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A Mouse Model of Mosquito Allergy for Study of Antigen-Specific IgE and IgG Subclass Responses, Lymphocyte Proliferation, and IL-4 and IFN- γ Production

Key Words

Mosquito Allergy
Mouse model
Aedes aegypti
IgE
IgG1
IgG2a
IL-4
IFN- γ
rAed a 1
rAed a 2

Abstract

To date, there are no ideal animal models for study of natural sensitization leading to IgE- and lymphocyte-mediated hypersensitivities. We established such a model in which four BALB/c mice were each sensitized by exposure to at least 16 mosquito *Aedes aegypti* bites, twice a week for 4 weeks. Four non-exposed control mice were also studied. Mosquito *A. aegypti* head and thorax extract, saliva, and two recombinant salivary allergens (rAed a 1 and rAed a 2) were used in vitro as antigens. Intradermal tests were performed. Serum mosquito antigen-specific IgG, IgG1, IgG2a, and total IgE were measured by ELISA; specific IgE was measured by passive cutaneous anaphylaxis (PCA). IgE responses to each antigen in the saliva were analyzed using Western blotting. Spleen lymphocyte proliferation assays were performed to determine the cell-mediated hypersensitivity response. Antigen-induced IL-4 and IFN- γ production in the spleen lymphocytes were evaluated using ELISA. After 4 weeks, all 4 mosquito-sensitized mice developed a positive immediate wheal 20 min after skin tests with mosquito antigens, and a positive delayed papule 24 h later, while control mice did not. Also, the sensitized mice had a positive PCA response, which correlated significantly with total IgE levels ($r = 0.84$, $p < 0.05$), confirming the presence of antigen-specific IgE, while none of control mice had a positive response. Antigen-specific IgG1, but not IgG2a, was increased in the sensitized mice ($p < 0.01$). Western blotting showed that 5 of the 8 antigens which elicited mouse IgE responses, including 2 major antigens, also elicited human IgE responses. The mean lymphocyte proliferation response to mosquito antigens also elicited human IgE responses. The mean lymphocyte proliferation response to mosquito antigens was significantly increased in the sensitized mice ($p < 0.05$). IL-4 production was significantly increased and IFN- γ production was decreased, further suggesting that a Th2 immune response predominates despite the development of the delayed skin reaction. This new model of natural sensitization without using an adjuvant is potentially useful for the study of other allergic disorders as well as mosquito allergy.

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Introduction

Mosquito bites cause immediate and delayed local cutaneous reactions and, occasionally, systemic reactions [1–4] by specific sensitization to mosquito saliva; initial exposure to species to which the subjects have not been previously exposed causes no reaction [5, 6]. In humans, mosquito salivary gland- or saliva-specific IgE antibodies have been identified in serum [7–9] and found to correlate with mosquito bite-induced wheals and flares [4, 8]. The lymphocyte proliferation response to mosquito antigens is increased in mosquito-allergic subjects and correlates with bite-induced delayed skin reactions [4, 8]. Recently, mosquito salivary gland-specific IgG has been found to be elevated in mosquito-allergic subjects and to correlate with skin mosquito bite reactions and specific IgE levels [4]. In a longitudinal study, in parallel with the development of skin reactions, IgG was increased as well as IgE [6], suggesting that IgG, too, is involved in the development of mosquito allergy.

Naturally acquired desensitization to mosquito bites has been observed in adults during long-term exposure to the bites, as documented in a cross-sectional study in which the size of the skin reactions to mosquito bites correlated inversely with the duration of exposure [4]. This was confirmed by our recent prospective study in which regular, continuous high-dose exposure to mosquito bites eventually leads to desensitization, defined as complete loss of immediate and delayed responses [6]. These data support the use of immunotherapy injections with relevant allergens to induce tolerance in patients with IgE-mediated hypersensitivities including mosquito allergy [3, 10]. However, most human adults have been sensitized by more than one mosquito species. Cross-reactivity among mosquito antigens and other insect antigens makes it difficult to study the mechanisms of sensitization and subsequent desensitization in humans. Development of an animal model of mosquito allergy will be useful for further elucidating the immunological mechanisms involved.

To date, reactions to mosquito bites have been studied only in two animal models: rabbits and guinea pigs [11–13]. In these species, the immune responses are not well understood and species-specific immunological reagents are not readily available. In contrast, the availability of well-characterized inbred mouse strains provides unique opportunities to study the role of a single compartment of the immune system and many species-specific reagents for immunological studies are commercially available.

Induction of the IgE response in mice has been achieved in most studies by injections of antigen with adjuvant such

as A1(OH)₃ [14, 15] and complete Freund's adjuvant [15, 16]; however, the adjuvants themselves induce nonspecific T cell and inflammatory responses and affect some cell functions [16]. In a few studies, in which increased IgE was induced by injections of antigens without adjuvant [17–18], the route of antigen administration differed from the natural route of sensitization in humans and this may have affected the immune responses. In one study, IgE was induced by exposure to aerosolized antigen [19].

