

Effect of prolonged venom immunotherapy on serum venom-specific IgE and IgG

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Summary

Serum venom-specific IgE and IgG were monitored in twenty-three patients receiving venom immunotherapy for more than 3 years. Two response patterns of IgE antibody were found. Following initiation of therapy, seven patients had a rise in serum venom-specific IgE, peaking at one year, then decreasing. Sixteen patients had a persistent fall in IgE antibody titres following initiation of therapy. At the end of 3 years, levels of serum venom-specific IgE in both groups were comparable. The presence of atopy may have influenced the rising IgE antibody response.

Serum venom-specific IgG either rose or remained elevated if the pretreatment titres were high. After several years of therapy, there was generally a decrease in serum venom-specific IgG.

Introduction

Venom immunotherapy provides protection against recurrent anaphylaxis in insect-sting-sensitive patients. For several years, this therapy has been available for routine use. The purpose of this study was to monitor the immunologic effects as determined by measurements of serum venom-specific IgE and IgG in patients who have been receiving venom immunotherapy for more than 3 years. The results of these observations revealed two patterns of IgE antibody response following venom immunotherapy: an initial rise followed by a decline, and a persistent decline. IgG antibody levels either remained elevated, or rose.

Materials and methods

Serum venom-specific IgE (RAST)

Specific IgE antibodies (V-IgE) to bee and yellow jacket venoms were measured by the

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RAST, as previously described (Light *et al.*, 1976). One milligram of venom protein was used to couple to 200 cyanogen bromide-activated discs. Results are expressed in percentage of net binding of a serum pool from ragweed-sensitive patients to ragweed discs. A net binding to the venom discs $>10\%$ is regarded as significant. The background activity bound when normal human serum was assayed was 0.7% for ragweed discs, and varied between 0.7% and 1.5% of the total activity added for the venom discs. The maximal binding of the ragweed standard went up to 15% of the total activity.

Serum venom-specific IgG (radioimmunoassay)

Serum venom-specific IgG (V-IgG) was determined by a modification of a solid-phase radioimmunoassay using ^{125}I -labelled protein A (Hamilton, Sobotka & Adkinson, 1979; Mueller *et al.*, 1981). The same venom-coupled discs used for the IgE antibody assay were used. A serum with a high titre of yellow jacket venom-specific IgG and another serum with high-titred bee venom-specific IgG were used as positive standards. Fifty microlitres of a 1:50 dilution of the standard serum or the test sera in borate-buffered saline containing 0.2% ovalbumin and 1% Tween, were added to each tube containing a venom-coupled disc. After incubation for 2 hr at room temperature, the discs were washed three times in buffered saline. Fifty microlitres of radio-labelled protein A were then added. After 90 min incubation at room temperature, the discs were washed three times with buffered saline and radio-activity bound determined in a gamma counter. The background activity bound for normal human serum was about 1% of the total activity added. The binding of the standard serum at a 1:50 dilution was about 13% of the total activity. Results are expressed in arbitrary units, based upon the percentage net binding of the total activity.

Study design (Table 1)

Serum venom-specific IgE and IgG were monitored in twenty-three patients. All patients had experienced a systemic reaction to an insect sting and had had at least one positive venom skin test. They had been on continuous venom immunotherapy for 3 years. Sequential blood samples were available for analysis over this time period.

These twenty-three patients received fifteen bee venom treatments and nine yellow jacket venom treatments. Therapy was accomplished by using a traditional dosing method. The starting dose was $0.01\text{ }\mu\text{g}$ and weekly injections were given for 4 to 6 months until a top dose of $50\text{ }\mu\text{g}$ was reached. We have found this top dose to be

Table 1. Details of venom immunotherapy

No. of patients	23
Venom therapy	
Honey bee	15
Yellow jacket	9
Therapy:	
Traditional dosing regimen	
Starting dose (μg)	0.01
Top dose (μg)	50.0
Maintenance (weeks)	every 4
Duration (years)	>3

effective in preventing subsequent systemic reactions to stinging insects. Therapy was maintained at 4-week intervals for at least 3 years. Since this study was begun, we have switched to a more rapid method of initial venom immunotherapy (Clayton *et al.*, 1983). Sequential sera were analysed at the same time.

Results

Based upon the results of the serum venom-specific IgE titers, patients could be divided into two groups. One group (Group A) consisted of sixteen patients. Following initiation of venom therapy, they showed a consistent decrease in serum venom-specific IgE. The second group (Group B) consisted of seven patients. Following initiation of venom therapy, there was an initial rise in serum venom-specific IgE, peaking at one year, then followed by a subsequent decrease. These mean changes in IgE antibody levels are shown in Fig. 1.

