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Seasonal Increase in Human IgE and IgG4 Antisaliva Antibodies to *Aedes* Mosquito Bites

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Abstract

Background: Mosquito bite-sensitive subjects frequently have circulating IgE and IgG4 antibodies to *Aedes* mosquito saliva proteins. **Methods:** In the present study we examined the antibody response during a mosquito season in 14 subjects living in Finnish Lapland. Immunoblotting was performed with *Aedes communis* saliva and the 22- and 36-kD antial saliva antibody bands were analyzed. **Results:** The preseason sera showed IgE antibodies to the main saliva antigens in 12, IgG4 antibodies in all 14 and IgG1 antibodies in 12 subjects, and the post-season sera in all but 1 subject. The postseason sera showed significantly more intense IgE ($p < 0.05$), IgG4 ($p < 0.001$) and IgG1 ($p < 0.01$) antibody bands than the preseason sera. **Conclusion:** These results show that seasonal exposure to mosquito bites leads to an increased IgE, IgG4 and IgG1 antibody response, a phenomenon similar to that occurring e.g. in pollen allergy.

Key Words

Mosquito allergy
Immunoblotting
Saliva
IgE antibodies
IgG antibodies

Introduction

Mosquitoes are a common nuisance in many areas of the world by causing harmful cutaneous bite reactions. These range from immediate wheal-and-flare reactions to delayed bite papules, and it seems that most people exposed to mosquito bites start to react early in childhood [1]. When mosquitoes feed, they inject saliva into the skin, and experimental evidence in rabbits and man suggests that bite reactions are due to specific sensitization to mosquito saliva [2–4]. Recent immunoblot studies have demonstrated a frequent occurrence of IgE and IgG4 antibodies to *Aedes communis* saliva proteins in Finnish children and adults [1, 5]. Similar results have been obtained in Canada when examining IgE and IgG antibodies to *Aedes vexans* salivary glands [6, 7]. *Aedes* mosquitoes can appear in huge numbers in Scandinavian countries and North America and the bite reactions tend to be more severe at the onset of the mosquito season [4, 7]. However, it is not known whether mosquito antibod-

ies show a seasonal fluctuation similar to that reported e.g. in pollen allergy [8, 9].

In the present study we collected pre- and postseason serum samples from people living in Lapland and examined the occurrence of IgE and IgG subclass antibodies to the main saliva antigens of *A. communis*.

Materials and Methods

Subjects

Fourteen adult volunteers (13 males and 1 female; mean age 38.4 years, range 19–47 years) from the personnel of the Frontier Guard District of Finnish Lapland were chosen for the study. Two subjects had allergic rhinitis and 1 had asthma. All subjects had lived in Lapland since childhood and their profession in the frontier guard involved regular outdoor activities. Serum samples were collected before the onset of the mosquito season in April to May and postseasonally in late August. At the onset of the mosquito season in June the subjects were exposed to the bites of a local *Aedes* mosquito species collected in the vicinity. The diameter of the bite lesion was measured

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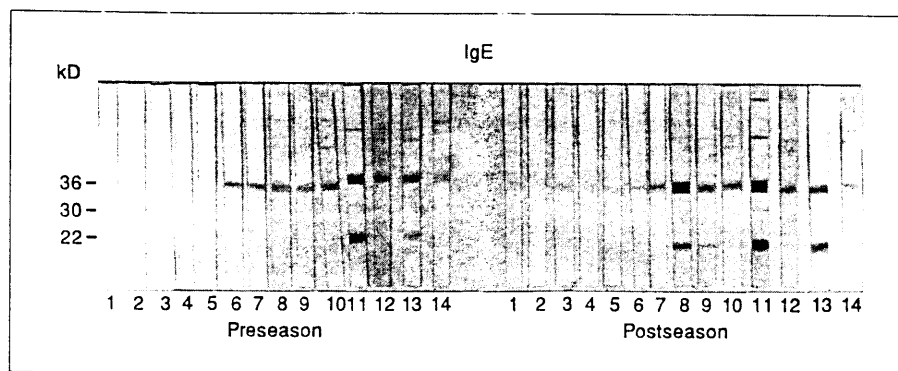
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Fig. 1. IgE immunoblots of antisaliva antibodies to *A. communis* in the pre- and post-season sera of 14 subjects. The post-season blots show more intense 36- and 22-kD antibody bands.



