

Anti-IgE autoantibodies and bee-sting allergy

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Serum anti-IgE autoantibodies (aaIgE) were investigated by dot immunobinding assay in bee-allergic patients in comparison with nonallergic beekeepers, healthy blood donors, and atopic subjects. Elevated serum levels of aaIgE – either free or combined with IgE – were found in both bee-allergic patients and atopic subjects as compared with beekeepers and healthy donors. With regard to a possible significance of aaIgE for the protective mechanism induced by specific allergen immunotherapy, we estimated aaIgE in bee-allergic patients before, during, and after bee-venom immunotherapy (BVIT) in relation to the outcome of a provocation test with a living bee (PT). During the first year of BVIT, there was no significant change in either free or combined aaIgE, but aaIgE decreased during protracted BVIT over 3–7 years. By using two monoclonal anti-IgE antibodies with different epitope specificity (Le27 and BSW17), we were able to detect two kinds of IgE/aaIgE immune complexes. Our data show that during and after BVIT the levels of one kind of IgE-aaIgE immune complex (the non-BSW17 type) tended to decrease in PT-negative patients but stayed elevated in PT-positive patients. The levels of the other kind of immune complex (the non-Le27 type) were similar in treatment failures and successfully treated patients. These data suggest that there are various kinds of aaIgE with different *in vivo* functions related to their epitope specificity. Some of them (non-BSW17 type) might be associated with BVIT failure.

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Anti-IgE autoantibodies (aaIgE) have been detected in sera of patients with allergic diseases such as asthma, allergic rhinitis, atopic dermatitis, and hay fever, as well as patients with hyper-IgE syndrome and allergic arthralgia (1, 2, 5, 13, 14, 20–24, 26, 29, 31). Most of the above reports showed that a large proportion of human IgE was present in the form of immune complexes with aaIgE. Several *in vitro* studies suggest that these autoantibodies can trigger histamine release from human basophils and tissue mast cells (4, 10, 14). They can also recognize membrane IgE of B_e cells, induce a cytotoxic reaction (3), and suppress IgE synthesis by human B cells (25, 27). On the basis of *in vitro* studies using different monoclonal anti-IgE antibodies, it is claimed that aaIgE are heterogeneous in function: some of them can trigger IgE-mediated histamine release from human basophils and thus are anaphylactogenic; others are not (11). Some can remove IgE from the surface of CD23-positive cells; in contrast, others can bind more IgE to the latter cells (19). Furthermore, these autoantibodies seem to have a regulatory influence

on human IgE mRNA synthesis as well as interleukin (IL)-4-induced proliferation of human mononuclear cells (28). All these observations suggest that aaIgE might play an important role in the pathogenesis of IgE-mediated allergy.

In this study, bee-sting allergy was chosen as a model to study the role of aaIgE *in vivo* for several reasons: 1) bee-sting allergy is a well-defined allergic disease; 2) bee venom (BV) is available in pure form in gram quantities, and its main allergenic components are known and some of them have been synthesized; 3) bee-venom immunotherapy (BVIT) is effective in most patients; 4) the efficacy of BVIT can be evaluated by a provocation test (PT) (sting challenge) with a living bee; 5) there exists a heavily exposed nonallergic population, i.e., beekeepers, which can be used as a control.

The major goals of our study were to investigate the prevalence of aaIgE in bee-sting allergy and to evaluate their relevance in the protective mechanism induced by BVIT.

