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Frequent Occurrence of IgE and IgG4 Antibodies against Saliva of *Aedes communis* and *Aedes aegypti* Mosquitoes in Children

Abstract

We examined the prevalence of IgE and IgG4 class antibodies to the saliva of *Aedes communis* and *Aedes aegypti* mosquitoes in the sera of three groups of exposed children using a sensitive immunoblot method. The frequencies of IgE antibodies to the major 36-kD *A. communis* and *A. aegypti* saliva antigens ranged from 82 to 90% in the 20 Finnish, 17 Kenyan, and 20 Mexican children. The corresponding IgG4 antibody frequencies were 85, 41, and 20%, respectively. The nonexposed 20 Icelandic children did not show IgE or IgG4 antisaliva antibodies. Several of the Finnish children showed also IgE and IgG4 antibodies to a 22-kD *A. communis* saliva antigen. The Finnish children abnormally sensitive to mosquito bites had frequently IgE and IgG4 antibodies to the 22-kD *A. communis* saliva antigen, suggesting that these antibodies play a role in the pathogenesis of immediate cutaneous mosquito bite reactions. In contrast to this, no increase was found in the *A. aegypti* antibody frequencies in the Kenyan and Mexican children with papular urticaria, suggesting that humoral immune response to *A. aegypti* saliva is not involved in the development of this disorder. The present results show that humoral IgE and IgG4 immune responses to *Aedes* mosquito saliva antigens is common in children living both in temperate and tropical zones. The IgE antibodies seem to be involved in the immediate mosquito bite whealing, and the occurrence of the IgG4 subclass antisaliva antibodies might be an indicator of intense mosquito bite exposure.

Key Words

IgE antibodies
IgG4 antibodies
Immunoblotting
Mosquito saliva
Type I reactions, children

Introduction

Immediate hypersensitivity reactions to bee and wasp stings are common in many countries. Allergic patients have IgE antibodies against the venom proteins, and high IgG4 antibody levels are found especially after desensitization treatment [1, 2]. Mosquitoes, too, cause frequently harmful bite reactions such as immediate wheals and delayed bite papules [3–5]. In children, a condition called papular urtica-

ria is common, especially in tropical countries, and seems to be caused by insect bites [6]. In contrast to bee and wasp sting allergy, little is known about the immune response against mosquito bites. Experimental evidence suggests that people exposed for the first time to *Aedes aegypti* mosquitoes are first nonreactive, but after repeated bites appears a delayed and then an immediate cutaneous reaction, and finally after long-lasting bite exposure a stage of nonreactivity, i.e., tolerance, could be achieved [4]. Several serological

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studies have found IgG class mosquito antibodies, providing evidence that also a humoral immune response occurs against mosquito bites [7–11]. However, the specificity of these antibodies is not well known because whole-body or salivary gland extracts, but not pure saliva, have been used as mosquito antigens. Recently, we developed a sensitive immunoblot method to measure mosquito saliva specific antibodies [see ref. 13]. We used saliva from *Aedes communis* mosquito, the most frequent man-biting mosquito in southern Finland [12], and found that almost all examined adults with cutaneous bite reactions had IgE and IgG4 antibodies against the 36- and 22-kD saliva antigens [13].

In the present study, we examined saliva-specific IgE and IgG4 antibodies to *Aedes* mosquitoes in four groups of children from Europe, Africa, and Central America to determine the prevalence of these antibodies. To examine whether these mosquito antibodies would be of clinical importance, we compared antibody patterns between the mosquito bite sensitive and nonsensitive Finnish children and also between the Kenyan and Mexican children with and without papular urticaria.

Subjects and Methods

Subjects

Sera were collected from four groups of children. These were 20 Finnish children (mean age 6.1, range 0.5–12 years) from Tampere, 17 Kenyan children (mean age 8.2, range 0.5–16 years) from Nairobi, 20 Mexican children (mean age 3.3, range 1–6 years) from Mexico City, and 20 Icelandic children (mean age 11.3, range 6–15 years). The sera were transported to Finland in dry ice and stored at -20°C until used for immunoblotting.