at 15 min and at 24 h as previously described [10, 11]. Eight subjects had small 15-min bite reactions; the mean diameter was 1.0 mm (range 0–3.5 mm) and that of histamine (10 mg/ml) prick 3.6 mm (range 3–4 mm). Six subjects had larger bite reactions; the mean diameter was 7.1 mm (range 6–8 mm) and that of histamine prick 3.4 mm (range 2–5 mm). The mean diameter of the 24-hour bite reactions was in the first group 0.5 mm (range 0–3 mm) and in the second group 2.7 mm (range 0–10 mm). During the study period the subjects received an estimate of at least 500 mosquito bites.

Immunoblotting

The pre- and post-season serum samples were examined for IgE class and IgG subclass antibodies using an immunoblot technique as previously described [5, 10]. Briefly, sodium dodecyl sulfate-polyacrylamide gels were loaded with 2.9 $\mu\text{g}/\text{mm}^2$ of *A. communis* mosquito saliva (protein concentration 100 $\mu\text{g}/\text{ml}$) diluted 1:2 in a sample buffer and electrophoresed at 200 V for 45 min. The antigens were electroblotted (1 h at 40 V, 1 h at 100 V) to polyvinylidene difluoride membranes which were cut into 3-mm strips and incubated overnight with the serum samples diluted 1:100 for IgG1, 1:50 for IgG2, 1:20 for IgG3, 1:10 for IgG4 and 1:4 for IgE. The strips were then incubated with biotinylated mouse monoclonal anti-human IgG subclass antibodies (Zymed, diluted 1:250) and biotinylated goat anti-human IgE antibodies (Vector, diluted 1:250) and stained with alkaline phosphatase conjugated to streptavidin (diluted 1:1,000) and its substrate. Serum from a rabbit immunized with *A. communis* mosquito bites [3] and serum from a child with intense IgE and IgG4 antibody bands were used as positive controls [11]. As a negative control immunoblotting was performed without human serum [10]. To examine the sensitivity of the immunoblot method, one serum was further diluted 2-fold up to 1:64 for IgE and up to 1:640 for IgG4. The specificity of the IgE binding was examined by an inhibition experiment where 1:32 diluted serum was incubated with 50 μg of saliva antigen for 1 h at room temperature before immunoblotting.

The pre- and post-season serum samples of each subject were always examined in the same immunoblot experiment. The intensities of the 36- and 22-kD antibody bands were analyzed by a densitometric method using a scanner (UMAX PowerLook II) with a ScanPack 2.0 program (Biometra, Biomedizinische Analytik, Göttingen, Germany). This calculated a numeric value for each band by analyzing the mean intensity of staining.

Statistics

The differences in the intensities of the antibody bands between the pre- and post-season sera were analyzed by the paired t test.

Results

IgE Antibodies

Twelve subjects showed IgE antibodies to the 36-kD saliva antigen in the pre-season and all 14 subjects in the post-season sera (fig. 1). Three subjects had antibodies to the 22-kD saliva antigen in the pre-season and 6 in the post-season sera. When the intensities of the IgE antibody bands in the pre- and post-season sera were compared, a significant increase ($p < 0.05$) was observed in the intensities of the 36- and the 22-kD antibody bands (fig. 2, table 1). The intensities of the 36- and 22-kD IgE antibody bands did not differ between the 8 subjects with small and the 6 subjects with large immediate bite reactions. The mean intensity values of the 36-kD bands in the pre-season sera were 1,457 and 2,311 ($p = 0.17$), and those of the 22-kD bands 0 and 1,142 ($p = 0.09$), respectively.