Material and methods

Serum samples

For investigating the prevalence of aaIgE in bee-sting allergy, serum samples were collected from 30 patients with bee-sting allergy with a mean age of 37 (21–69) years and a sex ratio (F/M) of 10/20. Four of the 30 bee-allergic patients had a history of hay fever, asthma, or both and positive skin tests to at least one of nine common inhalants (house-dust mite; pollen of grass, birch, hazel, and mugwort; dander of cat and dog; *Cladosporium*; and *Alternaria*). The 83 controls included 30 beekeepers, 30 healthy blood donors (kindly provided by Prof. A. Morell, central laboratories of the Swiss Red Cross, Bern), and 23 atopic subjects (kindly collected by Drs M. Bauer and E. Jensen-Jarolim, Institute of Clinical Immunology, Bern). All of the bee-allergic patients had a history of severe systemic reactions to bee stings (grade III or IV) (15, 16), and also a positive intracutaneous skin test with BV (Pharmacia Diagnostics AB, Uppsala, Sweden) at 0.1 µg/ml or less. Twenty-four of them had also a positive RAST ($\geq$ class 2; Pharmacia Diagnostics). None of the controls had a history of bee-sting allergy. All of the atopic subjects had allergic symptoms, such as asthma, rhinitis, or both with positive skin prick tests to grass pollen, house-dust mite, or both. None of the bee-allergic and atopic patients had received either corticosteroids or hyposensitization therapy before serum collection.

To evaluate the relevance of aaIgE in the protective mechanism induced by BVIT, we studied patients undergoing this therapy. All had a history of severe reactions to bee stings and a positive intracutaneous skin test to BV, as well as a positive RAST to BV, before starting BVIT. A first group of 31 patients underwent rush BVIT. Patients were hospitalized and given up to four daily injections of BV, starting at 0.1 µg. As a rule, the maintenance dose of 100 µg was reached on day 4. Afterwards, the interval between injections was gradually increased to 4 weeks. In this group, serum samples were collected from each patient before BVIT and on days 3, 16, and 110 (51–390) during BVIT. A second study group consisted of 51 patients who underwent conventional BVIT with weekly injections of BV up to the same maintenance dose after 7 weeks. The treatment was continued for 3–7 years. A bee-sting PT was done in every patient before stopping BVIT and also 1 year after stopping the treatment. Serum samples were collected before starting BVIT and before each PT. All serum samples were kept at -20°C until use. The PT was done as described before (17). It was defined as positive if a systemic reaction of grade I or higher according to Mueller (15) occurred. All patients who had a positive PT

either before stopping BVIT ($n = 9$) or 1 year after stopping ($n = 14$) were chosen for this study. Twenty-eight patients with negative PT both before stopping BVIT and 1 year after stopping it, matched for age, sex, reactions to bee stings, skin test, and RAST with the PT-positive patients, were selected as a control group.

Dot immunobinding assay

Anti-IgE autoantibodies. Dot immunobinding assay for detection of free aaIgE and IgE-aaIgE complexes was done as described previously (30) with some modifications. Purified IgE (50 ng/dot) from myeloma patient PS (a kind gift of Drs T. and K. Ishizaka, La Jolla, CA, USA) was used to detect free aaIgE. Murine monoclonal anti-IgE antibodies Le27 (500 ng/dot) and BSW17 (500 ng/dot) were used to detect IgE/aaIgE complexes. Natural anti-IgE antibodies, if able to bind to the absorbed solid-phase IgE, were termed “free” aaIgE, whereas IgE-aaIgE complexes were caught by the solid-phase monoclonal anti-IgE antibodies. Two different monoclonal antibodies were used to catch immune complexes. If Le27 monoclonal antibody recognized IgE, the immune complexes were termed “non-Le27 type” to indicate that this epitope was still accessible. The “non-BSW17-type” immune complex was termed in the same way. The specificity of these two monoclonal antibodies for IgE has been shown elsewhere (11).