The purpose of this study was to establish a mouse model of mosquito allergy using natural exposure to mosquito bites without an adjuvant, and to study the relevant immune responses including antigen-specific IgE, IgG1 and IgG2a, T lymphocyte proliferation to antigens, and Th1 and Th2 cytokine production.

Materials and Methods

Animals

The BALB/c mice (female, 8–10 weeks old) used for sensitization, and the female Sprague-Dawley rats (250 g each) used for passive cutaneous anaphylaxis (PCA), were obtained from The Central Animal Care Services, University of Manitoba. All animals were maintained under identical conditions in one room at the Service facility. The animal experiments were approved by the Animal Care and Use Committee, University of Manitoba and the investigators adhered to Canada Council Animal Care (CCAC) guidelines for humane treatment of animals.

Sensitization of Mice

4 BALB/c mice were sensitized by natural exposure to 16 mosquito bites each, twice a week for a total of 4 weeks. After confirming the development of skin reactions at week 4, the mice were sacrificed and the assays were performed.

Each mouse was put into a purpose-built 2.8 cm×11.5 cm round transparent tube with a 2.5 cm×6 cm window covered with plastic mesh to allow exposure to the mosquitoes. Then four mouse-loaded tubes were set into a cage containing 80 female mosquitoes that had been fed water without sugar for over 10 h. The cage, kept from bright light, sound, and movement for about 40 min, was closely observed to ensure that at least 16 or more mosquitoes were feeding on every mouse, or that about 80% of the mosquitoes had full abdomens. After 40 min, the tubes were removed from cage and the mice were released. 7 days after confirming the development of skin reactions at week 4 (after 8 mosquito exposures), the mice were sacrificed, sera were collected, and the spleen lymphocyte suspensions were prepared. 4 mice never exposed to any mosquito bites were included as controls.

A pooled serum from 4 mice immunized 3 times with recombinant Aed a 2 (rAed a 2) in complete Freund's adjuvant was included in the measurements of rAed a 2-specific IgG and its subclasses (fig. 3), to compare differential antibody responses between injections with antigens in an adjuvant and natural exposure.

Mosquitoes and Mosquito Preparations

The *Aedes aegypti* colony was obtained from the Department of Entomology, University of Manitoba, and maintained in our laboratory. 4- to 12-day-old female adult mosquitoes were used for the experimental mice challenges, saliva collection, and preparation of the head and thorax extract. Mosquito saliva was collected and prepared (0.25 mg/ml) as described previously [20], and used in ELISA and Western blot analysis. In the passive cutaneous anaphylaxis tests, lymphocyte proliferation assays, and antigen-induced cytokine production, a relatively large amount of antigen extract is required. Because collection of mosquito saliva is extremely time-consuming and labour-intensive, a mosquito head and thorax extract (4 mg/ml) was prepared as described previously [21] for use in these experiments.

Recombinant Mosquito Salivary Allergens, rAed a 1 and rAed a 2

Two genes of mosquito *A. aegypti*, encoding for Aed a 1, a 68-kDa salivary protein identified as an apyrase, and Aed a 2, a 37-kDa protein of unknown function, previously named D7, were cloned by James and his colleagues [22, 23]. In our laboratory, the 2 recombinant proteins were expressed using a baculovirus expression vector system and purified by ion exchange (DEAE Sephacel) and gel filtration (Sephacryl SH-100) chromatography. We found that the two recombinant proteins were allergens which elicited skin reactions in subjects with skin reactions to mosquito bites and bound to the IgE in sera from mosquito-allergic humans [24, 25].

Intradermal Tests

50 µl of mosquito head and thorax extract was injected intradermally into the shaved back of each mouse. 50 µl of a mast cell degranulating compound 48/80 (1.0 µg/ml, Sigma Chemical Co.) was injected as a positive control, and 50 µl of PBS was injected as a negative control 2 cm apart from the other injection sites. The immediate reaction was read 20 min after the injections by measuring the largest and orthogonal diameters of the wheal using a micrometer (Mitutoyo MFG, Tokyo, Japan). The delayed reaction was read 24 and 48 h later by measuring the induration. The results were expressed using the average diameter of the wheal or the induration. A diameter ≥ 5 mm was considered to be a positive test.

