

Short communication

Detection of mosquito saliva-specific IgE antibodies by capture ELISA

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We developed an IgE-capture ELISA and measured mosquito saliva-specific IgE antibodies in 27 children sensitive to mosquito bites. Children with large 15-min bite wheals had significantly higher ($P < 0.0005$) mosquito saliva-specific IgE levels than children with small wheals. In the latter group, the saliva-specific IgE level was significantly higher ($P = 0.031$) than the levels of six infants never exposed to mosquitoes. A positive correlation ($r = 0.65$; $P = 0.0002$) was found between the size of the 15-min wheal and the mosquito saliva-specific IgE antibody levels. These results further support the role of mosquito saliva-specific IgE antibodies in the pathogenesis of mosquito-bite whealing. Compared to immunoblotting, IgE-capture ELISA provides a quantitative method to measure mosquito saliva-specific IgE antibodies.

The role of mosquito saliva-specific IgE antibodies in the pathogenesis of immediate mosquito-bite reactions seems evident (1). IgE antibodies have been demonstrated against mosquito saliva or salivary gland extracts (2–7), and mosquito-bite hypersensitivity can be transferred with saliva-specific IgE antibodies to nonreactive recipients (8). Children begin to react to mosquito bites early in childhood, and almost all Finnish children show immediate whealing and/or delayed papular reactions to *Aedes* mosquito bites. Recently, we have shown by immunoblotting that mosquito-bite sensitive children frequently have saliva-specific IgE and IgG4 antibodies (9).

Immunoblotting is a sensitive method to demonstrate IgE antibodies, and it is also suitable for antigen characterization. However, only qualitative analysis of IgE antibodies can be performed by immunoblotting. Therefore, we developed an IgE-capture enzyme-linked immunosorbent assay (ELISA) and quantitated mosquito saliva-specific IgE antibodies in 27 mosquito bite-sensitive children.

Material and methods

Subjects and mosquito challenge

Sera from 27 mosquito bite-sensitive children were examined. The forearm of each child was exposed to one or two *Aedes aegypti* mosquito bites, as previously described (8). The size of the bite reaction was measured at 15 min. At the same time, histamine prick (10 mg/ml; ALK A/S, Hørsholm, Denmark) and physiologic saline controls were done. The children were divided into two groups according to their immediate mosquito-bite reactivity. In the first group, 16 children (mean age 5.3, range 2–10 years) had large bite reactions (wheal diameter > 5 mm or larger than the size of histamine prick); in the second group, 11 children (mean age 6.0, range 2–11 years) had small bite reactions (wheal diameter < 5 mm, or the same as or smaller than histamine prick). Sera from six infants (aged 6–8 months) never exposed to mosquitoes served as negative controls.

IgE-capture ELISA

Sera (50 µl) were diluted 1:2 in dilution buffer (Immunotech, Marseille, France) and incubated for 30 min at room temperature in commercial microtiter plate wells precoated with mouse antihuman IgE monoclonal antibody (10) (Immunotech, Marseille, France). All incubations were done at room temperature on a shaking platform. Wells were washed three times with phosphate-buffered saline (PBS)-Tween (0.05%). *A. communis* saliva (100 µl, total protein concentration [8] 1 µg/ml), collected from adult mosquitoes, as previously described (2), was added in 5% milk PBS. Only 5% milk-PBS without mosquito saliva was added to parallel wells. After 1-h incubation, wells were washed as before, and 100 µl serum (1:1000) in 5% milk PBS from a rabbit immunized with *A. communis* mosquito bites was added (11). After 1-h incubation, the wells were washed again three times, and 100 µl alkaline phosphatase-conjugated goat antirabbit IgG antibodies (1:1000; Zymed, San Francisco, CA, USA) was added in 5% milk PBS. Incubation was for 1 h. Wells were washed as before, and 200 µl of phosphatase substrate (*p*-nitrophenyl phosphate, Sigma, St Louis, MO, USA) in carbonate buffer (pH 9.8) was added. After 90-min incubation, absorbances were read at 405 nm against blank substrate (Multiscan®, Labsystems, Helsinki, Finland). The results were calculated by subtracting the absorbance of non-saliva-sample wells from the parallel mosquito saliva-containing sample wells. All ELISA absorbance data were converted to saliva-specific IgE units by interpolating optical density from the multidilution reference calibration curve. The sensitivity and quantifiable range of the assay was calculated with this calibration curve. Sera were studied in two consecutive ELISA runs. One of the serum samples with high saliva-specific IgE antibody levels was used as an internal standard to calibrate separate assays. Reproducibility was evaluated by studying one of the positive samples in four successive ELISA runs.