IgG Antibodies

IgG antibodies to the 36-kD saliva antigen were frequent in the pre-season sera. All 14 subjects had IgG4 (fig. 3), 12 IgG1, 10 IgG2 and 6 IgG3 antibodies to this antigen. The post-season sera showed IgG4 antibodies to the 36-kD antigen in all 14 subjects, IgG1 antibodies in 13, and IgG2 and IgG3 antibodies in 10 subjects. The pre-season sera showed IgG4 antibodies to the 22-kD antigen in 9 and IgG1 antibodies in 1 subject, and the post-season sera in 12 and 9 subjects, respectively. In addition, 1 subject developed IgG2 and IgG3 antibodies.

When comparing the intensities of the IgG4 antibody bands in the pre- and post-season serum samples, the increase was highly significant ($p < 0.001$) in the 36- and 22-kD antibody bands (fig. 4, table 1). The IgG1 bands showed also significant changes in the 36-kD ($p < 0.001$) and 22-kD ($p < 0.01$) antibody bands. In contrast, no significant changes were seen in the IgG2 and IgG3 antibodies.

Fig. 2. Densitometric analysis of individual IgE immunoblots of antisaliva antibodies to *A. communis* in the pre- and postseason sera.

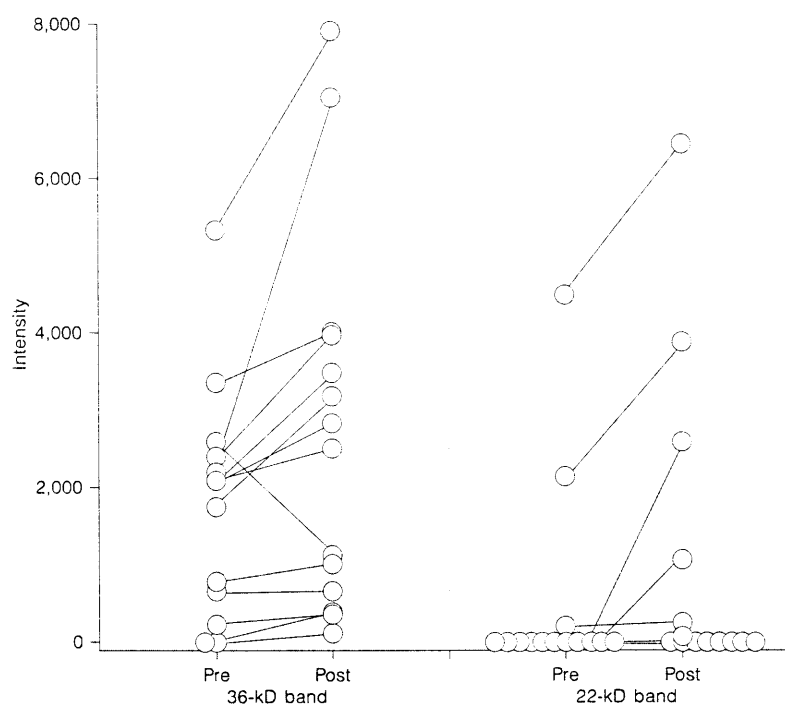


Table 1. Pre- and postseason intensities of IgE and IgG antibody bands to *A. communis* 36- and 22-kD saliva antigens in the sera of 14 subjects

Antibodies	Preseason	Postseason	p value ¹
<i>IgE</i>			
36 kD	1,823±1,453*	2,757±2,435	0.016
22 kD	490±1,238	1,023±1,967	0.024
<i>IgG4</i>			
36 kD	5,563±2,615	8,447±1,806	0.000076
22 kD	1,654±2,173	2,460±2,751	0.000074
<i>IgG1</i>			
36 kD	1,233±1,837	2,393±2,143	0.00055
22 kD	176±658	686±1,129	0.0026
<i>IgG2</i>			
36 kD	855±1,233	1,068±369	0.20
22 kD	0±0	125±469	0.17
<i>IgG3</i>			
36 kD	1,011±1,393	1,066±1,129	0.39
22 kD	0±0	14±53	0.17

* The intensities of the antibody bands in the immunoblots were analyzed by a densitometric technique. Values represent mean ± SD.

¹ Paired t test.