PCA Tests

Due to the large amount of specific IgG in mouse serum which completely inhibits the binding of the small amount of specific IgE to the antigens coated on the ELISA microplates, serum-specific IgE cannot be measured in mice by using a conventional ELISA. Therefore, PCA was performed to detect mosquito antigen-specific IgE levels [19]. Test mouse sera were diluted 1:2 in 0.02 M PBS, pH 7.2, then 0.1 ml of the diluted serum was injected intradermally into the shaved back of a rat. Compound 48/80 was injected as a positive control and PBS was injected as a negative control 2 cm away from other injection sites. After 24 h, the rat was challenge-exposed by intravenous injection of 1.2 mg of mosquito head and thorax antigens in 1 ml of 1% (w/v) Evan's blue dye in PBS. After 30 min, the rat was sacrificed, the skin was inverted, and the response, in terms of the infiltration of the blue dye rings around the injection sites, was read by measuring the largest and orthogonal diameters of the blue area. The results were expressed as the size of the blue area. A diameter ≥ 5 mm was considered a positive response.

ELISA Assays for Measurement of Total IgE and Antigen-Specific IgG, IgG1, and IgG2a

ELISA assays were developed to measure antibodies and cytokines in the study according to our ELISA techniques described previously [4]. Optimal conditions were chosen by checkerboard titration with reference to the manufacturer's instructions. Each determination was performed on at least 2 dilutions and each dilution was tested in duplicate or triplicate. All antibodies were purchased from PharMingen (San Diego, Calif., USA) except alkaline phosphate-conjugated monoclonal rat antibodies against mouse IgG, IgG1, and IgG2a which were obtained from Jackson Immuno Research Laboratories (West Grove, Pa., USA).

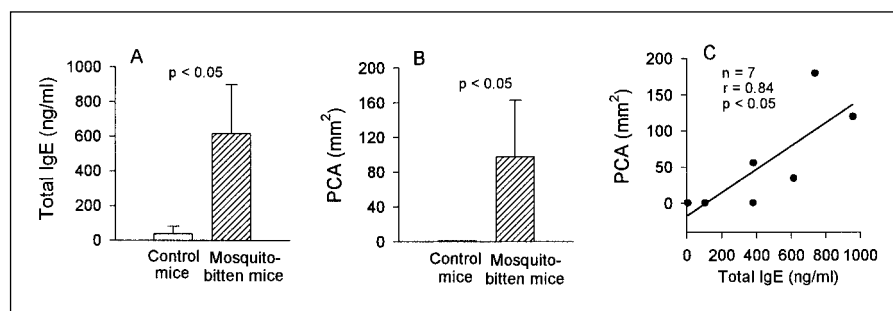
For detection of total serum IgE, the plates were coated with 1 µg/ml of monoclonal rat antimouse IgE and then sequentially incubated with test samples (1:20, 1:200) or a standard mouse IgE (1.95–500 ng/ml), biotinylated monoclonal rat antimouse IgE (1 µg/ml), and streptavidin-alkaline phosphate conjugate (1:1,000). The sensitivity of this assay was 1.95 ng/ml.

In the measurement of serum-specific IgG, IgG1, and IgG2a to mosquito saliva, rAed a 1 and rAed a 2, the plates were coated with 0.14 µg/ml of mosquito saliva and 2.0 µg/ml of rAed a 1 or rAed a 2, respectively. The plates were then incubated with serum samples (1:20 and 1:80 for IgG and IgG1; 1:10 and 1:20 for IgG2a) or a reference serum (1:80 to 1:20,480), followed by incubation with alkaline phosphate-conjugated monoclonal rat antibodies against mouse IgG (1:1,500), IgG1 (1:1,000) or IgG2a (1:1,500). The values of mosquito saliva-specific antibodies were calculated from the dilution curve of a reference serum pooled from four mosquito-sensitized mice and defined as 1,000 U/ml for saliva IgG, IgG1 and IgG2a, respectively. The sensitivity of the assay was 0.2 U/ml for IgG and IgG1 and 3.1 U/ml for IgG2a. The values of rAed a 1- or rAed a 2-specific antibodies were expressed by the optical absorbance after deduction of the PBS control.

Western Blot

To determine whether the IgE response to mosquito salivary antigens is similar in mice and humans, Western blotting was performed [20, 26] using the sensitized mouse serum and a pooled mosquito-allergic human serum from our previous studies [5]. 1.2 mg of mosquito saliva proteins prepared in a reducing buffer were loaded onto each well and separated by 12% gel SDS-PAGE using a Bio-Rad Mini-Protean II apparatus. The separated proteins were electrotransferred onto nitrocellulose membranes. Free binding sites on the membranes were blocked by 3% BSA in Tris buffer. In the analysis of mouse IgE response, the membrane was immunoblotted with a pooled mosquito-sensitized mouse serum (1:10) at room temperature for 2 h, followed by incubation with biotinylated rat mAb antimouse IgE (PharMingen) at room temperature for 1 h. After washing, the membrane was incubated with peroxidase-conjugated streptavidin (Sigma Chemical Co.) at 37°C for 1 h. In the analysis of human IgE response, the membrane was immunoblotted with a pooled mosquito-allergic human serum (1:10) followed by incubations with monoclonal mouse antihuman IgE (clone 7.12, ascites, a gift from Dr. Andrew Saxon, University of California, Los Angeles, Calif., USA) and then peroxidase-conjugated goat antimouse IgG. The membranes were finally incubated with ECL detecting reagents (Amersham, Arlington Heights, Ill., USA) and exposed to X-Omat film (Kodak, Rochester, N.Y., USA). Negative controls were normal mouse serum and a residual human serum provided by the Health Sciences Centre Clinical Chemistry Laboratory from a 5-month-old infant who had never been bitten by mosquitoes.

