

Human IgE and IgG antibodies to mosquito proteins detected by the immunoblot technique

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Immunoblot technique was used in this study to detect IgE and IgG antibodies in human sera against mosquito antigens. Mosquito proteins were separated on SDS-polyacrylamide gels and transferred electrophoretically to nitrocellulose papers. After incubation with sera from different individuals, the precipitated bands were analyzed with enzyme-labeled goat anti-human immunoglobulins. Distinct patterns of antigen were recognized by IgE and IgG antibodies.

INTRODUCTION

The human response to mosquito bites varies from local mild immediate to severe late even systemic reactions.¹⁻⁸ Though it is thought that the usual mild, immediate local wheal and flare reaction is primarily due to the pharmacologic activity of the mosquito salivary secretions,⁸⁻⁹ some unusual rather severe reactions after mosquito bite may also be through an immunologic mechanism.^{1,3,5,10} At the present time, however, the nature of the antigens and the antibodies involved is still not clear.

In this study, we report that IgG and IgE antibodies against antigens of the whole body and the salivary gland extracts of mosquitoes can be detected in man by immunoblot assay. The molecular weights of these antigens or allergens were determined. The possible significance of these antibodies in relation to different clinical manifestations is also discussed.

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MATERIALS AND METHODS

Mosquito Antigen

Five local species of mosquitoes were used in the present study, namely *Aedes aegypti*, *Aedes albopictus*, *Aedes togoi*, *Armigeres subalbatus*, and *Culex quinquefasciatus*. The mosquitoes were obtained from the Taiwan Provincial Institute of Infectious Diseases in 1982 and maintained in continuous laboratory colony in our insectary. Five- to seven-day-old female mosquitoes were used in all tests. Until used in experiments, adult mosquitoes had access to 10% sucrose only. Mosquitoes were anesthetized by putting them into a test tube and chilled on ice for 20 minutes. Whole body extracts were made by grinding 100 mosquitoes of each species in 2 mL phosphate-buffered saline (PBS) with a glass tissue grinder in an ice bath. The homogenate was sonicated at 40 W for 60 S (Sonifier Cell Disruptor Model W140, Ultrasonics Inc) and centrifuged at 7500 g for 30 minutes at 4°C (Sigma 4MK). The supernatant was filtrated through a 0.45- μ m filter (Millipore) and the filtrate was divided into small aliquots, and stored at -70°C. The salivary gland of *A. albopictus* was also used as a source of antigen. The chilled mosquitoes were transferred onto the chilled platform of a dissecting microscope and their salivary glands were dissected, removed, and chilled in PBS. The salivary gland extract was pre-

pared in the same way as that of the whole body extract except that 100 pairs of salivary glands were ground in 0.4 mL PBS.

Serum Samples

Ten milliliters of serum was collected from each of 15 individuals and stored in aliquots at -70°C. Five individuals (case numbers 1-5, Table 1) developed different degrees of rather severe cutaneous reactions after mosquito biting. The remaining ten individuals (case numbers 6 to 15, Table 1) responded mildly to mosquito bites as does most of the general population.

Immunoblot Analysis

Twelve microliters of the mosquito antigen extract was used for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as described by Laemmli¹¹ and separated on 10% polyacrylamide gels with a 5% stacking gel. Low molecular weight standards (Pharmacia, Uppsala, Sweden) were used to determine the molecular weight of the antigen components. When electrophoresis was completed, the gels were removed and the separated proteins were either stained with Coomassie blue R-250 or electrophoretically transferred to nitrocellulose paper (NCP, 0.45 μ m; BA 85; Schleicher & Schuell, Dassel, FRG) essentially as described by Towbin et al.¹² The NCP and gel was electrophoresed in transfer buffer (25 mM Tris, 192 mM glycine and 20% [vol/vol] methanol, pH 8.3) under constant

Table 1. Clinical Manifestations of Mosquito-Biting in Man

Case No.	Sex	Age	Clinical Manifestations
1	F	18	Moderate local immediate reaction severe late local reaction with vesicle formation and systemic fever, headache
2	M	38	Severe local immediate reaction with large wheals and itching
3	M	28	Ditto
4	F	40	Moderate local immediate reaction severe late reaction with large area of local swelling and redness
5	F	29	Mild local immediate reaction severe late bullous reaction
6-15	young	adults	Mild local reaction with transit swelling and/or slight itching