Control Experiments

When a control was performed without human serum, immunoblotting was always negative for IgE and IgG1–4 antibodies. The serum dilution experiments showed that the intensities of the IgE antibody bands decreased in a dose-dependent way. The 36-kD IgE band decreased 100% (from 2,192 in 1:4 dilution to 0 in 1:64 dilution) and the 22-kD IgE band 90% (from 2,980 in 1:4 dilution to 295 in 1:64 dilution). The corresponding decrease of the 36-kD IgG4 band was 95% (from 6,524 in 1:10 dilution to 298 in 1:640 dilution), and the decrease of the 22-kD IgG4 band 99% (from 7,976 in 1:10 to 34 in 1:640).

The inhibition experiment confirmed the specificity of IgE binding. The 36-kD IgE antibody band (intensity value 609) and the 22-kD IgE antibody band (intensity value 948) disappeared totally when the serum was preincubated with the saliva antigen.

Discussion

Our previous immunoblot study showed that after 40–60 *A. communis* bites rabbits developed IgG class antimosquito antibodies together with papular skin reactions [3]. These antibodies which were mainly against the 22-kD and 36-kD

Fig. 3. IgG4 immunoblots of antisaliva antibodies to *A. communis* in the pre- and postseason sera of 14 subjects. The 36- and 22-kD antibody bands in the postseason blots are more intense than those observed in the IgE blots.

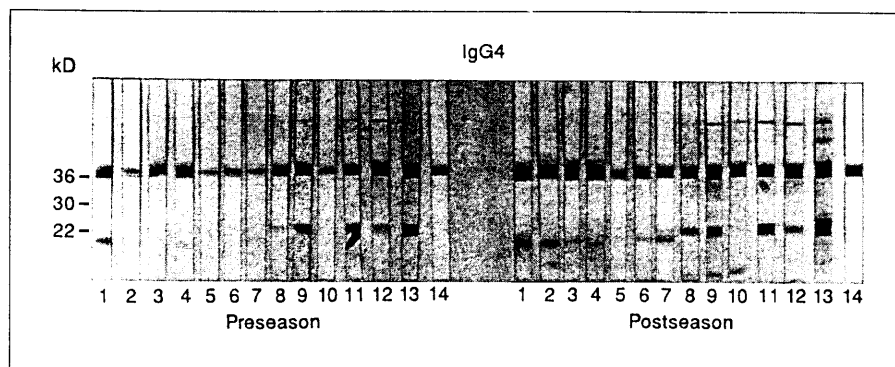
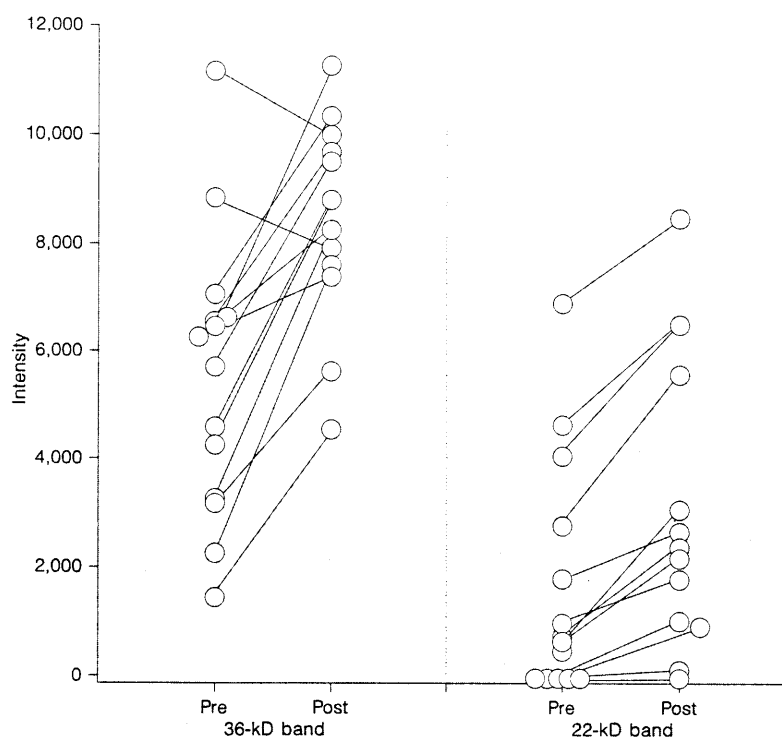


