

Detection of mosquito saliva–specific IgE and IgG₄ antibodies by immunoblotting

Henrikki Brummer-Korvenkontio, MSc^a [\[MEDLINE LOOKUP\]](#)

Pekka Lappalainen, MSc,^a [\[MEDLINE LOOKUP\]](#)

Timo Reunala, MD,^b [\[MEDLINE LOOKUP\]](#)

Timo Palosuo, MD^a [\[MEDLINE LOOKUP\]](#)

Helsinki, Finland

Sections

- [Abstract](#)
- [Methods](#)
- [Results](#)
- [Discussion](#)
- [References](#)
- [Publishing and Reprint Information](#)
- [Articles with References to this Article](#)

Abstract

IgE and IgG subclass antibodies against *Aedes communis* mosquito saliva were studied by immunoblotting in 12 adults with immediate and/or delayed skin reactions to mosquito bites. Four antigenic proteins, with molecular weights of 22, 30, 36, and 64 kd, were found in the mosquito saliva. Almost all subjects (11 of 12) had anti-mosquito saliva–specific IgE antibodies directed against the 36 kd protein. The IgG antibody response appeared to be restricted mostly to IgG₄ (11 of 12) and IgG₁ (8 of 11) subclasses against the same 36 kd antigen. Ten of the 12 subjects had both IgE and IgG₄ antibodies to the 36 kd protein. No anti-mosquito antibodies were found in pooled sera of five infants never exposed to mosquito bites. These results show that most persons with immediate skin reactivity to *A. communis* mosquito bites have both IgE and IgG₄ antibodies that recognize the 36 kd antigen present in the mosquito saliva, suggesting that anti-saliva antibodies may play a role in the pathogenesis of mosquito bite reactions. (J ALLERGY CLIN IMMUNOL 1994;93:551-5.)

MW	Molecular weight
TBS	Tris-buffered saline
WBE	Whole-body extract

Key words: [IgE](#), [IgG₄](#), [IgG subclass](#), [allergy](#), [mosquito saliva](#), [immunoblotting](#)

Many insect species cause allergic reactions in human beings by stinging or biting or via the inhalant route. Mosquitoes are ubiquitous in many parts of the world, and persons may respond to bites by developing immediate wheals and/or delayed bite papules. ¹ These reactions are considered immunologic, but little is known about the mosquito antigens, the antibodies involved, or their pathogenetic significance.

We showed earlier with immunoblotting that rabbits immunized with *Aedes communis* mosquito bites, the most common human-biting mosquito species in Finland, develop IgG anti-mosquito antibodies. The use of *A. communis* whole-body extract (WBE) as antigen frequently reveals similar IgG antibodies in adult Finns but not in infants who have never been exposed to mosquitoes. ² Other studies have similarly shown IgG class anti-mosquito antibodies in human beings by using WBE or extracts made from the salivary glands by means of immunoblotting or ELISA. ³⁻⁷

In the present study we have used pure *A. communis* mosquito saliva as antigen and have characterized IgE and IgG subclass anti-mosquito saliva antibodies in the sera of adult Finns by immunoblotting. The results show that most subjects have both IgE and IgG₄ antibodies that recognize a major 36 kd antigen present in mosquito saliva.

METHODS

Mosquito saliva and WBE

A. communis mosquito larvae were collected and reared as described earlier. ² After the adults emerged, the saliva was collected from the females by placing their mouthparts into small capillary tubes containing 20 µl sterile water. Salivation was induced by topical application of 0.5 µl 0.25% malathion in acetone to the thorax region as described by Boorman. ⁸ After 1 hour, the water with the saliva was collected, pooled, and stored at –70° C before use. WBE was made and stored as described previously. ²

Human sera

IgG subclass and IgE anti-mosquito saliva antibodies were studied in the sera of 12 healthy adults from southern Finland challenged with *A. communis* bites as previously described. ⁹ Eleven of them had immediate (within 15 minutes) skin reaction (mean wheal size = 9.3 mm, SD = 5.3 mm), and all 12 subjects had delayed 24-hour bite papules (mean size = 5.2 mm, SD = 3.7 mm). Pooled sera from five infants aged 6 to 8 months who had never been exposed to mosquito served as controls.