voltage at 90 V at 4 °C for one and one-half hours using TE 50 Transphor (Hoefer, San Francisco, CA). After electrophoresis, the NCP was stained either immunochemically or with Coomassie blue R-250. Immunochemical staining was performed by first blocking the NCP overnight with 3% (wt/vol) gelatin in tris-buffered saline (TBS; 20 mM Tris, 500 mM NaCl, pH 7.5) at 4 °C. All subsequent manipulations of the NCP were done at room temperature. After washing three times with TTBS (0.05% Tween 20 in TBS), the blots were incubated with human serum diluted either 1:5 or 1:50 in TTBS for 16 hours or one hour, respectively, to detect IgE or IgG antibodies in the serum which reacted with the mosquito antigens. For control experiments, cord serum (for IgE) or PBS (for IgG) was used separately. The blots were washed three times with TBS for ten minutes each and then incubated in optimal dilutions in TTBS of affinity purified, horseradish peroxidase-labeled goat anti-human IgE or anti-human IgG antibodies (KPL, Gaithersburg, MD) for six hours (IgE) or one hour (IgG), respectively. The blots were then washed as described above. The bound peroxidase was detected by the addition of a freshly prepared solution of 0.06% (wt/vol) 4-chloro-1-naphthol (Bio-Rad, Richmond, CA) and 0.01% (vol/vol) H₂O₂ in TBS. After 30 minutes, the blots were rinsed in distilled water and air dried. Antigen-antibody binding

was identified by deposition of purple stain.

RESULTS

Protein Profile of Mosquito Antigen Extracts

For analysis of the protein components recognized by human IgE and IgG antibodies, whole body extracts of *A. albopictus*, *A. aegypti*, *A. togoi*, *A. subalbatus*, *C. quinquefasciatus*, and the salivary gland extract of *A. albopictus* were separated on SDS-polyacrylamide gels. Figure 1A depicts the SDS-PAGE pattern and Figure 1B shows the electrophoretically transferred patterns of the six mosquito extracts on nitrocellulose membrane. The whole body extracts of five different mosquito species showed a similar SDS-PAGE pattern (Fig 1A, lanes 1-5). There were multiple components in these extracts and components with molecular weights (MWs) of about 88, 55, 49, 45, 42, 38, 34, 30, 26 and 22 K were most intensely stained. In the salivary gland extract (Fig 1A, lane 6), however, only a few faint bands were found.

IgE Antibodies Detected by Immunoblotting

Figure 2 demonstrates the immunoblot patterns of six individuals (case numbers 1 to 5 and no. 11 in Table 1) using the enzyme-labeled anti-IgE antiserum. The specific IgE-binding patterns differed markedly in these cases. From one to ten IgE-binding components were identified in each case, and none of them

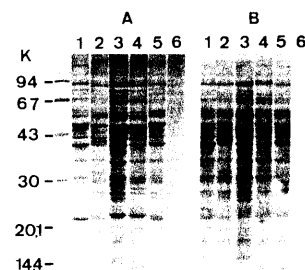


Figure 1. Fractionation of mosquito extract of SDS-PAGE separated components to nitrocellulose and stained with Coomassie blue R-250. Mosquito antigens used were whole body extracts of *Aedes albopictus* (lane 1), *Aedes aegypti* (lane 2), *Aedes togoi* (lane 3), *Armigeres subalbatus* (lane 4), *Culex quinquefasciatus* (lane 5), and salivary gland extract of *Aedes albopictus* (lane 6). The molecular weight standards (Pharmacia) are phosphorylase b (94,000), bovine serum albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), soybean trypsin inhibitor (20,100), and α -lactalbumin (14,400).

was common for all these patients. Furthermore, different mosquito species showed somewhat different reaction patterns to the serum of the same patient. Serum no. 1 exhibited very strong IgE-binding activity towards multiple components of the mosquito extracts. Among them, the components with MWs of 62, 55, 49, 42, 34, 30, 26 to 24 and 21 K were more commonly seen (Fig 2A). Serum no. 2 showed positive IgE activity towards the 36-K and the 34- to 30-K antigens of different mosquito species respectively (Fig 2B), while serum no. 3 reacted very strongly with a 38-K antigen of all the species tested (Fig 2C). The sera of cases no. 4 (Fig 2D), no. 5 (Fig 2E), and the other ten cases (case no. 11, Fig 2F) showed limited IgE binding. Of all the fifteen sera assayed in this study, only serum no. 1 exhibited strong IgE-binding activity to antigens in the salivary gland extract of *A. albopictus* (Fig 2A, lane 6). No anti-mosquito IgE antibodies were observed in the con-

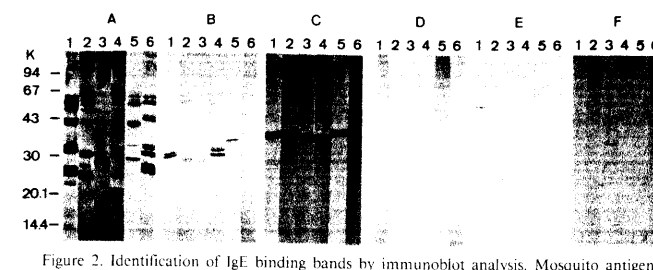


Figure 2. Identification of IgE binding bands by immunoblot analysis. Mosquito antigens (lanes 1 to 6, the same antigen preparations as shown in Fig 1) were separated by 10% SDS-PAGE and electrophoretically transferred to nitrocellulose paper. Blots of panels A through F were incubated separately with sera from six individuals (case numbers 1 to 5 and 11 in Table 1). The presence of IgE antibodies against the mosquito antigens was then detected immunochemically.

