

Immunotherapy with Yellow Jacket Venom

A Comparative Study Including Three Different Extracts, One Adsorbed to Aluminium Hydroxide and Two Unmodified

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Thirty-two patients with previous systemic allergic reaction to yellow jacket stings were randomly allocated to three groups receiving immunotherapy with different preparations of yellow jacket venom: 1) extract adsorbed to aluminium hydroxide (Alutard®-SQ), 2) Pharmalgen® extract or 3) non-adsorbed extract from Allergologisk Laboratorium (ALK aq.). Regular examinations showed a decrease in skin prick test size in nearly all patients. Specific IgE-antibody (RAST and CRIE scores) showed a similar, but not significant tendency to decrease in all three groups. Specific IgG-antibody increased considerably in the Alutard group only; after 2 years, however, no difference could be detected between the three groups. During dose increase, patients treated with ALK aq. generally had smaller local reactions to injections than those treated with Pharmalgen. Few systemic reactions occurred in all three groups. Nineteen patients treated for 2½-3½ years were challenged in-hospital with stings from yellow jackets. No systemic and only minor local reactions occurred. Consequently, with the dose regimens applied all three extracts seem effective even though no common changes in either specific IgE or IgG could be demonstrated.

Key words: aluminium hydroxide adsorbed extract; CRIE; immunotherapy; specific IgE; specific IgG; sting challenge; yellow jacket.

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CLINICAL ASPECTS

Immunotherapy with different insect venom extracts in aqueous solutions has previously proved effective in insect allergy. In the present study an extract containing aluminium hydroxide as an adsorbent was shown to give an equal protection against systemic reactions even with intervals extended from 4 to 6 weeks between injections at maintenance dose. The aluminium hydroxide did not protect against systemic side effects, but these were few and no medical treatment had to be instituted.

Immunotherapy with Hymenoptera venom has shown effect in several studies with different dose regimens (1, 4, 9, 10, 20, 23). Previously, aqueous solutions of extracts without adjuvants or modifying additives have been employed. When treating for allergy against other sources, aluminium hydroxide ($\text{Al}(\text{OH})_3$) has been used as adsorbent, or the allergens have been coupled covalently to the inert matrix to obtain a sustained release with a decreased risk of

severe, immediate systemic reactions and with a possibility of extending dose intervals (16). The aim of the present study was to compare the efficacy and feasibility of immunotherapy with different preparations of yellow jacket (YJ) venom, one being adsorbed to aluminium hydroxide and two without such additive in aqueous solution. The efficacy was evaluated by clinical as well as immunological parameters.

MATERIAL AND METHODS

Patients

At two allergy clinics, 32 adult patients with previous systemic reactions (Table 1) and allergy to YJ venom (skin prick test with 100 µg/ml > diluent reaction and/or RAST ≥ class 2) were randomized to immunotherapy with one of three venom extracts. None of the patients had been given insect venom therapy prior to the study, and during the study no other immunotherapy was introduced.

Allergen extracts

The following extracts were used for treatment: 1) Pharmedgen® YJ venom reconstituted in albumin-containing saline diluent (Pharmacia, Uppsala, Sweden), 2) YJ venom without low molecular components (cut off 1,000 D) in aqueous solution with albumin and saline (ALK aq.) (Allergologisk Laboratorium A/S (ALK), Copenhagen, Denmark) and 3) Alutard®-SQ YJ venom containing the allergens of ALK aq. and Al(OH)₃ as adsorbent (ALK, Copenhagen, Denmark).

Skin testing was performed with Soluprick® venom extract containing the allergen material from ALK aq. in glycerol solution 50 % v/v.

All four extracts employed for treatment or *in vivo* testing were produced from raw venom obtained from Vespa laboratories Inc., Spring-mills, PA, U.S.A. The YJ raw venom from this source was furthermore used in determination of specific antibodies (anti YJ IgE and IgG).