Fig. 1. Serum total IgE (**A**) and specific IgE (**B**) levels and the correlation between them (**C**).



Lymphocyte Proliferation Assay

The lymphocyte proliferation assay was carried out in the presence of mosquito antigens (head and thorax extract and rAed a 1, respectively) and Con A [4, 27, 28]. Mouse spleen cell suspensions were prepared as described previously [27]. 100 µl of the suspension at a concentration of 4×10^6 cells/ml in a culture medium consisting of RPMI-1640 (Gibco, Grand Island, N.Y., USA), supplemented with 5 mM Hepes buffer, 5×10^{-5} mM 2-mercaptoethanol, and 100 units/ml of penicillin-streptomycin, enriched with 10% heat-inactivated FCS, was pipetted into each well of a flat-bottomed tissue culture plate. 100 µl of the mosquito head and thorax extract (100 µg/ml diluted in complete medium) or rAed a 1 (10 µg/ml) was added into each well as a lymphocyte stimulant. Con A (5.0 µg/ml) and culture medium were used as positive and negative controls, respectively. All assays were performed in triplicate. Cells were cultured in 5% CO₂ at 37°C for 4 days. [³H]-Thymidine was added at 0.5 µCi per well 16 h before harvest. The cells were then collected on glass fibre filters with an automatic harvester (Flow Laboratories). Radioisotope incorporation was determined in a β-counter (Beckman Liquid Scintillation Counter), and the results were expressed as the mean counts per minute (cpm) ± standard deviation.

IL-4 and IFN-γ Production by Spleen Lymphocytes

Mouse spleen cell suspensions were prepared as described previously [28] and then cultured in triplicate to a final concentration of 2×10^6 cells/ml (total 200 µl/well) in the presence of Con A (a final concentration of 5.0 µg/ml), mosquito saliva (5.0 µg/ml) or rAed a 1 (5.0 µg/ml) in 96-well tissue culture plates diluted in complete medium at 37°C in a humidified atmosphere with 5% CO₂. Culture with the medium alone served as an unstimulated control. Culture supernatants were harvested at 24, 48, 72 or 96 h and stored at -70°C until cytokine analysis.

The levels of IL-4 and IFN-γ in the culture supernatants were determined by ELISA as described above, with reagents purchased from Pharmagen. The plates were coated with purified monoclonal rat antimouse IL-4 or antimouse IFN-γ (1.0 µg/ml) and then incubated with test culture supernatants (undiluted and 1:2) or a recombinant standard (IL-4, 0.25–250 pg/ml; IFN-γ, 1.0–1,000 pg/ml). This was followed by incubations with biotinylated monoclonal rat anti-mouse IL-4 or antimouse IFN-γ (1.0 µg/ml), and finally streptavidin-alkaline phosphatase (1:1,000). The sensitivity of the assay was 2 pg/ml for IL-4 and 15.6 pg/ml for IFN-γ.

Statistical Analysis

Immunoglobulin and IL-4 or IFN-γ concentrations were expressed by calculating the mean ± 1 standard deviation (SD). Groups were compared using two-tailed Student t tests. The χ^2 square test was used to compare skin test positivity rate between groups. Linear regressions were used for the analysis of correlation between PCA results and total serum IgE levels.

Results

Skin Tests

All four mosquito-sensitized mice developed a positive wheal reaction (7–12 mm in diameter) 20 min after testing with mosquito antigens and with compound 48/80, but not with PBS. Control mice developed a positive wheal only after testing with compound 48/80, but not with mosquito antigens or PBS ($p < 0.01$). 24 h later, in the 4 sensitized mice but not the control mice, a positive indurated area smaller than the preceding immediate wheal was palpated manually, suggesting that delayed skin reactions had developed ($p < 0.01$).