Nitrocellulose (0.45-mm) membranes (Millipore, Bedford, MA, USA) were used as the solid phase. One milliliter each of purified IgE PS and the two antibodies were dotted on the solid phase in duplicate. The membranes were dried overnight at room temperature and blocked with 0.15% casein (Fluka Chemie AG, Buchs, Switzerland) in TRIS-buffered saline (TBS) for 4 h with constant shaking at 50 rpm. Subsequently, the nitrocellulose membranes were cut into single strips and then incubated with test serum samples diluted 1:4 with TBS-casein in reservoir trays (Dynatech Laboratories, VA, USA, overnight with gentle shaking. After three washings with TBS, strips were incubated with peroxidase-conjugated monoclonal anti-IgG antibody (BS17, 1:1000, Biotest AG, Frankfurt, Germany) for 4 h. The strips were then washed four times with TBS and developed with substrate solution of 16.6% (v/v) of 4-chloro,1-naphthol (Merck, Darmstadt, Germany: stock solution 3 g/l in methanol), and 0.042% (v/v) hydrogen peroxide (stock solution 30% in water) in TBS for 30 min. After washing with distilled water, the strips were dried, and the optical density (OD) of the dots was measured with a hand-held reflection densitometer (Gretag, Regensburg, Switzerland) at 600 nm to obtain relative densitometric units.

IgM-type rheumatoid factors which might interfere with the estimation of the IgG anti-IgE autoantibody were sought by latex slide test (BIO Mérieux SA, Lyon, France). Only one sample from a beekeeper was positive, with a titer of 1:20.

Serum specific IgE and specific IgG. Serum specific IgE and specific IgG against BV (5 µg/dot; V-3375, Sigma, Chemical Co, St Louis, MO, USA), phospholipase A₂ (5 µg/dot; P-9279, Sigma), and melittin (5 µg/dot; M-7129, Sigma) were detected by the same method as described above. Serum samples for detecting specific IgE were diluted to 1:4; for IgG, to 1:50. The peroxidase-conjugated antibody for detecting specific IgG was BS17 (see above). For specific IgE, we used peroxidase-labeled monoclonal anti-IgE antibody BSW17 (1:1000).

Quality control. The positive control consisted of a serum pool from 15 subjects and contained high levels of BV-specific IgE (> 17.0 PRU/ml) and specific IgG (> 40.0 µg/ml), as well as aaIgE. The negative controls were the dots incubated directly with peroxidase-conjugated antibodies without adding sera before. There was no background OD for specific antibodies. Background values for aaIgE were very low. Anti-IgE autoantibodies and specific IgG were estimated by comparison with IgG standard dots after subtracting the OD value of the negative control. Specific IgE was estimated by comparison with IgE standard dots. For these standards, known amounts of myeloma IgE (PS) and human IgG (Nordic Immunologic Laboratories, Tilburg, The

Netherlands) were dotted in serial dilution and developed with the same labeled anti-IgG (BS17) or anti-IgE (BSW17), respectively. Values are given in ng/ml. The lowest concentration which could be recognized by the densitometer for IgE was 2.5 ng/ml; for IgG, 1 ng/ml.

To determine the specificity of the monoclonal antibodies, we incubated them with human IgG subclasses, IgA, IgM, and IgE, respectively. There were no cross-reactions of mouse antihuman IgG or mouse antihuman IgE with the other immunoglobulins (Fig. 1).

The intra-assay variation coefficients were 3.4% and 5.2% for IgE-aaIgE complexes (non-Le27-type and non-BSW17-type, respectively), 36% for free aaIgE, 11.3% for specific IgE against BV, and 4.1% for BV-specific IgG. The inter-assay variation coefficients were 21.9% for specific IgE, 21.6% for specific IgG, and 37.5% for IgE-aaIgE complexes. All samples to be compared in statistical tests were run in the same assay.

Statistical analysis

The distribution of individual values for each variable was analyzed by histograms. A skewed distribution was found for all variables. Medians instead of arithmetic mean values are therefore given, and a nonparametric test (Mann-Whitney U test) was used to compare each variable among groups. Differences were considered to be significant for *P* values < 0.05. Correlations among various antibodies were analyzed by Spearman's rank test.