Table 1

Reactions to stings by yellow jacket (YJ) prior to treatment in the participating patients. (All patients had experienced at least one systemic reaction)

Reaction to YJ sting	Extracts for treatment		
	Alutard	ALK aq.	Pharm-algen
Large local	4	4	4
Urticaria/angioedema	11	7	8
Respiratory symptoms	7	3	6
CNS symptoms	9	7	5
Total No. patients	12	10	10

Treatment

During the phase of dose increase, patients receiving aqueous extracts visited the clinic twice a week. Injections were given subcutaneously in the dorsal part of the forearms. Until local swelling exceeded 5 cm in diameter, more than one injection was given per visit (Table 2). At maintenance dose, i.e. 100 µg, or the dose four times giving local swelling > 5 cm, intervals were increased to 4 weeks ± 1 week.

The albumin adsorbed extract was administered once a week (Table 3) until maintenance dose: 100 µg, or the dose four times giving local swelling > 8 cm. The intervals were then extended to 6 ± 2 weeks.

Table 2

Dose schedule employed for immunotherapy with aqueous extracts. Initial dose: 0.2 ml of a concentration 1:1,000 less than the one giving a reaction in skin prick test equivalent to the histamine control

Serial No.	Inj. No.	Conc. (µg/ml)	Vol. (ml)
1	1	0.001	0.2
	2	-	0.4
	3	-	0.8
2	4	0.01	0.2
	5	-	0.4
	6	-	0.8
3	7	0.1	0.2
	8	-	0.4
	9	-	0.8
4	10	1.0	0.2
	11	-	0.4
	12	-	0.8
5	13	10	0.2
6	14	10	0.3
7	15	10	0.4
8	16	10	0.6
9	17	10	0.8
10	18	100	0.1
11	19	100	0.15
12	20	100	0.2
13	21	100	0.3
14	22	100	0.4
15	23	100	0.6
16	24	100	0.8
17	25	100	1.0

Exceeded intervals or adverse effects caused a modification of the subsequent dose. Swelling > 5 or 8 cm, respectively in the two groups would prevent the dose increase, and in both groups the dose would be reduced if swelling exceeded 10 cm in diameter.

Measurements of the parameters mentioned below were followed at 3, 6, 12 and 24 months. Continuation of treatment was then offered until a deliberate sting challenge could be arranged.

Skin prick test

Skin prick test (SPT) was performed in singular using a canule (6) or an ordinary blood lancet (14) in the two allergy centers, respectively. Half log dilutions from 1000 to 1 µg/ml of extract were used. After 15 min the size of the wheals was recorded as the mean diameter (half maximum length + half perpendicular). Skin prick tests with histamine dihydrochloride 1 mg/ml and isotonic saline in 50 % glycerol were used to check reactivity and as (negative) control.

Table 3

Dose schedule employed for immunotherapy with Al(OH)₃ adsorbed extract

Inj. No.	Conc. (µg/ml)	Vol. (ml)
1	0.1	0.2
2	-	0.4
3	-	0.8
4	1.0	0.2
5	-	0.4
6	-	0.8
7	10	0.2
8	-	0.3
9	-	0.4
10	-	0.6
11	-	0.8
12	100	0.1
13	-	0.15
14	-	0.2
15	-	0.3
16	-	0.4
17	-	0.6
18	-	0.8
19	-	1.0

Specific IgE

Radioallergosorbent test (RAST) was performed as described (2) with allergen-coupled paper discs as allergosorbent. A pool of sera from 1000 normal individuals (unselected with respect to insect reaction) was tested as control. Crossed radioimmuno-electrophoresis (CRIE) was performed as described (22). The patients' specific IgE was bound to immunoprecipitates of the YJ venom antigens precipitated by rabbit anti-YJ venom in crossed immunoelectrophoresis. Autoradiography was performed after incubation with ¹²⁵I-labelled anti-human IgE. These were evaluated by a scoring of the degree of radiostaining of the individual precipitates (13).

Specific IgG

Specific IgG was determined by a radioimmunoassay using labelled protein A from *Sta-*

Table 4

Number and age of patients in treatment groups

Patients	Extracts for treatment		
	Alutard	ALK-aq.	Pharmalgen
Number included	12	10	10
completing (2 years)	9	10	7
Initial age, median and (range)			
included	41 (29-79)	40 (24-60)	46 (21-62)
completing (2 years)	42 (30-79)	40 (24-60)	45 (21-62)

Table 5

Reasons for leaving the study before challenge

Treatment	Duration of treatment (months)	Reason
Pharmalgen	8	immunotherapy with bee venom
	17	local + systemic side effects
	21	other disease
	24	lack of time
Alutard	3	psychic reactions
	7	other disease
	12	unknown
	27	emigration