Total IgE and PCA Tests

The mean serum total IgE level was significantly higher in mosquito-sensitized mice (614.4 ± 283.7 ng/ml) than in control mice (38.09 ± 45.01 ng/ml, $p < 0.05$; fig. 1A). The PCA results showed that all 4 sensitized mice gave a positive response (blue areas 180, 120, 56, and 35 mm²), while none of the control mice gave a positive response (no blue spots were found) (fig. 1B); for 1 control mouse, there was not enough serum to perform PCA testing. The increased total IgE levels and PCA responses in the parasite-free and monosensitized mice suggest that total IgE consists primarily of mosquito antigen-specific IgE. This was further supported by the significant correlation between the sizes of the PCA reactions and the levels of total IgE ($r = 0.8379$, $p < 0.05$; fig. 1C). These results indicate that mosquito antigen-specific IgE can be induced in the mice by natural ex-

posure to mosquito bites, and that in the mouse model, specific IgE levels can be estimated by measuring total serum IgE levels.

Mosquito Antigen-Specific IgG, IgG1 and IgG2a

As illustrated in figure 2, serum mosquito saliva-specific IgG was higher in the mosquito-bitten mice than in the control mice (185.36 ± 81.5 vs. 11.066 ± 4.1 U/ml, $p < 0.001$). Specific IgG1 isotype antibodies were the dominant specific-IgG subset (313.05 ± 171.3 vs. 2.56 ± 1.97 U/ml), while IgG2a was undetectable. These data indicate that mosquito bites can stimulate host B cells to secrete specific IgG1 antibodies.

A similar pattern of IgG and its subclass responses was found when recombinant mosquito salivary antigens, rAed a 1 and rAed a 2, were used in the ELISA (fig. 3). rAed a 1 and rAed a 2 have been demonstrated to be important anti-

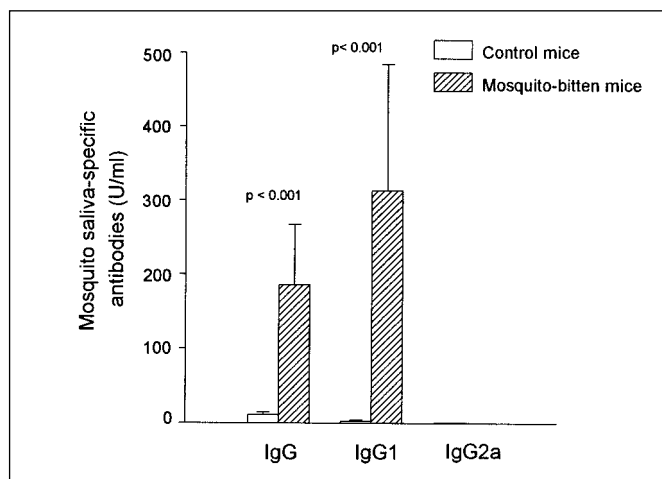


Fig. 2. Mean serum mosquito saliva-specific IgG, IgG1, and IgG2a levels measured by ELISA.