Rabbit sera

Paired serum samples from one rabbit before and after immunization with 60 *A. communis*

mosquito bites were used as a positive control. The animals were maintained according to guidelines of the institutional animal care and use committee. All serum samples were stored at -20°C before examination.

Immunoblotting

Electrophoresis and blotting to nitrocellulose membrane were carried out as described previously, ² except that the sample was diluted 1:2 into an electrophoresis sample buffer, from which 2.9 $\mu\text{l}/\text{mm}$ ² gel was loaded (corresponding to 3.3 μl saliva-water solution per strip). The molecular weights (MWs) of the proteins in mosquito saliva were calculated with the use of 4% to 20% gradient and 10%, 12%, and 16% single-concentration gels. The blotted membrane was cut into 3 mm wide strips. One of the strips was stained for total proteins by biotinylating the transferred proteins and visualizing them by using streptavidin-alkaline phosphatase conjugate and nitro blue tetrazolium 5-bromo-4-chloro-3-indolyl phosphate as substrate (Amsterdam International Ltd., Little Chalfont, Bucks, U.K.).

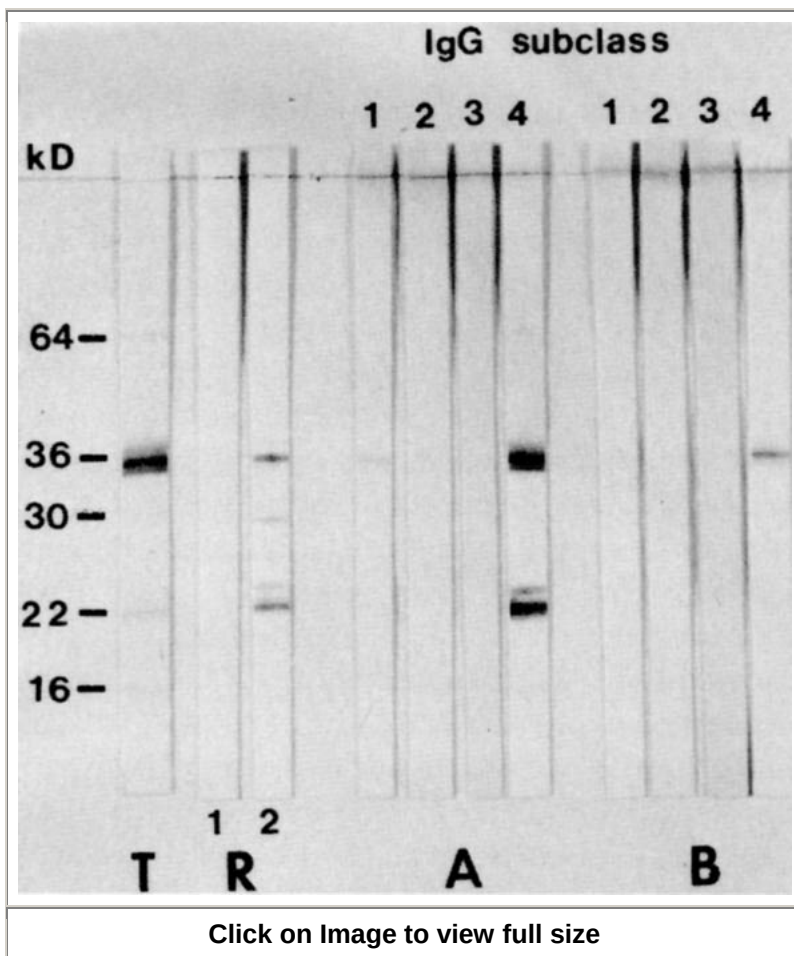
Other strips were washed three times for 5 minutes in 0.1% Tween-Tris-buffered saline (TBS) and incubated for 45 minutes in a blocking buffer containing 5% nonfat milk powder in TBS. Human serum samples (final dilutions: IgG, 1:100; IgG₁, 1:100; IgG₂, 1:50; IgG₃, 1:20; IgG₄, 1:10; and IgE, 1:4) or rabbit serum (final dilution, 1:500) were added and incubated overnight at room temperature with continuous shaking. The strips were washed twice for 1 minute and three times for 5 minutes in 0.1% Tween-TBS. Biotinylated mouse monoclonal anti-human (1:250) IgG subclass antibodies (Zymed, Inc., Camarillo, Calif.), goat anti-human (1:250) IgE antibodies (Kirkegaard & Perry Laboratories, Gaithersburg, Md.), and donkey anti-rabbit (1:250) IgG antibodies (Amersham International Ltd.) were added in 5% milk in TBS and incubated for 30 minutes at room temperature with shaking. The strips were then washed twice for 1 minute and three times for 5 minutes in 0.1% Tween-TBS. Alkaline phosphatase-conjugated streptavidin (1:1000) in TBS was added and incubated for 30 minutes at room temperature. The strips were washed as before, and the substrate (nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate) for alkaline phosphatase was added. The reaction was stopped after 15 minutes by rinsing the blots in tap water.