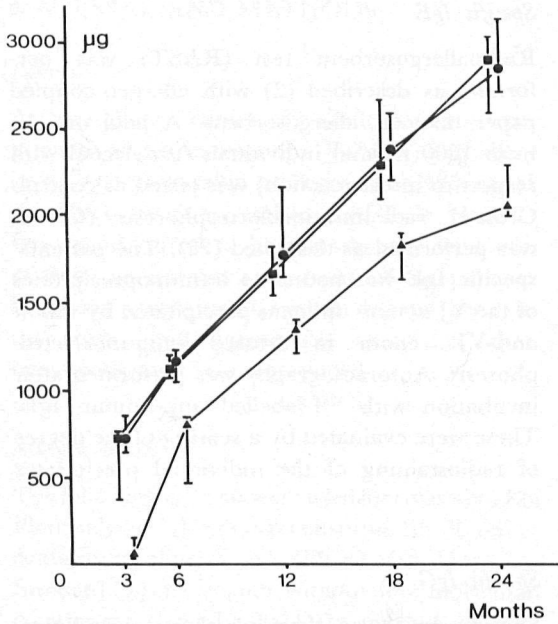


Fig. 1. Accumulated doses of yellow jacket venom administered during treatment with Alutard (▲), ALK aq. (●) and Pharmalgen (■). Medians and 25 % percentiles.

phylococcus aureus as previously described (9). Paper discs were used instead of sepharose as the solid support to which YJ venom was coupled. The sera were preabsorbed with solid-phase-coupled anti-IgE to avoid binding of this antibody to the discs. Results were expressed in percent of binding relative to a selected (positive) serum (9). The amount of specific IgG in this serum was $44.2 \pm 3.8 \mu\text{g/ml}$ as determined by double antibody method (personal communication with R. G. Hamilton, Baltimore, M.D., U.S.A.).

Sting challenge

Nineteen of the 24 patients still on treatment after 2½ to 3 years were exposed to a sting challenge with YJ performed in the clinic. Nine patients had received ALK aq., seven and three had been treated with Alutard and Pharmalgen respectively. Four patients were not invited, one due to (old) age, another had disseminated cancer and two because of practical misunderstandings. Only one patient had no interest.

Statistics

Wilcoxon signed rank test was used to evaluate differences within the separate groups during treatment. Comparisons between groups after identical treatment periods were evaluated by the Mann-Whitney test except for evaluation of local side effects for which the Chi-squared test was applied. All probabilities (*P*) less than 0.05 were considered significant (two-tailed tests).

RESULTS

Feasibility

Twenty-six of 32 patients completed the first 2 years of treatment (Table 4). Only one of the drop-outs was with certainty related to the treatment (Table 5). During the subsequent 6–18 months, before challenge could be performed, two patients stopped treatment due to emigration and lack of time, respectively.

The median accumulated doses are shown in Fig. 1. Due to a dose schedule with more frequent injections, higher doses of extract were administered when giving aqueous preparations. No statistically significant differences in accumulated doses, number of injections or visits were observed between the two groups receiving aqueous extracts (Table 6). Among patients continuing treatment for 2 years, the median number of injections was 30–72 (lower in the Alutard treated group than in the other patients (*P* < 0.05) (Table 6)).

During the phase of dose increase, systemic side effects of possible allergic origin were seen

Table 6

Number of visits and injections given during 2 years' treatment, median and (range)

	Extracts for treatment		
	Alutard	ALK aq.	Pharmalgen
Visits/patient*	41 (36–67)	43 (30–70)	47 (38–56)
Injections/patient	41 (36–47)	49 (42–72)	52 (42–62)
----- <i>P</i> = 0.05 -----			
----- <i>P</i> < 0.05 ----- ----- <i>P</i> > 0.05 -----			

* No significant difference between groups.