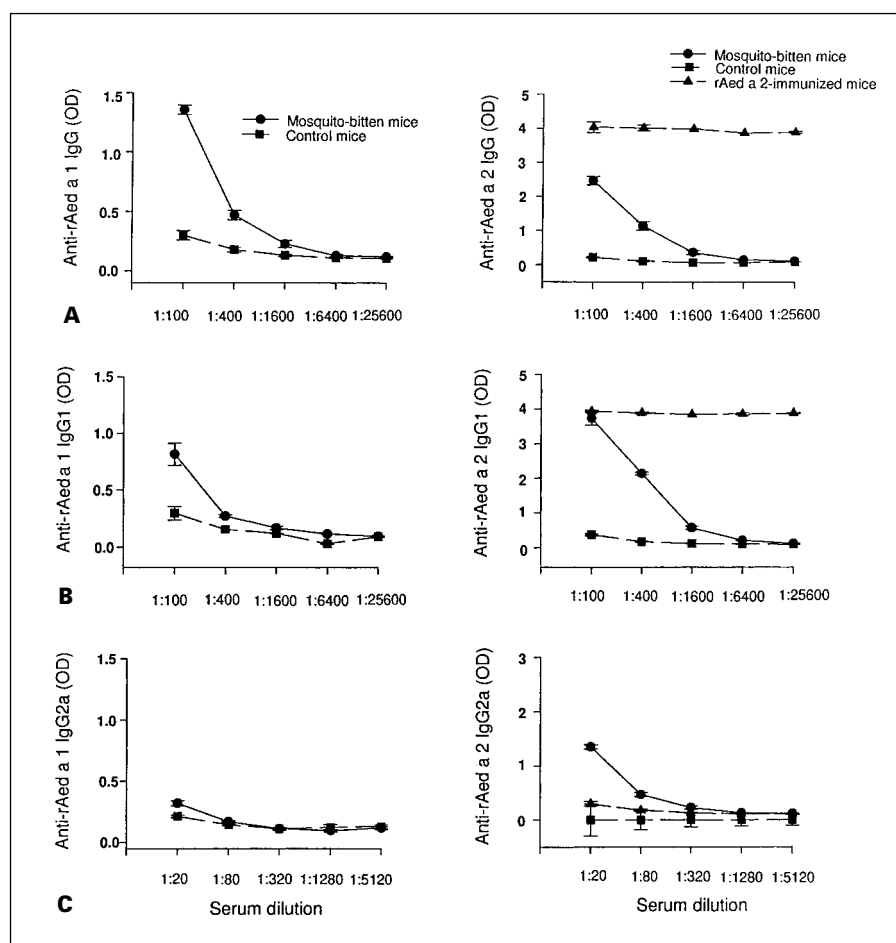


Fig. 3. Serum specific IgG (A), IgG1 (B) and IgG2a (C) responses to recombinant mosquito salivary antigens, rAed a 1 and rAed a 2 measured by ELISA.

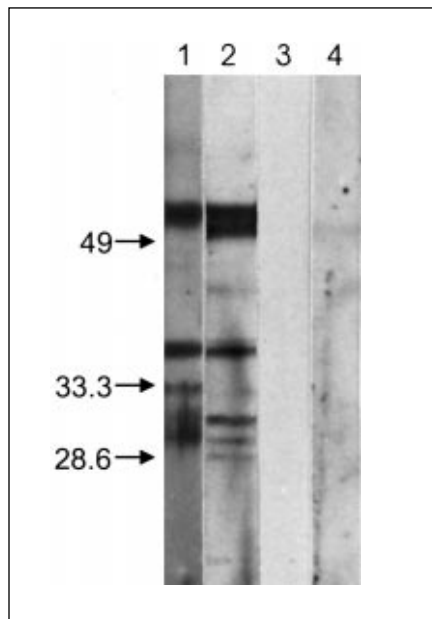


Fig. 4. Western blot analysis of mosquito salivary antigens. Mosquito saliva proteins were separated by 12% gel SDS-PAGE and then transferred onto nitrocellulose membranes. The membranes were incubated with a pooled mosquito-allergic human serum (lane 1) or a pooled mosquito-bitten mouse serum (lane 2). This was followed by sequential incubations with relevant anti-IgE antibodies and conjugates as described in the Methods. Serum from an infant never exposed to mosquitoes (lane 3) and normal mouse serum (lane 4) were used as negative controls, respectively.

gens in human mosquito allergy [24]. The increased rAed a 1- and rAed a 2-specific IgG antibodies suggest that the native mosquito salivary Aed a 1 and Aed a 2 induce an antibody response which can be measured using their recombinant forms, and that the 2 recombinant antigens have identical antigenicity to their native forms. In the rAed a 2-specific antibody response, the serum from immunized mice exhibited much higher levels of specific IgG and IgG1 than the sera from mosquito-bitten mice.

Western Blotting

The human and murine IgE responses to each mosquito saliva antigen were compared using Western blotting. Figure 4 shows that the IgE in mosquito-sensitized mouse serum (lane 2) reacted with 8 mosquito salivary proteins (the 28-, 30-, 32-, 34-, 37-, 46-, 50-, and 52-kDa saliva ones). 5 of the 8 proteins (the 30-, 32-, 34-, 37-, and 52-kDa ones) including 2 major antigens were also recognized by the IgE in human mosquito-allergic serum (lane 1), suggesting the IgE responses to mosquito salivary antigens in the mouse model are similar to those in humans.

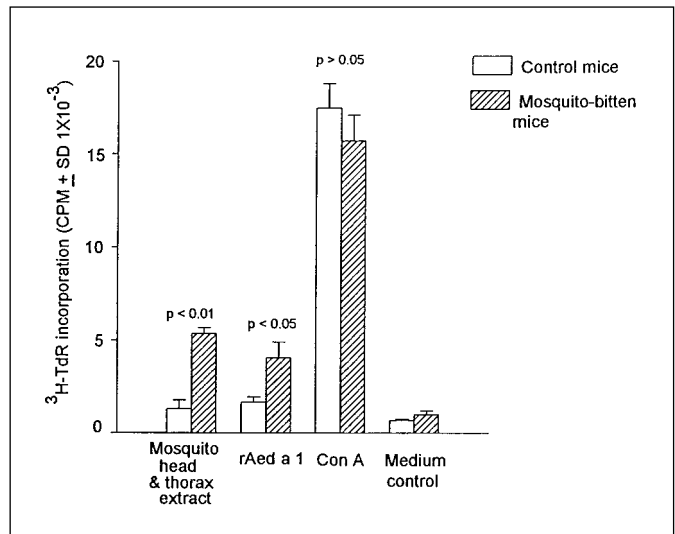


Fig. 5. Mosquito allergen-specific lymphocyte proliferation response. Lymphocyte proliferation was induced by mosquito head and thorax extract and rAed a 1, respectively, as antigen-specific stimulation, and Con A as a non-specific positive control. The data expressed as ^3H -thymidine incorporation, are the mean $\pm$ SD of the results on day 4 of an in vitro cell culture.