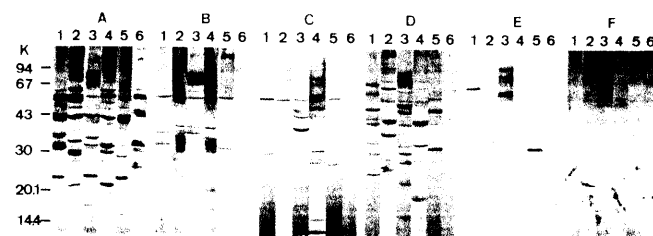


Figure 3. Identification of IgG-binding bands by immunoblot analysis. Mosquito antigens (lanes 1 to 6, the same antigen preparations as shown in Fig 1) were separated by 10% SDS-PAGE and electrophoretically transferred to nitrocellulose paper. Blots of panels A through F were incubated separately with sera from six individuals (case numbers 1 to 5 and 11 in Table 1). The presence of IgG antibodies against the mosquito antigens was then detected immunochemically.

trol cord serum by the immunoblot method.

IgG Antibodies Detected by Immunoblotting

Figure 3 shows the immunoblot patterns of six samples from case numbers 1 to 5 and 11 using the enzyme-labeled anti-IgG antiserum. Multiple IgG-binding components were identified in all the immunoblots, but the patterns differed both qualitatively and quantitatively. Generally speaking, the staining of bands precipitated by sera no. 1 to 5 were heavier than those obtained by sera no. 6 to 15. Serum no. 1 showed very strong IgG binding activity towards antigens with MWs of 78, 62, 55, 42, 34 to 31, 29 to 28, and 23 to

21 K in the extracts prepared from the whole mosquito body and from the salivary gland (Fig 3A). In other serum samples, only antibodies that reacted with components in the whole body extracts were detected. Sera no. 2 and no. 3 reacted moderately to components with MWs of 78 to 76, 64 to 62, 55, 38 to 36, and 34 to 29 K (Fig 3B and 3C). Sera no. 4 and no. 5 exhibited strong IgG-binding activity towards many components with MWs of 88, 82, 64 to 62, 49, 45, 42, 36, 31 to 29, 26, 21, 18 and 15 K (Fig 3D) and few components with MWs of 88, 78, 62, 58 to 55 and 30 K (Fig 3E), respectively. The immunoblot pattern of the other ten cases (numbers

11 to 15) showed limited IgG-binding towards mosquito antigens as shown in Fig 3F (no. 11).

DISCUSSION

The whole body extract of mosquitoes contains many protein components, most of which can induce IgE as well as IgG immune responses in man through mosquito bites. By using the immunoblot technique, the antigenic components were identified by human sera containing IgE and IgG anti-mosquito antibodies.

IgE antibodies recognized protein components with MWs of 62, 55, 49, 42, 38, 36, 34 to 30, 26, and 21 K. Immunoblot patterns varied individually, no one single component was common in all cases. Only three of the 15 serum samples exhibited strong reactivity to different numbers of mosquito proteins. It indicates that the IgE response to mosquito bites is less common and not uniform.

All the serum samples contained IgG antibodies against mosquito components. Proteins with MWs of 78, 64 to 62, 55, 45, 42, 38 to 36, 34 to 29, and 23 to 21 K occurred more frequently and stained more heavily in immunoblot analysis. Most of the antigens recognized by IgE antibodies also reacted with IgG antibodies.

Although it is generally considered that the usual wheal and flare reaction after mosquito bites is mediated through pharmacologic activities in the mosquito saliva, the rather severe cutaneous reactions may be results of various immunologic responses. Among individuals (case numbers 6 to 15) who exhibited little or no IgE and moderate IgG antibody responses against mosquito antigens, pharmacologic activities of the mosquito saliva seemed to be responsible for their usual mild skin reactions. The low-titered antibodies observed would have been generated after mosquito biting but did not develop noticeable clinical symptoms. This view is

consistent with the report that low-titered anti-mosquito IgG antibodies frequently occur in normal human population (Ailus et al¹³). In case number 2 and no. 3, since the IgE antibody response was relatively strong, type 1 hypersensitivity seemed to be involved in their severe local immediate reaction after mosquito bites. Both patients also had a history of allergy to some other allergens. Rockwell and Johnson reported that a significant population of individuals who developed severe mosquito-bite reactions had personal and/or family history of other hypersensitivity reactions.³ For case numbers 4 and no. 5, who showed little or no IgE reactions but strong IgG responses against mosquito antigens, type 3 Arthus reactions seemed to be involved in their severe late reactions after mosquito biting. For case no. 1, who showed both strong IgE and IgG reactivity, a mixed reaction of type 1 and type 3 hypersensitivity seemed to be involved in her severe clinical manifestations. Alternatively or concurrently, the severe reaction to mosquito bites could also be the effect of a delayed type hypersensitivity.¹⁰

In conclusion, both IgE and IgG anti-mosquito antibodies in human can be detected and can be used to characterize the corresponding antigens by immunoblot analysis. The

occurrence of these antibodies may be related to the clinical expression and the pathogenesis of human reactions to mosquito bites.

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