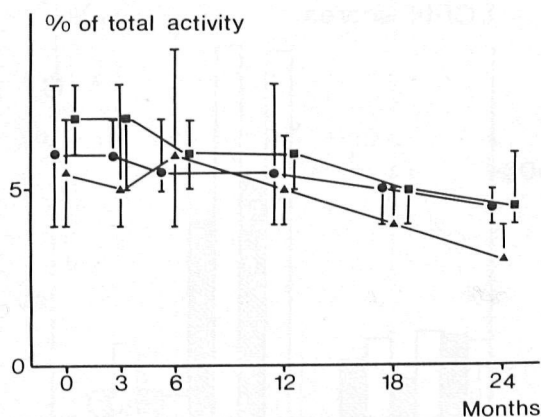


Fig. 2. Specific IgE (RAST) during treatment with Alutard (▲), ALK aq. (●) and Pharmalgen (■). Expressed as per cent of total radioactivity bound to sorbent. Medians and 25 % percentiles.

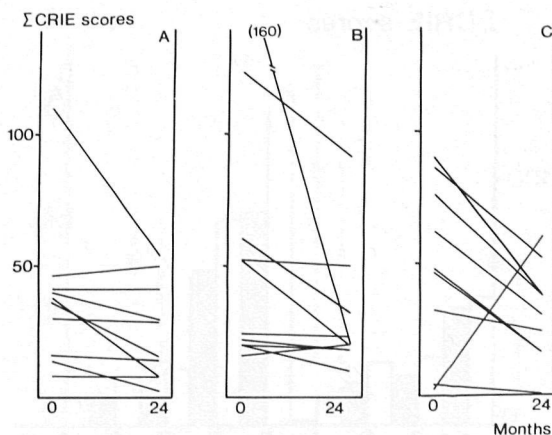


Fig. 3. Specific IgE expressed in accumulated CRIE scores against yellow jacket venom initially and after immunotherapy for 2 years with Alutard (A), ALK aq. (B) and Pharmalgen (C).

in three patients (Table 7). One had generalized urticaria, one experienced light throat symptoms with impaired swallowing in two instances, and the last patient had headache and nausea subsequent to an injection. All three patients received $\text{Al}(\text{OH})_3$ adsorbed extract. During the later phases generalized symptoms were seen in one patient in each group. One patient receiving Pharmalgen repeatedly had large local reactions accompanied by general malaise, nausea, and sweating, and due to these side effects the treatment had to be stopped after 17 months. All other symptoms were slight and no medical treatment of side effects had to be instituted in any patient. Among those receiving aqueous extracts several afterwards

complained of severe tiredness during the rest of the day (Table 7). Subsequent unreduced doses did not cause any side effects in these patients.

Venom-specific IgE

Venom-specific IgE as evaluated by RAST showed a decreasing tendency in most patients. In the group receiving Pharmalgen (in which the initial level of specific IgE was highest) this response was statistically significant after 24 months. However, when comparing the IgE RAST value from the various treatment groups at all the fixed time points, no statistically significant differences were observed.

Table 7

Adverse reactions during immunotherapy. Number of episodes in parenthesis

Phase	Extracts for treatment		
	Alutard	ALK aq.	Pharmalgen
Dose-increase	throat symptoms (2)	tiredness (4)	tiredness (2)
	generalised urticaria (1)		
	headache + nausea (1)		
Maintenance dose	malaise (1)	headache + near fainting (1)	malaise + nausea + sweating (2)
		tiredness (1)	tiredness + nasal obstruction (1)