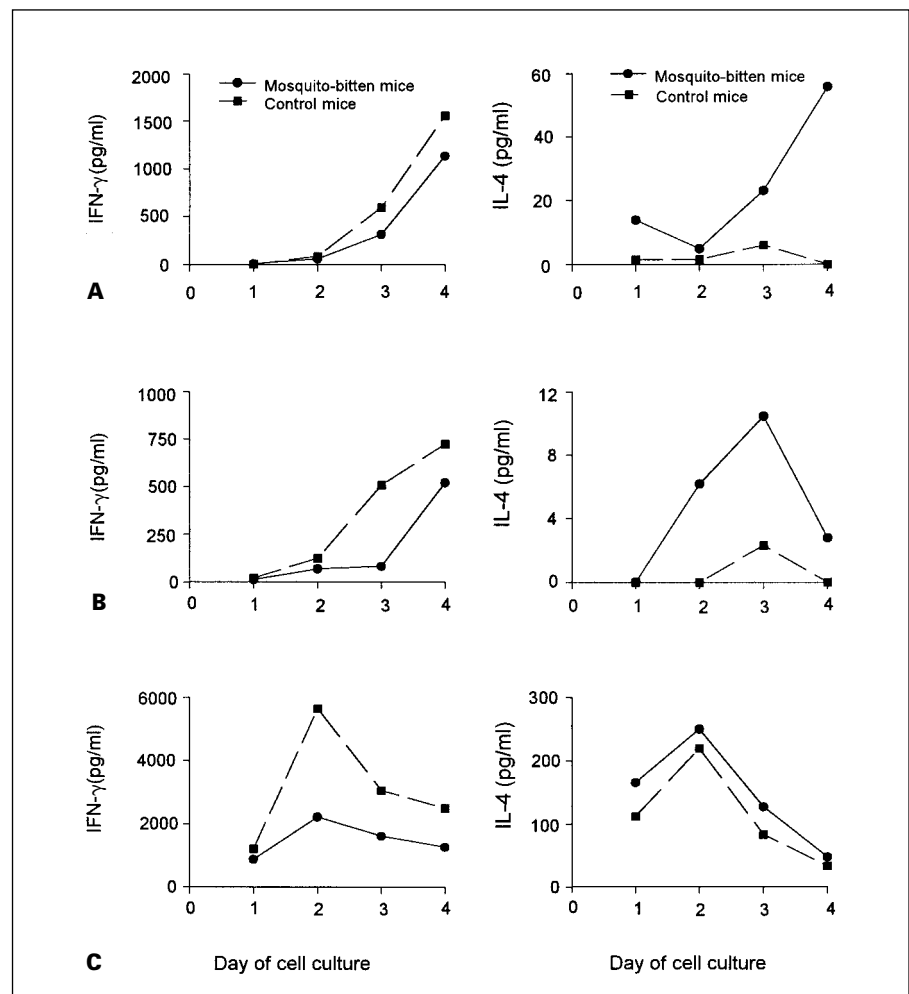
Mosquito Allergen-Specific Lymphocyte Proliferation Assay

The mean lymphocyte proliferation (cpm) after stimulation by mosquito antigens was significantly higher in mosquito-bitten mice (5,379 $\pm$ 316 for mosquito head and thorax extract, 4,075 $\pm$ 844 for rAed a 1) than that in the control mice (1,331 $\pm$ 460 for mosquito head and thorax extract, 1,668 $\pm$ 310 for rAed a 1, p<0.01; fig. 5), suggesting that lymphocytes are involved in the development of immunological reactions to mosquito saliva.

IL-4 and IFN- γ Production by Spleen Lymphocytes

The profiles of cytokine secretion in response to mosquito antigens or T cell mitogen Con A stimulation were analyzed to determine the ratio of the Th1 and Th2 responses in the mouse model. As shown in figure 6, the levels of IL-4 in the supernatants of cultured spleen cells stimulated by mosquito saliva, rAed a 1, or Con A were higher in mosquito-bitten mice than those in the control mice. In contrast, the levels of IFN- γ were lower in the sensitized mice than in the control mice. The increased IL-4 and de-

Fig. 6. T cell cytokine (IL-4 and IFN- γ) production by spleen cells from mosquito-bitten mice and normal mice. Spleen cell suspensions were cultured with mosquito saliva (A), rAed a 1 (B), and Con A (C) as described in the Methods. Supernatants were harvested on various days, and the levels of IFN- γ and IL-4 in the supernatants were measured by ELISA.



creased IFN- γ protein levels after antigen-specific or non-specific stimulation suggest that after being exposed to mosquito bites, a predominant population of Th2 cells developed in the mouse model, in agreement with the findings of increased serum IgE and IgG1 levels in the mosquito-bitten mice.