The main characteristics of the two groups are shown in Table 2. The mean age of patients in the two groups was similar. Group A contained a few more older individuals. The ratio of male to female patients was similar in both groups. There were more atopic patients in Group A than in Group B. In Group A, four of sixteen patients were atopic; in Group B, five of seven were atopic. The ratio of honey bee to yellow jacket treatments was the same for both groups.

The mean serum venom-specific IgE titres for each group over the 3-year period are shown in Table 3. The paired *t*-test was used to determine significant differences. Patients in Group A who experienced a constant fall in IgE antibody levels showed a significant difference between the initial mean value of 84, and a 3-year value of 30

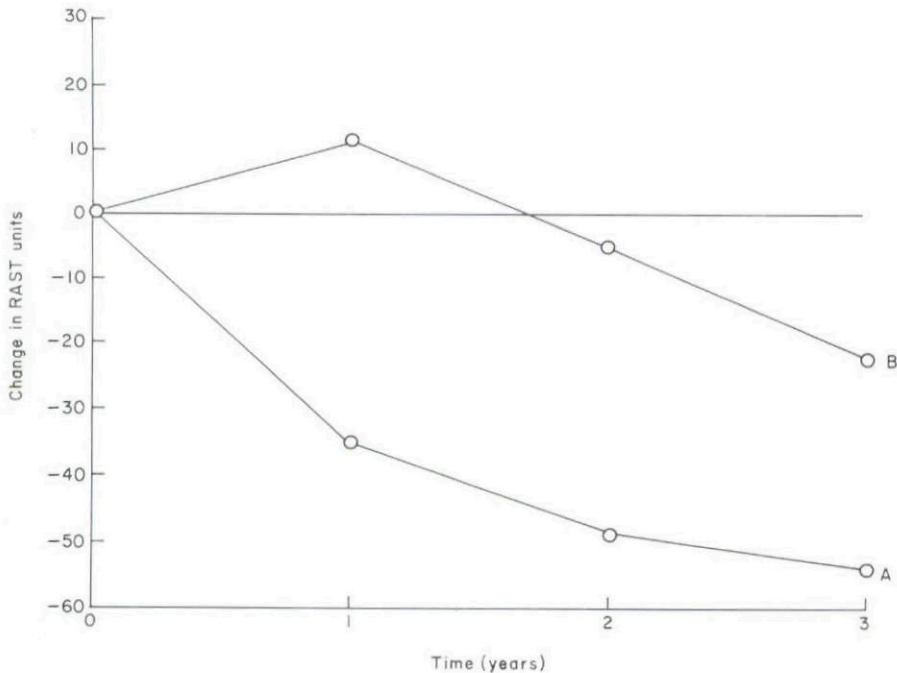


Fig. 1. Mean change in serum venom-specific IgE over 3 years. Patients were grouped depending upon initial IgE antibody response.

Table 2. Characteristics of patients in Group A (persistent decline in V-IgE) and Group B (initial rise, then fall in V-IgE)

	Group A	Group B
Age (mean)	27	18.5
Range	3-69	7-32
Sex		
Male	9	4
Female	7	3
Atopic history		
Yes	4	5
No	12	2
Venom therapy		
Honey bee	10	5
Yellow jacket	6	3

($P < 0.001$). Patients in Group B who had a lower initial mean serum venom-specific IgE level (45), also demonstrated significant differences between the 3-year level (28), and the initial level ($P < 0.02$). The two groups differed significantly in their initial values (84 and 45), but did not differ in their 3-year values (30 and 28).

Group A contained several patients with serum venom-specific IgE values above 100%, accounting for the elevation in the mean RAST for the group. In order to show that these patients did not skew our data, patients in Group A whose initial serum IgE

Table 3. Changes in serum mean venom-specific IgE titres expressed as percentage of positive standard

	Group A	Group B
Initial	84	45 ($P < 0.001$)
1 year	47	63
2 years	36	41
3 years	30	28 (NS)
Initial vs 3 years	$P < 0.001$	$P < 0.02$

antibody levels fell into the same range as Group B patients were compared. This comparison is shown in Fig. 2. All Group A patients had a fall in IgE levels; all Group B patients had a rise. For this comparison, patients in Group A had a mean initial serum venom-specific IgE RAST level of 49, which fell to 19. In Group B patients, the initial RAST level was 45, and rose to 63.