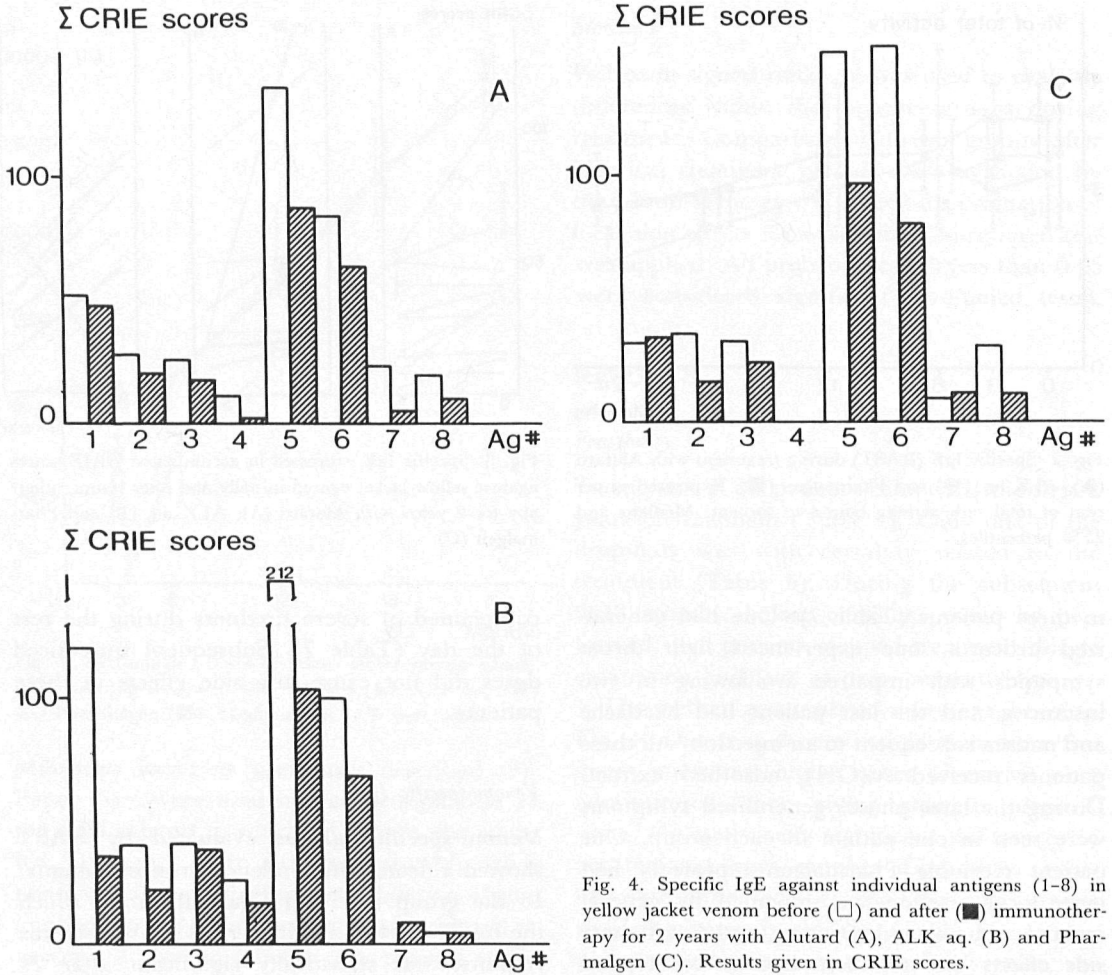


Fig. 4. Specific IgE against individual antigens (1-8) in yellow jacket venom before (□) and after (▨) immunotherapy for 2 years with Alutard (A), ALK aq. (B) and Pharmalgen (C). Results given in CRIE scores.

Likewise, when evaluating the IgE response to individual allergens by CRIE, a decrease was observed after 2 years in several patients (Fig. 3). For some of the individual allergens the reduction in CRIE scores before and after 24 months of treatment was statistically significant. This was the case with IgE towards Ag 1, Ag 2, and Ag 5 for the group of patients treated with ALK aq. and for IgE towards Ag 6 among the group of patients treated with Pharmalgen. In the Alutard treated group no significant changes were observed. Neither before or after 24 months of treatment could any significant differences in CRIE scores for the individual allergens be seen when comparing the three treatment groups (Fig. 4).

Venom-specific IgG

Changes in venom-specific IgG were observed (Fig. 5). Statistically significant increases were found for specific IgG levels in the group of patients treated with Alutard when comparing before treatment with 6 months, 12 months, and 18 months of treatment. In the group of patients treated with ALK aq. no such differences were found. In the group of patients treated with Pharmalgen statistically significant increase was found when comparing before treatment with 3 months of treatment. When comparing treatment with the various extracts, statistically significant differences in patients' IgG-response were found for 12 and 18 months