RESULTS

Proteins in mosquito saliva

Five different protein bands were found in the mosquito saliva (■Fig. 1). The approximate MWs were 16, 22, 30, 36, and 64 kd. Two of them, those weighing 22 kd and 36 kd, seemed to be clusters of two to three bands.

FIG. 1. IgG subclass immunoblots against *A. communis* mosquito saliva in two individuals (A and B). Both show positive reactions in IgG₄ subclass against the 36 kd protein; individual A also had a positive reaction against the 22 kd protein. T, Total protein stain of the saliva; R, paired serum samples from one rabbit before (1) and after (2) immunization with 60 *A. communis* mosquito bites.



IgE antibodies to mosquito saliva proteins

IgE antibodies were directed mostly against the 36 kd (11 of 12) and 22 kd saliva antigens (7 of 12) (Table I). In addition, faint IgE blots against the 64 kd antigen were seen in five serum samples and against 30 kd protein in one sample. Ten of 11 subjects with immediate skin reactivity to *A. communis* bites had IgE anti-mosquito saliva antibodies. Four positive IgE and IgG₄ immunoblots are shown in Fig. 2. No IgE anti-mosquito saliva antibodies were found in the sera of the infants.

FIG. 2. IgE and IgG₄ immunoblots (Subjects: C, D, E, and F) to *A. communis* mosquito saliva. Positive reactions against the 22 kd and the 36 kd proteins can be seen.

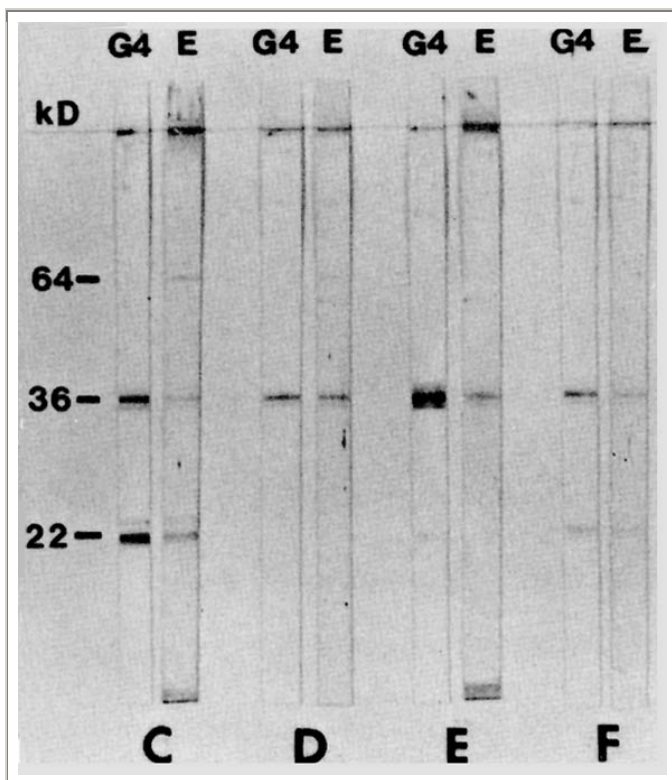


TABLE I. Distribution of mosquito saliva-specific IgG and IgE antibodies in sera from 12 mosquito-exposed subjects

Ig class	<u>Saliva proteins</u>			
	22 kd	30 kd	36 kd	64 kd
IgG ₁	5*	0	8	0
IgG ₂	0	1	0	0
IgG ₃	1	0	2	0
IgG ₄	6	0	11	0
IgE	7	1	11	5

*Number of subjects with positive immunoblot reaction.