The specificity of the rabbit antisaliva IgG antibody was confirmed both by immunoblotting (2) and by measuring the saliva-specific IgE antibodies from the serum of one child with large bite reactions. In this serum, there were 279 saliva-specific IgE antibody units with the rabbit postimmune sera. The rabbit preimmune serum gave a value of 1.5 units. The total serum IgE content was determined from the serum of all 27 children by commercial ELISA (Immunotech, Marseille, France). The possible effect of nonspecific IgE on the capture ELISA was studied by adding increasing amounts of an IgE myeloma protein (100–100 000

units, Chemicon International, CA, USA) to two mosquito saliva-positive sera.

Immunoblotting

An immunoblot technique to detect IgE antibodies against the 36- and 22-kDa *A. communis* saliva antigens, described in detail previously, was used (2). Briefly, sodium dodecyl sulfate-polyacrylamide gel-electrophoresed *A. communis* mosquito saliva was electroblotted to polyvinylidene difluoride membranes, and the binding of IgE-class mosquito saliva-specific antibodies from each sample was demonstrated with biotinylated goat antihuman IgE and alkaline phosphatase conjugated to streptavidin.

Results

The distribution of IgE mosquito saliva-specific antibodies is shown in Fig. 1. Mosquito saliva-specific IgE units were significantly higher ($P=0.0005$, Student's *t*-test, one-tailed) in the 16 children with large mosquito bite wheals (140.6 ± 130.1 ; mean $\pm$ SD) than in the 11 children with small wheals (mean 8.0 ± 7.4). Moreover, mosquito saliva-specific IgE units were significantly higher ($P=0.031$, Student's *t*-test, one-tailed) in the children with small wheals than in the six infants never exposed to mosquitoes (mean saliva specific IgE units 3.1 ± 2.4).

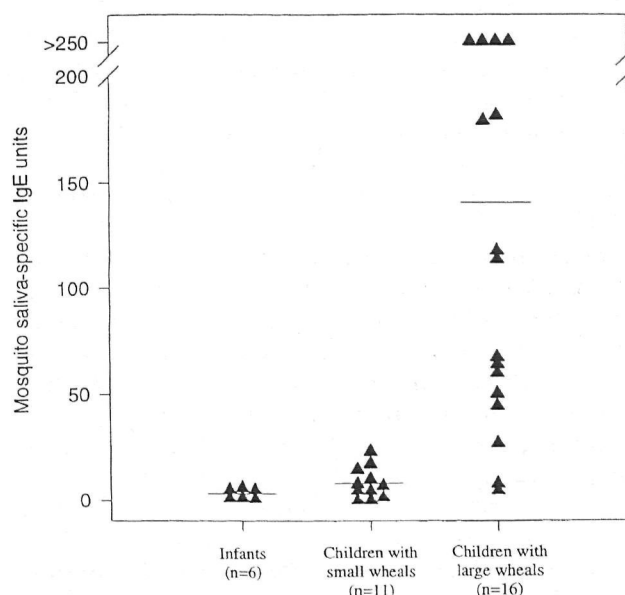


Fig. 1. Distribution of *Aedes communis* mosquito saliva-specific IgE measured by capture ELISA in 27 mosquito-bite-sensitive children and six unexposed infants. Diameter of wheals was measured after challenge with *A. aegypti* mosquito bites. Mean mosquito saliva-specific IgE antibody units are shown as horizontal bars.

A significant positive correlation ($r=0.65$; $P=0.0002$, Spearman rank, two-tailed) was found between saliva-specific IgE units and the size of the mosquito-bite wheals in the 27 children. No significant correlation ($r=0.3$; $P=0.13$, Spearman rank, two-tailed) was found between the saliva-specific IgE units and the total IgE units in the 27 children's sera. The mean of the total IgE units was not statistically different ($P=0.148$, Student's t -test, one-tailed) in 16 children with large mosquito-bite wheals (mean 786 U/ml, range 25–8200) from that in the 11 children with small wheals (mean 221 U/ml, range 25–910).

The mean coefficient of variation in four successive ELISA runs was 11.8%. The effect of irrelevant IgE added to the capture ELISA was studied in sera from two children with slightly elevated mosquito saliva-specific IgE antibody levels (118 and 114 units, respectively). A dose-dependent decrease in IgE antibody values was demonstrated in both sera starting at 100–300 units, but the values became negative (below 22.8 units; the cutoff level for positivity, representing the mean of saliva-specific IgE in children with small wheals + 2 SD) only after addition of as many as 10 000 units of the myeloma IgE. The sensitivity of the assay was two saliva-specific IgE units and the quantifiable range 2–300 units of saliva-specific IgE.

Immunoblotting showed IgE antibodies to the 36-kDa saliva protein in all 16 children with large mosquito-bite wheals and in 10 of the 11 children with small wheals. In most cases, the bands were clearly more intense in the children with large wheals and high levels of saliva-specific IgE units than in those with small wheals (Fig. 2). Nine of the 16 children with large wheals reacted also against the 22-kDa saliva protein (Fig. 2).