Table 4 shows the mean venom-specific IgG titres determined by the protein A radioimmunoassay and expressed as percent of positive standard. All patients showed a rise in serum venom-specific IgG or a maintenance of a significant pretreatment level. One patient in Group A was omitted from analysis because of a very high initial IgG antibody titre. The patients in Group A showed a peak titre at 1 year, followed by a

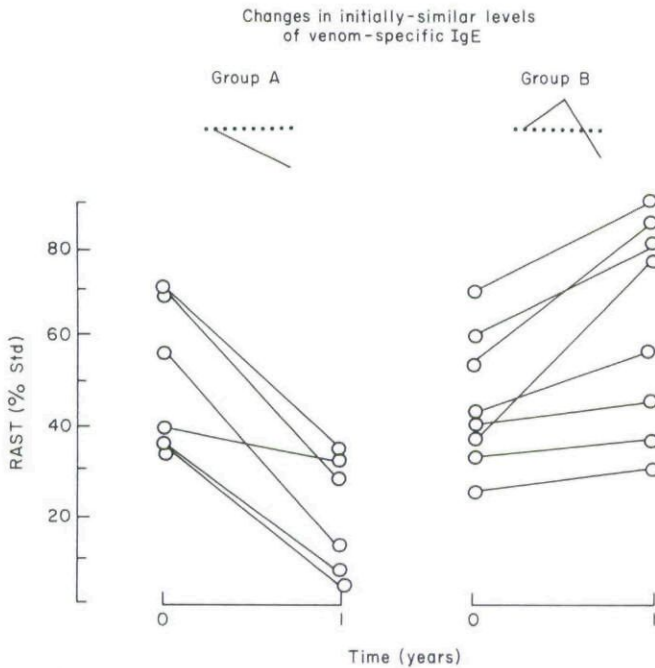


Fig. 2. Comparison of change of serum venom-specific IgE in patients in Group B with those patients in Group A whose initial pre-treatment titres were in the same range as Group B patients.

Table 4. Changes in serum mean venom-specific IgG titres expressed as percentage of positive standard

	Group A	Group B
Initial	5.7	3.1
1 year	7.2	9.2
2 years	6.9	11.3
3 years	5.1	9.5

Table 5. Effect of re-stings

	Group A	Group B
No. of patients re-stung	10	3
Clinical reaction	0	0
V-IgE anamnestic response	1	0

slow decline. The patients in Group B showed a more gradual rise, peaking at 2 years, with some decline in the third year of therapy.

Thirteen of twenty-three patients were re-stung (Table 5). Most patients believed they had been stung by the same insect which had caused the original reaction. None had a systemic reaction. One patient in Group A showed a subsequent venom-specific IgE anamnestic response.

Discussion

This study of twenty-three patients receiving venom immunotherapy for a period of 3 years revealed two different patient populations with regard to their serum venom-specific IgE response. Following venom immunotherapy, patients in Group A showed a

persistent decline in serum antibody titres. Patients in Group B had an initial rise, followed by a decline. There were no differences between the two groups at the end of the 3 years. A greater proportion of patients in Group B (rise, then fall) were atopic, suggesting perhaps that an atopic background may influence the IgE antibody response. Theoretically, an increase in antibody titre early in the treatment regimen might suggest increased clinical sensitivity.

The persistent fall in IgE antibody is the preferable immunologic response. We have used a decline of this antibody titre to insignificant levels in patients undergoing venom immunotherapy as a criterion for discontinuing treatment. To date, such patients have had no difficulties when re-stung following cessation of therapy. Fifteen re-stings in twelve patients resulted in no systemic reaction and five local reactions (Georgitis *et al.*, 1981).

In prior studies, we have measured the antibody response in patients treated with a traditional and a rapid venom-dosing regimen (Clayton *et al.*, 1983). Of fifty-two patients studied, only twelve demonstrated an initial rise in venom-specific IgE following the initiation of treatment. Four of nineteen patients in the rapid group and eight of thirty-three patients in the traditional group showed this rise, suggesting little difference between the venom immunization procedures.

Golden *et al.* (1980) have reported somewhat different results. They found an initial rise of venom-specific IgE in seventeen of twenty-three patients receiving a slow venom immunotherapy regimen, and in ten of sixteen patients treated by the rush method. They concluded that venom-specific IgE usually rises early in therapy and then declines, reaching baseline levels within 1 year. They have pointed out that this is an 'average and idealized response; the variation from patient to patient is extreme' (Lichtenstein, Valentine & Sobotka, 1979). We did follow venom-specific IgE levels at 1 to 2 month intervals, and we did not find this early rise in most patients. In a more recent study in children (Schubert *et al.*, 1982), a four-fold rise in venom-specific IgE titres in the first 6 months of treatment was found. This response was referred to as the 'expected response'. Our observations differed. The rise or decline in venom-specific IgE in children occurred with the same frequency as described for our total population.

In general, venom-specific IgG rose after initiation of therapy. In those patients who already had high pre-treatment levels, venom IgG antibodies were maintained. With continued therapy over a long period of time, there was some decline in antibody titres. This is in general agreement with the results published by others.

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