Thirteen of the Finnish children were classified as abnormally sensitive to mosquito bites because they had experienced large immediate wheals (diameter at 15 min ≥ 10 mm) and/or delayed cutaneous bite lesions (diameter at 24 h ≥ 20 mm). Sera were collected also from 7 children in whom the mosquito bite lesion was small (≤ 5 mm at 15 min and 24 h) or absent. These children were termed nonsensitive. The ubiquitous man-biting mosquito in southern Finland where the children lived is *A. communis* [12]. None of the Finnish children had visited any subtropical or tropical country and, therefore, had not been exposed to the bites of *A. aegypti* [14].

The Kenyan and 10 Mexican children suffered from papular urticaria due to which they had consulted either a dermatologist or a pediatrician. These children lived in the suburbs of Nairobi or Mexico City or in the surrounding rural areas. Mosquitoes or other insect bites were suspected to have caused their papular urticaria. The remaining 7 Kenyan and 10 Mexican children had no history of abnormal insect bite reactions or papular urticaria.

All 20 Icelandic children were healthy, most of them lived in rural areas, and their serum had been collected to control the efficacy of vaccinations. These children had not been abroad, and, because no blood-feeding mosquitoes exist in Iceland [15], their sera served as a control for the antibody studies.

Mosquitoes

A. communis mosquitoes were raised from larvae collected and reared as described earlier [11]. After adults had emerged, the saliva was collected from the females by placing their mouth parts in capillary tubes containing water. Salivation was induced by malathion as previously described [13].

A. aegypti mosquitoes were derived from strains reared for many years in the Laboratory of Protozoology, Institute of Tropical Medicine, Antwerp, Belgium. Newly hatched adult mosquitoes were transported to Finland where saliva was collected and stored as that of *A. communis*.

Immunoblotting for Antisaliva Antibodies

Mosquito saliva samples diluted 1:2 in a buffer were electrophoresed in sodium dodecyl sulfate-polyacrylamide gels, transferred into polyvinylidene difluoride membranes, and electroblotted as previously described [13]. Each gel was loaded with $2.9 \mu\text{l}/\text{mm}^2$ mosquito saliva. Strips were incubated with serum samples (diluted 1:4 for IgE and 1:10 for IgG4) and biotinylated goat antihuman IgE (diluted 1:250; Kirkegaard & Perry) and mouse monoclonal antihuman IgG and IgG4 (diluted 1:250; Zymed) antibodies. The strips were then stained with alkaline phosphate conjugated streptavidin (diluted 1:1,000) and its substrate (Amersham, UK) as earlier described [13].

The Finnish and Icelandic sera were examined for *A. communis* antibodies and those from Kenyan and Mexican children for *A. aegypti* antibodies. Positive controls included a reference IgE serum from a Finnish mosquito-allergic patient and polyclonal IgG antiserum from a rabbit immunized with *A. communis* bites [11, 13]. Immunoblot reactions in the Icelandic children were used for determination of cutoff limits for positivity. Only reactions that were clearly more intense than those seen in a few Icelandic sera with faint bands were regarded as positive.

Results

Prevalence of Antisaliva Antibodies

The IgE and IgG4 antibodies frequencies in the different groups of children are compiled in tables 1 and 2. IgE and IgG4 antibodies to *A. communis* saliva 36-kD antigen were found in 85% of the Finnish children (fig. 1, 2). The frequencies of IgE antibodies to *A. aegypti* 36-kD saliva antigen were similarly high (82–90%) in the Kenyan and Mexican children, whereas the frequencies of IgG4 antibodies were lower (41%) in the Kenyan and low (20%) in the Mexican children (fig. 3, 4). Some of the immunoblots from Icelandic children showed faint IgE and IgG4 staining to *A. communis* 36- and 30-kD saliva antigens. A cluster of bands with molecular weights from 60 to 65 kD was seen in almost all Finnish and Icelandic sera studied against *A. communis* saliva.

A 22-kD antigen was found only in *A. communis* saliva (fig. 1, 2). In the Finnish children, the frequencies of IgE and IgG4 antibodies against the 22-kD antigen in *A. communis* saliva were 45 and 75%, respectively (table 1). *A. aegypti*

Fig. 1. Immunoblotting showing IgE antibodies to 36-, 30-, and 22-kD *A. communis* saliva antigens. The mosquito-sensitive Finnish children (lanes 1–5) have more intense bands than the nonsensitive children (lanes 6–10). Serum samples from the nonexposed Icelandic children (lanes 11–15) are shown for comparison. C = Control serum from a rabbit immunized with *A. communis* bites.