IgG antibodies to mosquito saliva proteins

The results of immunoblotting are shown in Table I and Fig. 1. The IgG₄ antibodies were found as frequently as the IgE antibodies against the 36 kd antigen (11 of 12) and 22 kd antigen (6 of 12) (Table I). Other IgG antibodies directed against the 36 kd protein were in IgG₁ (8 of 12) and IgG₃ (2 of 12) subclasses, and against 22 kd protein in IgG₁ (5/12) and IgG₃ (1/12) subclasses. One serum sample showed IgG₂ antibodies against the 30 kd antigen. As a rule, the IgG₄ subclass blots were more intense than those of the other subclasses (Fig. 1). The rabbit serum sample taken after 60 mosquito bites showed IgG anti-mosquito saliva antibodies against the 22, 30, and 36 kd saliva proteins (Fig. 1). No anti-mosquito saliva antibodies in any IgG subclass were found in the sera of the infants.

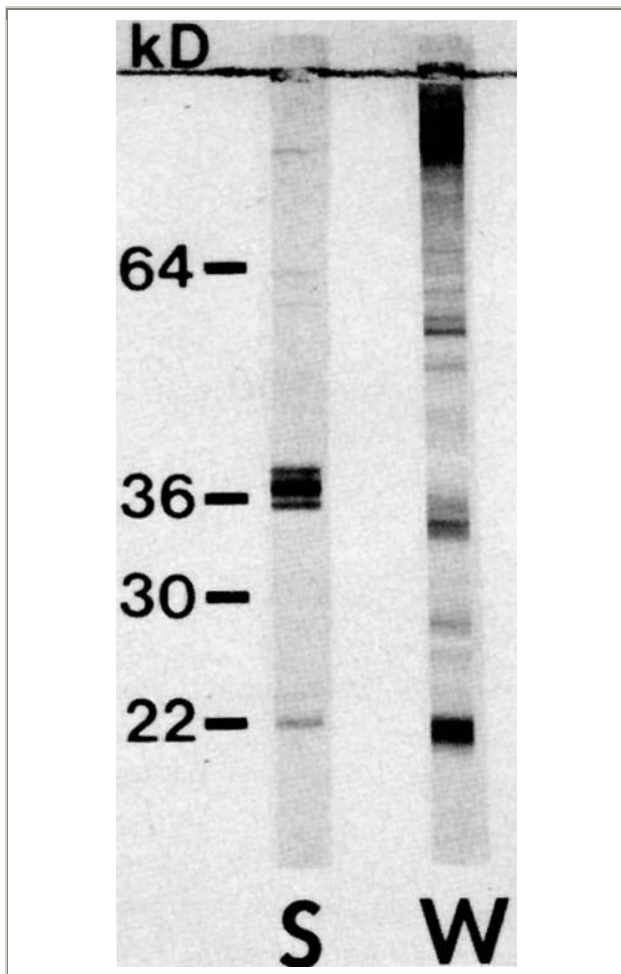
DISCUSSION

We found five proteins with MWs ranging from 16 to 64 kd in the saliva of *A. communis* mosquitoes. Four of these bound IgE and/or IgG₄ from human sera. Ten of the 12 adult subjects exposed to these mosquitoes had IgE and IgG₄ antibodies against 36 kd protein, suggesting that it could be a major saliva antigen of *A. communis* mosquitoes. The presence of anti-mosquito saliva antibodies in almost all examined subjects is not unexpected because *A. communis* mosquitoes are very common in Finland and all persons are seasonally bitten frequently by this mosquito from early childhood. In southern Finland, where our study was done, almost every adult subject reacts to *A. communis* bites with immediate whealing.

Previously, several proteins have been characterized from the salivary glands of different mosquitoes. [10-15](#) Some of these proteins have been shown to be enzymes such as apyrase, [11](#) esterases, [13](#) and α -glucosidase. [10](#) Recently, one female saliva-specific protein from *A. aegypti* with an MW of 37 kd has been cloned. [12](#) The possible relationship between the 37 kd and the 36 kd mosquito saliva-specific proteins and their functions are at present unknown.