Discussion

In previous studies, sensitization to mosquito antigens has been achieved by injection of *A. aegypti* antigens with [12, 13] or without adjuvants [29] or by *A. aegypti* bite challenges in rabbits and guinea pigs [11]. Although skin reactions to mosquito bites developed, because of the lack of availability of suitable reagents in these animal species, immunological studies consisted only of the measurement of IgG, precipitating antibody or PCA testing. In preliminary

studies, using Western blot analysis, we found that the antibody responses to mosquito saliva antigens in rabbits were quite different from the responses in humans and that several antibodies which reacted with mosquito saliva antigens were found in the sera of the rabbits *before* they were sensitized by mosquito bites (unpubl. data).

In our previous study, a human subject who received 100 mosquito bites every 2 weeks took 3 weeks to develop a skin response, and a rabbit that received 140 mosquito bites every week took 2–4 weeks to develop a skin response [6]. Therefore, in the mouse model we sensitized the mice with a relatively large number of mosquito bites twice each week for 4 weeks to ensure a good immune response.

Our mouse model has many potential advantages. There is a large body of knowledge about the mouse immune system and its functional compartmentalization. Furthermore, different mouse strains each with an identical genetic background have been characterized, providing a controlled

means of studying the immune system. No mosquito saliva-specific antibodies were found in the normal mouse serum (fig. 4, lane 4), the pattern of antigen-induced IgE responses was similar to that found in humans (fig. 4), and we were able to study IgE, IgG subclasses, and cytokine responses using commercially available reagents. Although the large confluent visible skin reactions to mosquito bites seen in human mosquito-allergic subjects were not observed in the mice (data not shown), immediate and delayed reactions were successfully induced by intradermal injection of mosquito antigens.

In the sensitized mice, both IgE and IgG1 responses to mosquito saliva proteins, as well as to the recombinant allergens (rAed a 1 and rAed a 2) were elicited by exposure to mosquito bites without concomitant adjuvant administration. The sensitization method used is identical to the natural sensitization that occurs in humans in areas infested with mosquitoes. The antibody isotype responses in the model were comparable to the findings in humans in which both mosquito-specific IgE and IgG were increased in mosquito-allergic subjects [4]. We further identified that the increased IgG was an IgG1 isotype, which like IgE is capable of sensitizing mast cells, but in a shorter sensitizing period (4 h) compared to IgE (>24 h) [30, 31].

CD4⁺ T helper (Th) cells secrete a variety of cytokines involved in immune regulation. In mice and humans, cloned CD4⁺ Th cells can be characterized into two subsets according to the specific cytokines they produce. Th1 cells secrete IFN- γ and IL-2 which promote a cell-mediated immune response characterized by expression of complement-fixing and opsonizing antibodies, such as IgG2a in the mouse, and macrophage activation. Th2 cells, in contrast, secrete IL-4, IL-5 and IL-6 which promote the development of selected humoral immune responses including the expression of IgE and of IgG1 in mice. The development of IgG1 and of IgG2a isotypes are dependent on IL-4 and IFN- γ expression, respectively. IL-4 promotes B cell proliferation and the isotype switch from IgM to IgE and IgG1, while IFN- γ stimulates the production of IgG2a and IgG3 [32–35] and suppresses IgG1 production [36–38].

In the sensitized mice, we were able to demonstrate increased IL-4 production and decreased IFN- γ production in spleen lymphocytes. This suggests that a Th2-dominant response developed in the sensitized mice, as occurs in humans with allergic disorders [39, 40]. This also could account, in part, for the differential effects of cytokines observed on the IgG subclass production and for the role of cytokines in altering the immune response through the production of dominant IgE and IgG1.

Antigen-specific lymphocyte proliferation was also increased in the mosquito-bitten mice as has been found in humans [4, 8], possibly because mosquito antigens are T cell-dependent antigens. The production of IgE or IgG1 requires IL-4 as confirmed in this study, and other T cell helper factors. Furthermore, the mosquito-bitten mice developed T lymphocyte-mediated delayed type skin reactions as found in humans, supporting the concept that lymphocytes are the central mediators involved in initiating, developing and effecting the hypersensitivity response [38].

In summary, our results demonstrate for the first time that, after natural exposure to mosquito bites without adjuvant, mice develop immediate and delayed skin reactions associated with increased IgE and IgG1 responses and T lymphocyte proliferation, suggesting a Th2-predominate response. This model will facilitate future human mosquito allergy studies and provide an excellent tool for in-depth study of the mechanisms of mosquito allergy and other allergic disorders.

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