Results

As shown in Table 1, the median values of aaIgE of either free or combined types were higher in both bee-allergic and atopic patients than in beekeepers and healthy donors (*P* < 0.01–0.05). The levels of

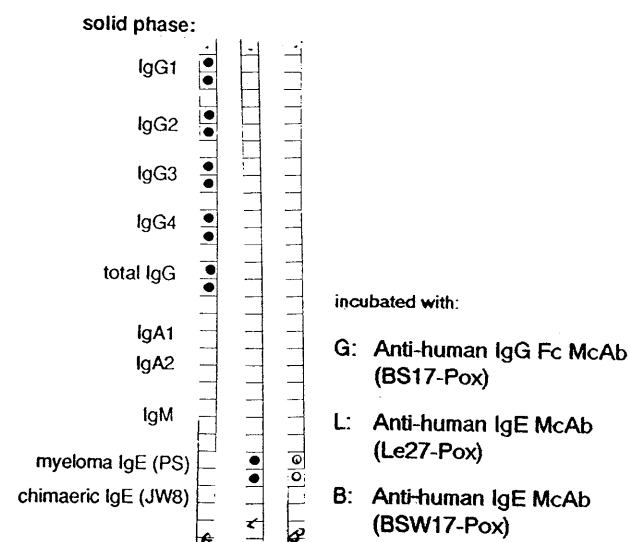


Fig. 1. Specificity of monoclonal antibodies (mAb): on all three strips, equal amounts of various human immunoglobulins (total IgG, IgG1–4, IgA1, IgA2, IgM, IgE PS, and IgE JW8) were dotted. Monoclonal antihuman IgG reacts only against IgG and its subclasses; monoclonal antihuman IgE only against IgE.

Table 1. Anti-IgE autoantibodies in bee-venom-allergic patients and controls

		Free aalIgE (ng/ml)	IgE-aalIgE complexes (ng/ml)	
			Non-Le27-type	Non-BSW17-type
Bee-allergic patients (n = 30)	Median	5.4*	58.5**	*44.9**
	Range	0–94.2	31.3–254.9	16.3–172.9
Beekeepers (n = 30)	Median	3.4°	51.6	28.1°°
	Range	0–54.8	13.0–163.0	0–136.8
Healthy donors (n = 30)	Median	3.8°	44.9°°	°37.6
	Range	0–26.6	11.0–89.8	2.4–72.0
Atopic patients (n = 23)	Median	5.0	63.9**	*53.1**
	Range	0–172.9	10.0–323.8	6.6–225.4

P < 0.05 between numbers marked * and °.

P < 0.01 between numbers marked ** and °°.

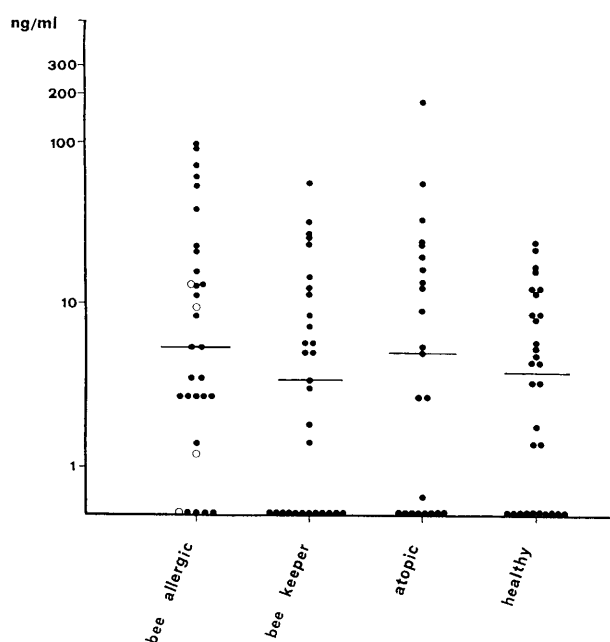


Fig. 2. Serum levels of free aalIgE in bee-allergic patients, beekeepers, atopic subjects, and healthy controls. Horizontal bars give medians. Difference between bee-allergic patients and healthy controls, as well as beekeepers, was significant ($P < 0.05$) with and without inclusion of atopic, bee-allergic patients (open circles).