Discussion

Previously, we have shown by immunoblotting the frequent occurrence of mosquito saliva-specific IgE and IgG4 antibodies in children and adults (2, 9). In the present study, we developed an IgE-capture ELISA to quantitate these IgE antibodies. Sera from 27 children were examined, and a positive correlation was found between saliva-specific IgE units and the size of the mosquito-bite wheal. Moreover, children with large wheals had significantly higher mean levels of mosquito saliva-specific IgE than children with small wheals. Since it is known that the total serum IgE level can influence the results of the IgE-capture assay, the effect of added irrelevant IgE was analyzed. Serum samples containing slightly elevated levels of mosquito saliva-specific IgE became negative only after high amounts of nonspecific myeloma

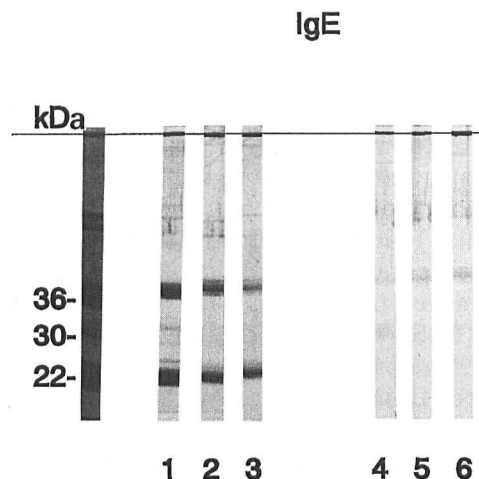


Fig. 2. IgE immunoblots against *Aedes communis* saliva in three children (lanes 1–3) with large wheals (corresponding to saliva-specific IgE units 429, 301, and 255) and in three children (lanes 4–6) with small wheals (corresponding to saliva-specific IgE units 0, 5, and 1). Note more intense bands to 22- and 36-kDa saliva proteins in children with large wheals. C=control sera from rabbit immunized with *A. communis* mosquito bites.

IgE were added. This suggests that the present IgE-capture ELISA does not give false-negative results due to competitive IgE binding. However, one child with a large mosquito-bite wheal and high total IgE level (8200 U/ml) had a low but positive saliva-specific IgE level (64 units).

Mosquito salivary-gland extracts, but not pure saliva, have previously been used as antigens in ELISA. In Japan, Oka (4) found a correlation between ELISA IgE scores and immediate bite reactivity to *A. albopictus* mosquitoes. In Canada, Peng et al. (7) used *A. vexans* salivary-gland ELISA and found in subjects with immediate bite reactions significantly higher antimosquito IgE antibody levels than in subjects without bite reactions. Our IgE-capture ELISA findings with pure saliva are in accordance with the results of these studies with salivary-gland extracts. However, Peng et al. (7) did not show a correlation between the size of the mosquito-bite wheals and salivary-gland specific IgE units.

The mosquito whole-body and salivary-gland extracts differ from pure saliva in total protein and antigen content (2, 12, 13). The low antigen content may explain why the mosquito RAST based on the whole-body extract does not detect well saliva-specific IgE antibodies (8). Saliva contains enough antigens for immunoblotting (2, 9), and, as shown in the present study, also for the capture-ELISA method. However, the antigen content of saliva was not high enough to be used in ordinary ELISA (data not shown). The low level of IgE-capture ELISA units in one of the children with a large wheal could be due to species differences. The

wheals were induced by *A. aegypti* mosquito bites, while *A. communis* saliva was used in the IgE-capture ELISA.

In vitro tests used to detect allergen-specific IgE antibodies in the serum hardly ever identify all skin-test-positive subjects (14–16). The levels of specific IgE antibody may be too low, or high concentrations of allergen-specific IgG antibodies may interfere with IgE binding (17–19). After immunotherapy in pollen and Hymenoptera venom allergies, IgG antibodies belong predominantly to the IgG4 subclass (20–22). In our previous immunoblotting studies, we have found that many mosquito-bite-sensitive subjects have saliva-specific IgE and IgG4 antibodies against the same saliva proteins (2, 9). The present IgE-capture ELISA allows the measurement of minute amounts of saliva-specific IgE antibodies and does not seem to be obstructed by saliva-specific IgG4 antibodies.

Aedes mosquito-saliva- and salivary-gland-specific IgE antibodies can be found in subjects living in different parts of the world (2–4, 7, 9). By the present IgE-capture ELISA, it seems to be possible to identify those subjects who react to the bites with large wheals; i.e., subjects who are abnormally sensitive to *Aedes* mosquito bites.

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