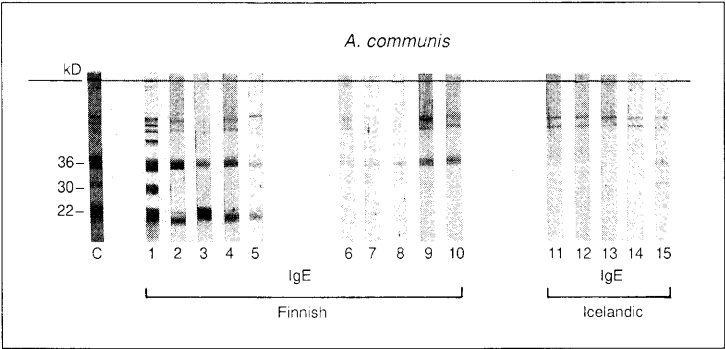


Fig. 2. Immunoblotting showing IgG4 antibodies to 36-, 30-, and 22-kD *A. communis* saliva antigens. The serum samples and symbols are the same as in figure 1.

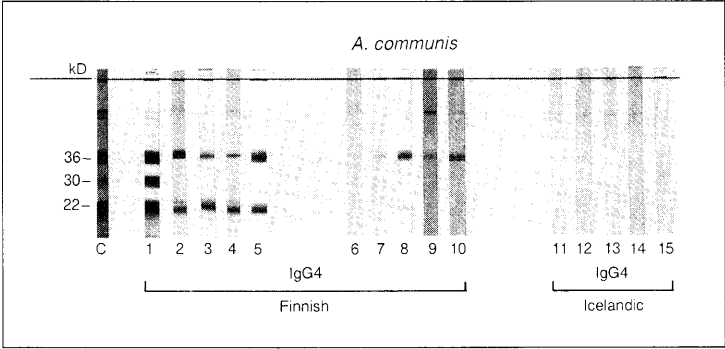
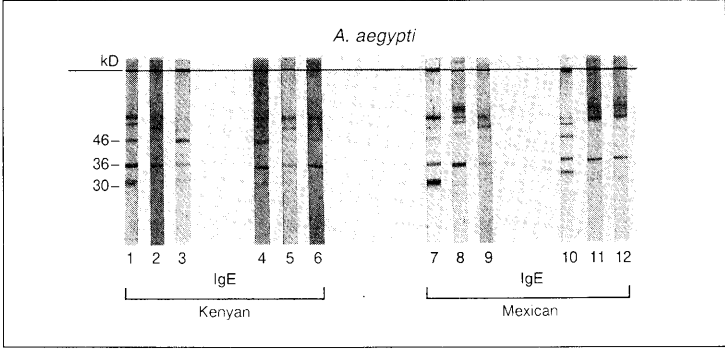


Fig. 3. Immunoblotting showing IgE antibodies to 46-, 36-, and 30-kD *A. aegypti* saliva antigens in the Kenyan and Mexican children. Serum samples are from children with (lanes 1–3 and 7–9) and without (lanes 4–6 and 10–12) papular urticaria.



saliva 30-kD IgE antibodies were found in 3 (10%) Kenyan and 4 (20%) Mexican children (table 2). One Mexican child had also IgG4 antibodies to the 30-kD antigen (fig. 4).

Antisaliva Antibodies in Mosquito Bite Sensitive and Nonsensitive Finnish Children

All 13 Finnish mosquito-sensitive and 4 of the 7 (60%) mosquito nonsensitive children had IgE and IgG4 antibod-

ies to *A. communis* 36-kD saliva antigen (table 3). Eight of the 13 (62%) sensitive children had IgE, and all had IgG4 22-kD saliva antibodies in contrast to only 2 (29%) non-sensitive subjects (table 3). The 36-kD antibody bands were somewhat more intense than the 22-kD bands, and the sensitive children had as rule more intense bands than the non-sensitive children (fig. 1, 2).