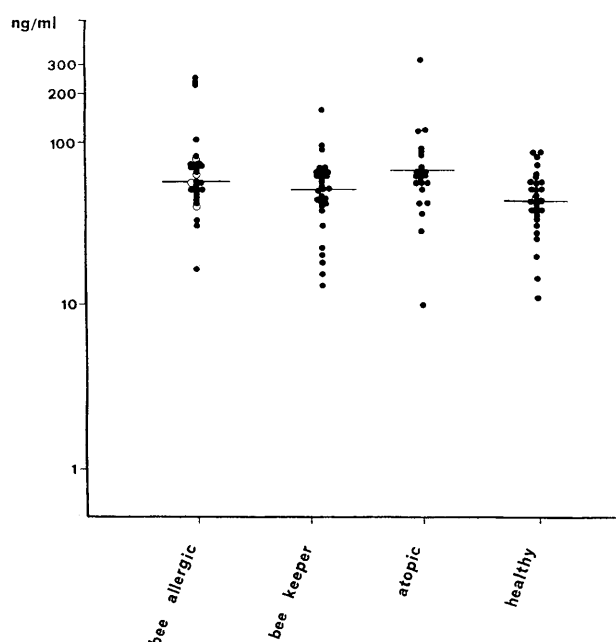


Fig. 3. Serum levels of non-Lc27 IgE-aalIgE complexes in bee-allergic patients, beekeepers, atopic patients, and healthy controls. Horizontal bars give medians. Difference between bee-allergic patients and healthy controls was significant ($P < 0.01$). After exclusion of four atopic, bee-allergic patients (open circles), difference between bee-allergic patients and healthy controls was still significant ($P < 0.03$).

Table 2. Specific IgE and IgG antibodies in bee-venom-allergic patients and controls

		Specific IgE (ng/ml)	Specific IgG (ng/ml)
Bee-allergic patients ($n = 30$)	Median	22.8**	853.0**
	Range	0–266.0	223.5–1257.5
Beekeepers ($n = 30$)	Median	0 ^{°°}	844.0**
	Range	0–104.2	260.5–1257.5
Healthy donors ($n = 30$)	Median	0 ^{°°}	153 ^{°°}
	Range	0–43.6	0–1517.0
Atopic patients ($n = 23$)	Median	0 ^{°°}	242.0 ^{°°}
	Range	0–122.8	17.0–1210.0

$P < 0.01$ between numbers marked ** and ^{°°}.

these autoantibodies were almost the same in bee-allergic and atopic patients, and there was no significant difference between the two nonallergic groups. A significant difference between bee-allergic and healthy donors was still observed after exclusion of four atopic, bee-allergic patients (Figs. 2–4). In view of the high variation coefficient for estimation of free aalIgE and the frequently negative (background) results, the statistical difference observed for these antibodies has to be assessed with some caution.

The serum levels of BV-specific IgE were significantly higher in bee-sting-allergic patients than in

