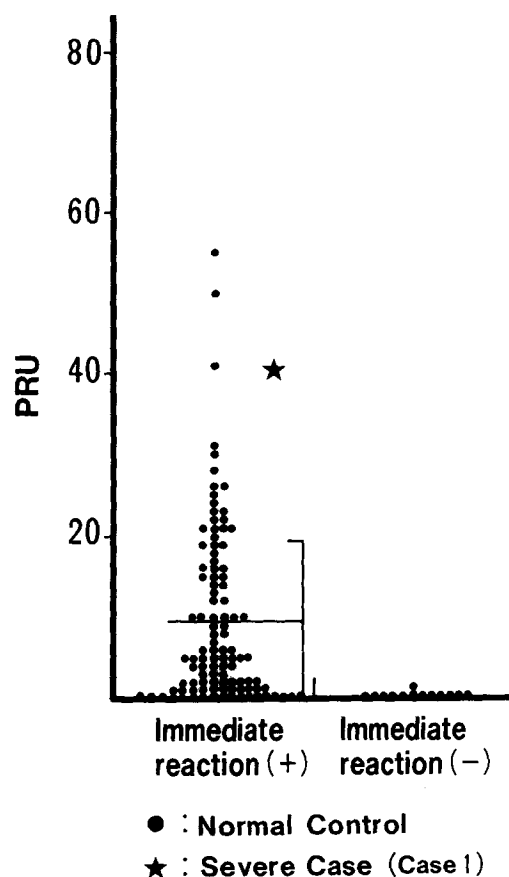


Fig. 3. Amount of IgE specific to SGE in the sera of 116 normal reaction volunteers and Case 1. Normal reaction subjects are divided into two groups; immediate reaction positive and immediate reaction negative. Other explanations are the same for Fig. 1.

undetectable.

Discussion

Reactions to mosquito bites can be divided into two types. One is a local reaction on the skin around the bitten site, that disappears, at most, within several days. This reaction is widely observed. The other is a severe skin lesion that develops into hemorrhagic bulla, necrosis, and ulcer with systemic symptoms such as high fever and hepatosplenomegaly in the worst cases.

Since the first case was reported in 1969 (18), more than 30 severe cases have been reported in Japan. Fifteen of these have died from malignant histiocytosis, malignant lymphoma, or other malignant diseases (10–13). Only two

severe cases have been reported in other countries: one from Taiwan (19) and the other from the United States of America (20).

Although the mechanisms of the intense reaction in severe cases has not been clarified, the following findings suggest that Type III allergy of Coombs and Gells may play an important role in the development of a hypersensitivity reaction to mosquito bites. As we reported earlier (5), histological features of the skin specimens of the intense reaction area can be classified as an Arthus type reaction (Type III allergy) with prominent bleeding, vasodilatation and necrotizing vasculitis accompanied by infiltrations of neutrophils and eosinophils. This argument is further supported by the findings that perivascular deposition of IgG has been detected in skin specimens by immunofluorescent studies (9) and an autopsied specimen of the kidney of a patient with a systemic severe reaction to mosquito bites showed glomerulonephritis with depositions of C1q and C₃ in the mesangial area of the glomerulus (10). It is generally considered in Arthus type reactions that specific IgG to antigen rather than IgE is primarily induced (21). In these studies, we have shown that the titers of IgG specific to salivary gland components in the serum of the four severe cases were considerably higher than those of the 116 volunteers with common reactions.

As shown in Figure 3, however, the specific IgE to SGE in one severe case is also higher than that of normal persons. This suggests that, in severe reactions, not only Type III allergy but also Type I allergy may be involved. It is important to note that the amount of the specific IgG₄ to SGE in severe cases was not higher than those in the normal group. It is widely claimed that, in allergy to hymenopteran venoms, IgG₄ works as a blocking antibody to prevent hypersensitive reactions (22, 23). If IgG₄ acts as a blocking antibody for mosquito saliva, a lower titer of IgG₄ in the sera of severe cases may not prevent hypersensitive reactions induced by a high titer of IgG.

It has been reported that increased titers of antibody to mosquito salivary gland were observed immunohistochemically in patients in-

fect by HIV (24). These authors speculated that this fact might be a consequence of nonspecific B-cell activation, a feature of AIDS. These patients had types of skin eruptions which differed from those in our severe cases; they had no cutaneous necrosis or no systemic symptoms to the mosquito bites.

In the severe cases, some kind of immunodeficiency may induce increases in serum IgG and IgE, but not in IgG₄ to mosquito salivary gland antigens. Several severe cases, including Case 1, have had high titers of antibody to Epstein-Barr virus (5, 9, 25). It has been claimed that chronic infection by EB virus of T-cells can result in malignant lymphoma (26). In the severe cases, a kind of immunological abnormality may have caused both a hypersensitivity to mosquito saliva component and an infection by EB virus. Death from malignancy, including malignant histiocytosis and lymphoma, may be based on some form of congenital immunodeficiency. There are several reports suggesting that natural killer cell lymphocytosis may have some relationship to this type of hypersensitivity and to prolonged infection of the EB virus, resulting in malignant histiocytosis (6, 8, 10). To reach a definite conclusion, however, further immunological studies need to be undertaken.

To prevent worsening of the symptoms in severe cases, it is very important to identify the causative species of mosquito. Usually skin tests using whole body extract, which is commercially available, are used for identification, but these results are ambiguous (5). A high titer of the specific IgG and IgE in severe cases opens the way to development of an *in vitro* test to identify causative species of the mosquito.

In common cases, the skin reaction to repeated bites develops from no reaction, delayed type reaction, both delayed and immediate type reaction, immediate type reaction only and finally, again, no reaction. This means that the obtained immunity against mosquito saliva antigen diminishes the skin reaction caused by the bites (1). We confirmed, with 162 volunteers, that this serial development of reactions was due to repeated bites of the same species of the mosquito (2).

Oka (3) suggested, by LIT test using mosquito antigen, that the cell mediated immune system may be involved in delayed reactions and that an immediate reaction is probably a Type I allergy; she found high IgE levels in the sera of the immediate reaction positive group. Recently, Reunala et al. (4) reported that the immediate reaction may be caused by histamine, because an anti-histamine agent inhibits it. We could not detect any histamine in the saliva of mosquitoes (17), but this does not conflict with Reunala's report, if specific IgE to mosquito saliva stimulates mast cells to release histamine.

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