Prior attempts to characterize human IgE and IgG antibody response to mosquitoes have been made with either WBE or salivary gland extracts by means of ELISA [5-7](#) or immunoblotting. [3, 4, 16](#) Using immunoblotting, Shen et al. [4](#) found IgG- and IgE-binding antigens in *A. albopictus* mosquito WBE in several subjects studied; however, only one subject also had antibodies against salivary gland extracts. Similarly, in the present study with mosquito saliva as the antigen, we detected four IgG- and IgE-binding proteins but more than 10 IgG binding antigens in WBE (■Fig. 3). The reason for the greater reactivity of human sera against WBE could be that anti-mosquito immune responses are directed to inhaled or ingested insect particles, as well as to mosquito bites. Antibody responses to inhaled insect allergens have been described previously, [17-19](#) and it is possible that some inhalant insect allergens are cross-reactive.

FIG. 3. Human IgG antibody (from one individual) binding to *A. communis* mosquito saliva (S) and WBE (W). Antibodies identify a larger number of proteins from the WBE than from the saliva.



Penneys et al. ³ used immunoblotting and salivary glands from five mosquito species (*Culex nigripalpus*, *C. quinquefasciatus*, *A. taeniorhynchus*, *A. aegypti*, and *Anopheles quadrimaculatus*) and found IgG class antibodies, which reacted against several proteins with MWs ranging from 14 kd to 126 kd. Serum from one of five studied individuals had antibodies against the 36.7 kd protein present in the salivary gland extracts of *C. quinquefasciatus* and *A. taeniorhynchus*. However, the antigen pattern identified from the five human sera studied was unique for each subject, because each individual serum sample recognized both species-specific and species-shared antigens. Using immunoblotting in one subject, Shen et al. ⁴ showed IgG antibodies against *A. albopictus* salivary gland proteins with MWs of 21 to 23, 28 to 29, 31 to 34, 42, 55, 62, and 78 kd. Some of them may be similar to the main antigens found in the present study in the saliva of *A. communis* mosquitoes with apparent MWs of 22 and 36 kd. All 12 subjects examined in the present study had IgG anti-mosquito saliva antibodies belonging mostly to the IgG₄ and IgG₁ subclasses. A similar IgG subclass distribution has been reported in subjects receiving allergen-specific immunotherapy. ^{20, 21}

To our knowledge, pure saliva has not been used previously as antigen in the study of IgE antibodies to mosquito bites, and only one previous immunoblot study demonstrates the existence of IgE anti-mosquito salivary gland antibodies. ⁴ The authors of this study found clear-cut IgE antibody reactions against several salivary gland proteins of *A. albopictus* mosquitoes, with MWs ranging from 24 to 62 kd in one of 15 subjects studied, no reactivity against the 36 kd protein was found. In the present study 10 of the 11 subjects with immediate skin reactions to *A. communis* mosquito bites had IgE class anti-mosquito

saliva antibodies against the 36 kd saliva protein. An interesting finding was that the same 10 subjects also had IgG₄ antibodies that recognized this same protein. Earlier studies have likewise shown coexisting IgE and IgG₄ antibodies directed against the same antigens in patients allergic to pollen and bee venom. ^{20, 22} Interleukin-4 has been shown to upregulate human B-cell IgE and IgG₄ production in vitro, ²³ and house dust mite-specific T helper cell (T_{H2}) clones producing interleukin-4 have been isolated from atopic patients. ²⁴ It would be of interest to evaluate the role of similar antigen-specific T_{H2} cells in the subjects in the present study who had immediate skin reactivity to *A. communis* mosquito saliva 36 and 22 kd antigens.

In summary, we have demonstrated the presence of IgE and IgG₄ antibodies against the 36 kd antigen present in *A. communis* saliva in 10 of 12 subjects exposed to this mosquito species, suggesting that these anti-saliva antibodies may be involved in the pathogenesis of immediate mosquito bite reactions.

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Zhikang Peng, MD^{a,b}, Hongbing Li, RT^a, F. Estelle R. Simons, MD^{a,b}

Winnipeg, Manitoba, Canada

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