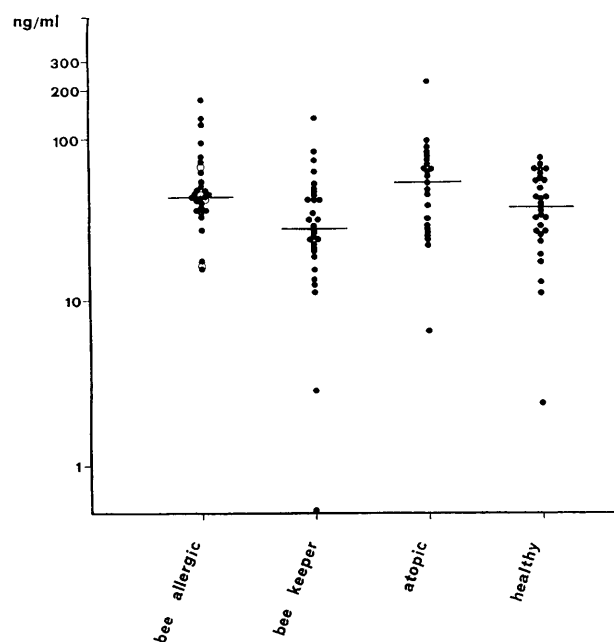


Fig. 4. Serum levels of non-BSW17 IgE-aalIgE complexes in bee-allergic patients, beekeepers, atopic patients, and healthy controls. Horizontal bars give medians. Difference between bee-allergic patients and healthy controls was significant ($P < 0.05$). After exclusion of four atopic, bee-allergic patients (open circles), difference between bee-allergic patients and healthy controls was still significant ($P < 0.05$).

Table 3. Clinical data of provocation test (PT)-positive ($n = 23$) and PT-negative ($n = 28$) patients

Provocation test		Positive ($n = 23$)	Negative ($n = 28$)
Sex	M/F	17/6	20/8
Age (years)	Mean (range)	29.37 (15–53)	29.08 (15–53)
	II	0	1
Reaction to sting	III	13	15
	IV	10	12
Skin sensitivity ($-\log$ EPC in g/l)	Mean (range)	4.83 (3–8)	4.76 (3–8)
	BV-specific IgE (before BVIT)	Mean (range)	42.4 (0–266.8)
			40.5 (0–324.8)

EPC = end-point concentration.

controls. BV-specific IgG levels were significantly elevated in the two most highly exposed groups, bee-allergic patients and beekeepers (Table 2).

The correlation between free aaIgE and the two kinds of IgE/aaIgE complexes was good ($r = 0.80$ – 0.87). Only weak correlations were found between BV-specific IgE and BV-specific IgG, while the correlation between aaIgE and BV-specific IgE or IgG was poor or absent.

During the first year of rush BVIT, specific IgE showed initial increase until day 16, and thereafter a slow decrease, while specific IgG showed a similar increase in the first 2 weeks of rush BVIT, but then remained elevated throughout the first year of treatment. No significant changes in either free or combined aaIgE were observed during the first year of rush BVIT.

In 51 patients on prolonged BVIT over 3–7 years, specific IgE decreased strongly, whereas specific IgG remained elevated. While free aaIgE stayed largely

unchanged, both types of IgE-aaIgE complexes tended to decrease. Of these 51 patients, 23 had a positive PT either during or after BVIT or both. They were compared with 28 patients with a negative PT at both occasions (Table 3). The two groups did not differ significantly in sex, age, severity of previous bee-sting reaction, pretreatment skin sensitivity, or BV-specific serum IgE. Fig. 5 shows aaIgE in relation to the result of the PT. Free aaIgE and IgE-aaIgE complexes of non-BSW17 type were higher in PT-positive than PT-negative patients, both during and 1 year after BVIT. However, this difference did not reach statistical significance. Non-Le27-type IgE-aaIgE complexes were similar in both groups.

Discussion

The measurement of anti-IgE antibodies is a technically delicate method. One of the greatest difficulties in measuring immune complexes by capturing IgE via a monoclonal antibody lies in the fact that some of the antibodies that bind may directly recognize the capturing antibody. For this purpose, we use an irrelevant monoclonal antibody of the same isotype as a negative control. This gives signals which are usually well below the signals obtained when dotting IgE. We decided not to subtract this signal from the overall anti-IgE response because of the earlier described existence (24) of panspecific anti-immunoglobulin antibodies. It is well established that among the pool of naturally occurring antibodies there are anti-isotype antibodies that may recognize even immunoglobulins from other species. Such antibodies can also recognize IgE (6), but we do not have a precise method to distinguish between exclusively isotype-specific anti-IgE antibodies and anti-IgE antibodies that also recognize other isotypes. A further complication lies in the fact that if one uses different myeloma IgE a large variability in the extent of the signals may be observed; this observation could be explained by the existence of anti-idiotypic antibodies (6). Despite all these technical problems, it is obvious that the levels of such naturally occurring autoantibodies may differ in different patient groups (5, 12, 20, 21, 23, 29).