Fig. 4. Densitometric analysis of individual IgG4 immunoblots of antisaliva antibodies to *A. communis* in the pre- and postseason sera.



saliva antigens disappeared when bite exposure was discontinued but reappeared in the following year after 20 mosquito bites. In agreement with these results, we have shown a frequent occurrence of IgE and IgG4 antibodies to the 22- and 36-kD saliva antigens in mosquito bite-sensitive children and adults in southern Finland [1, 5]. In the present study we examined whether IgE and IgG4 antisaliva antibodies show a seasonal response to mosquito bites. During the 3-month mosquito season the present subjects were exposed to hundreds of mosquito bites. The major man-biting mosquito in Lapland is *Aedes punctor* [12], but we used the saliva of *A. communis* in immunoblotting because the saliva antigens of these two species are cross-reactive [13].

In accordance with our observation in subjects living in southern Finland we demonstrated the frequent occurrence of IgE and IgG4 antisaliva antibodies in the preseason sera and, in addition, we found that IgG1 antibodies were common. The comparison of the pre- and postseason sera by densitometric analysis disclosed significant increases in the intensities of the IgE, IgG4 and IgG1 antibody bands to the main antigens in *A. communis* saliva. This obviously indicates that a seasonal exposure to *Aedes* mosquito bites causes an increase in the IgE and IgG4 antibody production similar to that observed during the pollen season in patients with allergic rhinitis or asthma [8, 9]. Unfortunately, no follow-up samples were available from our subjects in the win-

ter after the mosquito season. It can be expected, however, that as in pollen allergy [8, 9] the antisaliva antibody levels would decrease during the winter months and then increase again in the following mosquito season.

The finding that the increase in the intensity of the antisaliva antibody bands seemed to be more prominent in the IgG4 than in the IgE antibody class deserves a comment. The present subjects had been heavily exposed to mosquitoes when living in Lapland and 8 of them had no or rather small immediate and delayed bite reactions. They could thus represent people who are achieving tolerance to the bites after massive mosquito exposure [4, 14]. In agreement with this, high levels of IgG4 and low levels of IgE antibodies can be observed after successful immune therapy in pollen and bee venom allergy [15, 16].

It seems that the present immunoblotting and densitometric analysis is useful when evaluating changes in the IgG class antibodies. However, it may not be sensitive enough to recognize subtle changes in the IgE antibody levels. Supporting this view, we could not find any significant correlation between the intensities of the 36-kD and 22-kD IgE antibody bands and the size of the immediate mosquito bite reactions in the present subjects with small or large mosquito bite reactions. However, a larger series should be investigated before final conclusions can be drawn. A better method to measure IgE antisaliva antibodies seems to be an IgE capture ELISA, which in 27 Finnish mosquito bite-sensitive children showed a good correlation between IgE antisaliva antibody levels and the size of immediate bite reactions [17]. Similarly, Peng et al. [7] in Canada observed with ELISA that subjects with immediate bite reactions had

increased levels of IgE antibodies to *A. vexans* salivary gland antigens. The obvious benefit of the present immunoblotting and densitometric analysis is, however, that the IgE antibody response can be studied separately against several antigens such as the 22- and 36-kD saliva proteins.

In the present study we found significant changes in the antisaliva IgE, IgG4 and IgG1 antibodies, but no marked responses in the IgG2 or IgG3 subclasses. The simultaneous production of antisaliva IgE and IgG4 antibodies is most likely explained by the effects of IL-4 and IL-13 on B cells [18]. It is also known that immune therapy favours the production of IgG4 and IgG1 antibodies and that prolonged immunization leads to a shift in the IgG4/IgG1 ratio [19, 20]. In the present study the IgG4 antisaliva antibody response was more prominent than the IgG1 response, a finding that could be due to the massive bite exposure the study subjects experienced during the mosquito season.

In conclusion, the present study documented that a short but massive mosquito bite exposure causes an increase in the antisaliva IgE, IgG4 and IgG1 antibody production in man. The antibody responses to mosquito bites resemble those observed in other seasonal allergies and thus mosquito bites may offer a model for natural immune therapy.

Acknowledgements

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