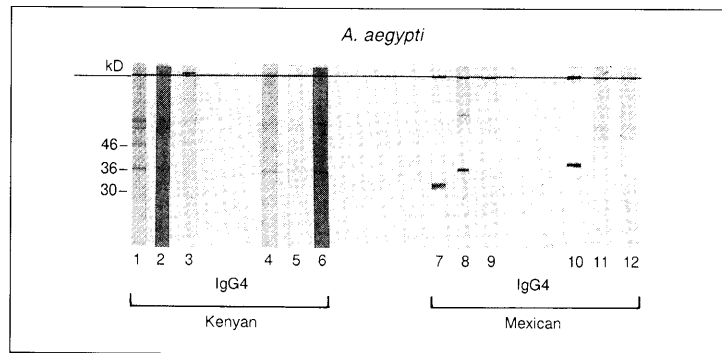


Fig. 4. Immunoblotting showing IgG4 antibodies to 46-, 36-, and 30-kD *A. aegypti* saliva antigens in the Kenyan and Mexican children. The serum samples are the same as those shown in figure 3.

Table 1. Frequency of *A. communis* mosquito saliva specific IgE and IgG4 antibodies in the Finnish and Icelandic children

Saliva antigen kD	Finnish children (n = 20)		Icelandic children (n = 19)	
	IgE	IgG4	IgE	IgG4
36	17 (85%)	17 (85%)	0	0
30	1 (5%)	1 (5%)	0	0
22	9 (45%)	15 (75%)	0	0

Table 2. Frequency of *A. aegypti* mosquito saliva specific IgE and IgG4 antibodies in the Kenyan and Mexican children

Saliva antigen kD	Kenyan children (n = 17)		Mexican children (n = 20)	
	IgE	IgG4	IgE	IgG4
46	4 (24%)	3 (15%)	3 (15%)	0
36	14 (82%)	7 (41%)	18 (90%)	4 (20%)
30	3 (18%)	0	4 (20%)	1 (5%)

Antisaliva Antibodies in the Kenyan and Mexican Children with Papular Urticaria

The antibody patterns to *A. aegypti* 30-kD saliva antigen in the Kenyan and Mexican children with and without papular urticaria are shown in table 4. No clear difference have been found between affected and nonaffected children either in the frequency of the antibodies or in the intensity of the immunoblots. The only possible difference was that the children with papular urticaria had somewhat lower frequencies of IgG4 antibodies than the nonaffected children (table 4).

Table 3. Frequency of *A. communis* saliva antibodies in the Finnish mosquito-sensitive and mosquito-nonsensitive children

Saliva antigen kD	Sensitive children (n = 13)		Nonsensitive children (n = 7)	
	IgE	IgG4	IgE	IgG4
36	13 (100%)	13 (100%)	4 (57%)	4 (57%)
30	1 (8%)	1 (8%)	0	0
22	8 (62%)	13 (100%)	1 (14%)	2 (29%)

Table 4. Frequency of *A. aegypti* 36-kD saliva antibodies in the Kenyan and Mexican children with and without papular urticaria (PU)

	Kenyan children				Mexican children			
	PU (n = 10)		no PU (n = 7)		PU (n = 10)		no PU (n = 10)	
	IgE	IgG4	IgE	IgG4	IgE	IgG4	IgE	IgG4
Frequency	8 (80%)	5 (50%)	6 (86%)	2 (29%)	8 (80%)	1 (10%)	10 (100%)	3 (30%)

Discussion

In our previous study [13] we showed the presence of IgE and IgG4 antibodies to *A. communis* saliva in sera from most of the examined adult Finns with cutaneous mosquito bite reactions. In the present study, we examined children reacting to *A. communis* bites and could confirm the frequent occurrence of these antibodies and especially of the antibodies reacting against the 36-kD saliva antigen. The fact that these antibodies were not found in Finnish infants in our previous study [13] shows that humoral IgE and IgG4 antisaliva antibody responses seem to be a specific phenomenon starting early in childhood. In southern Finland young adults are exposed to a variable amount of bites in a 3–4 month long mosquito season, and more than 100 bites can be achieved within 1 month [16]. All but 1 of the Finnish children in the present study had been exposed to mosquitoes in more than one mosquito season, and their sera were collected after the mosquito season during October and November 1992. Most of the children showed at this time IgE and IgG4 class antibodies to *A. communis* saliva antigens. Therefore, humoral IgE and IgG4 antibody responses to mosquito bites seem to resemble those known to occur in the pollen season in subjects with hay fever. The finding of weak reactivities to *A. communis* 36- and 30-kD saliva antigens in some of the nonexposed Icelandic children and the cluster of 60 to 65-kD antigens seen in almost all Finnish and Icelandic sera could be due to cross-reactivity with other insect antigens acquired by alimentary or inhalant route [17].