This study confirms the presence of aaIgE in the sera of atopic patients allergic to grass pollen, house-dust mite, or both. We found also a significant elevation of these autoantibodies of either free or combined type in the sera of bee-sting-allergic patients, while beekeepers had normal levels. However, the serologic difference between bee-allergic patients and beekeepers was more evident when specific IgE levels were compared. This raises a general question of the meaning of aaIgE determinations.

It is obvious from previous work (11, 19) that

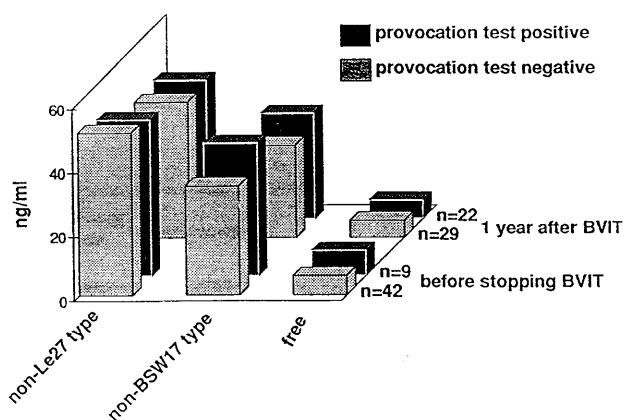


Fig. 5. Anti-IgE autoantibodies in relation to result of bee-sting provocation test (PT). Levels of non-BSW17-type IgE-aaIgE complexes were higher in PT-positive than PT-negative patients, both before and 1 year after stopping BVIT. However, difference did not reach statistical significance ($P > 0.05$).

aaIgE are functionally heterogeneous, but at present we cannot determine the biologic properties of these autoantibodies with serologic methods. A first step toward characterization of aaIgE is the use of two types of monoclonal anti-IgE antibodies to catch immune complexes: Le27 is a strongly anaphylactogenic antibody, while BSW17 does not release, or only weakly releases, histamine from normal, untreated basophils (11, 19). As in previous studies (28), elevated levels of IgE-aaIgE immune complexes were found in atopic patients. A similar pattern has been observed in bee-allergic patients, who, most often, are nonatopic (16). Interestingly, beekeepers were found to have the lowest serum levels of non-BSW17-type autoantibodies, which are anaphylactogenic *in vitro*.

To investigate further the significance of aaIgE in BV allergy, we studied patients on BVIT. While the expected changes in BV-specific IgE and IgG antibodies were observed (7, 8, 18), both free aaIgE and the two types of IgE-aaIgE complexes remained virtually stable during the first year or rush immunotherapy. During prolonged BVIT over 3–7 years, both types of IgE-aaIgE complexes decreased, while free aaIgE did not change significantly. However, when aaIgE were studied in relation to a sting PT, it was found that the non-BSW17-type IgE-aaIgE immune complexes were higher both during and 1 year after BVIT in PT-positive than in PT-negative patients. In PT-positive patients, these antibodies remained elevated during BVIT, while in PT-negative patients they decreased. Although these differences did not reach statistical significance, they point to a functional heterogeneity of aaIgE also *in vivo*, and indicate that aaIgE with an epitope specificity different from BSW17 may be associated with treatment failures in BVIT.

So far, only one paper has studied aaIgE in relation to Hymenoptera sting allergy (9). This paper found no correlation between serum levels of aaIgE and BV-specific IgE during immunotherapy. However, in contrast to our study, aaIgE were not analyzed in relation to the success of immunotherapy and with regard to different epitope specificities of aaIgE. Further investigations, including the estimation of the IgG subclass of aaIgE, as well as a more detailed analysis of the epitope specificities of these autoantibodies, are warranted.

Acknowledgments

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