IgE antibodies to *A. aegypti* saliva 36-kD antigen were found in the Kenyan and Mexican children in the same frequency as *A. communis* antibodies in the Finnish children. The corresponding frequencies of IgG4 antibodies were somewhat lower in the Kenyan and clearly lower in the Mexican children. *A. aegypti* mosquitoes are common in tropical countries, but an altitude of 6,000–8,000 ft is likely to limit their distribution [14]. However, we do not know how common *A. aegypti* or related species are in Nairobi or Mexico City areas and how frequently children are bitten there by these mosquitoes. Yet, the present finding of a high prevalence of IgE antisaliva antibodies shows that exposure seems to be rather general. In contrast to IgE antibodies, the IgG4 class *A. aegypti* mosquito antibodies were rather infrequent. It would be interesting to know whether these antibodies are more prevalent in people living at lower altitudes in tropical or subtropical areas where *A. aegypti* exposure would be more common than in the areas where the present children were living. Previous Korean [9] and Japanese [10] studies have suggested that IgG antibodies to *Aedes albop-*

ictus are common among people living in these countries. However, in these studies pure saliva was not used as mosquito antigen, nor were IgG subclasses examined. Therefore, the results cannot be compared with the antibody frequencies found in the present study.

Various insect bites are considered a major cause of papular urticaria [6]. In Nairobi 22% of the children visiting a dermatologist suffer papular urticaria [18], and this condition is common also in the Mexico City area [19]. We, therefore, compared the IgE and IgG4 mosquito saliva antibody patterns in the Kenyan and Mexican children with and without papular urticaria. However, we did not find any differences supporting the explanation that their papular urticaria was not associated with *A. aegypti* antisaliva antibodies. IgE antibodies cause, e.g. in bee and wasp sting allergy, cutaneous whealing and edema and also immediate systemic symptoms by releasing histamine and other mediators from the mast cells. Eleven of the present Finnish mosquito-sensitive children exhibited wheals > 5 mm, and 1 of them also had immediate systemic symptoms from mosquito bites. In agreement with their clinical history, we could show in the sera of most of these children IgE and also IgG4 antibodies to *A. communis* saliva antigens. These antisaliva antibodies were found also at a lower frequency in the sera from mosquito-nonsensitive Finnish children. It should, however, be remembered that, although these children were classified as mosquito nonsensitive, 5 of the 7 reacted with small wheals to the bites. The bands in the immunoblots seemed also to be more intense in the mosquito-sensitive than in the mosquito-nonsensitive children, also supporting the contention that the IgE antisaliva antibodies mediate cutaneous whealing after mosquito bites. The role of the IgG4 antisaliva antibodies is more obscure, but, as in Hymenoptera or pollen allergy [1, 20], the presence of high levels of this antibody subclass could be a marker of prolonged natural immunization to mosquito bites. The low frequency of *A. aegypti* saliva IgG4 antibodies in the Mexican children would indicate a low exposure level, whereas the more frequent occurrence of these antibodies in the Kenyan children would then reflect a higher exposure rate. The prevalence of IgG4 antibodies to various mosquito species should be examined in highly exposed populations. If the correlation between the increased IgG4 antisaliva antibody levels and a high rate of mosquito bite exposure can be confirmed, the IgG4 antibody measurements could be used to study how frequently people have been exposed to certain, e.g., disease-transmitting mosquito species. On the other hand, it would be interesting to know whether mosquito antisaliva antibodies play any role in malaria, yellow fever, or other disease transmission. The mosquito bite tissue reaction caused by IgE

antisaliva antibodies with subsequent accumulation of inflammatory cells such as eosinophils could be harmful to the disease agents injected into the skin with mosquito saliva [21].

In conclusion, the frequent occurrence of IgE and IgG4 antisaliva antibodies in the children from various geographical areas confirms that a humoral immune response to *Aedes* mosquito bites is a ubiquitous phenomenon. The present findings suggest that a 36-kD protein is a major antigen in both *A. communis* and *A. aegypti* saliva. Moreover, a 22-kD antigen seems to be specific to *A. communis* saliva, and antibodies to this protein are found especially in Finnish children exhibiting large cutaneous bite reactions.

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