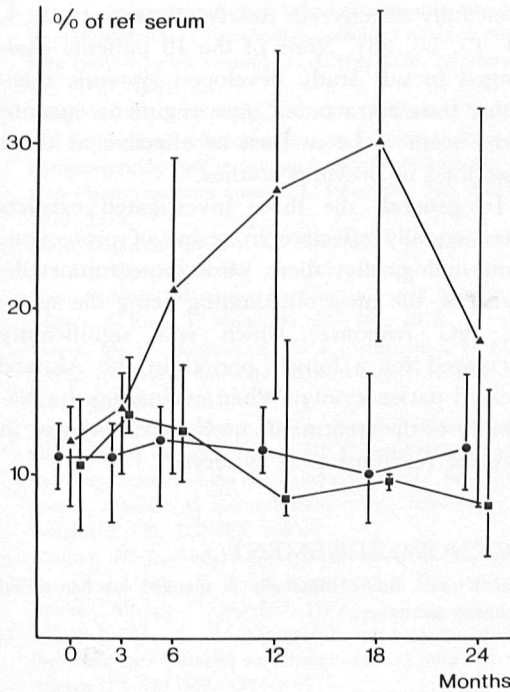


Fig. 5. Specific IgG against yellow jacket venom during treatment with Alutard (▲), ALK aq. (●) and Pharmalgen (■). 100 % corresponding to 44.2 µg/ml yellow jacket venom specific IgG. Medians and 25 % percentiles.

of treatment when comparing treatment with Alutard and Pharmalgen. Similarly, when comparing Alutard with ALK aq. statistically significant differences in responses for the groups were found after 6, 12, 18, and 24 months of treatment.

Skin sensitivity

In the Pharmalgen and the Alutard groups most patients showed a reduction in SPT after 24 months (Fig. 6).

Sting challenge

All patients challenged (Table 9) tolerated the stings well. Even though some of the patients had detectable amounts of specific IgE and low titer of specific IgG, none had systemic reaction and the local reactions were moderate.

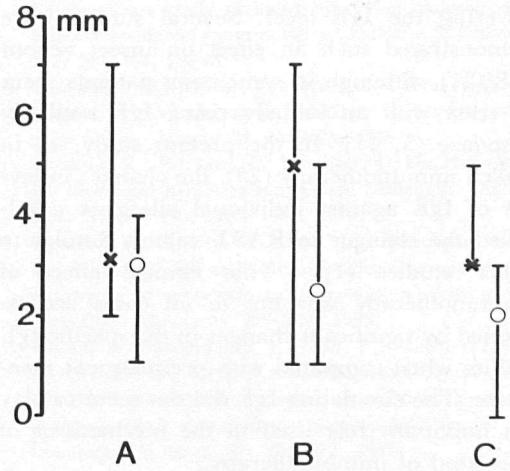


Fig. 6. Diameters of skin prick tests with yellow jacket venom (Soluprick) before (×) and after (○) treatment for 2 years with Alutard (A), ALK aq. (B) and Pharmalgen (C). Medians and 25 % percentiles.

DISCUSSION

Systemic side effects of immunotherapy in patients with inhalant allergy would often show up as deterioration of ongoing symptoms. In contrast, patients with venom allergy have only experienced few allergic episodes previously – and these reactions as well as the possible systemic side effects – could be very alarming. In insect allergy it is therefore of special importance to find extracts and dose regimens with a minimum of such side effects. Although a rush regimen of 6 h (23) seems to have local side effects only, systemic side effects would normally be a general finding in both fast and slow dose regimens (1, 19). The symptoms in the present study have not been very severe and they are comparable to those seen in other studies aiming at the same maintenance dose (1, 19).

A rather large number of the patients experienced local side effects. Since most of the swellings were only slightly larger than the arbitrarily defined limit of acceptable reactions (i.e. 5 or 8 cm in diameter) they meant little discomfort to the patients.

High levels of specific IgE in serum are generally considered to be indicative of clinical sensitivity to allergens (24). Consequently, successful immunotherapy could (in theory) act by

lowering the IgE level. Several studies have demonstrated such an effect on insect venom IgE (21), although in some cases patients seem to react with an initially rising IgE antibody response (5, 11). In the present study, as in pollen immunotherapy (25), the changes in levels of IgE against individual allergens paralleled the changes in RAST values. Similar to other studies (1, 4) the clinical effect of immunotherapy was not in all cases accompanied by significant changes in the specific IgE values when compared with pretreatment measures. The circulating IgE did not seem to play an important role itself in the mechanisms of this kind of immunotherapy.

Specific IgG has been postulated to act as "blocking antibody", probably preventing the IgE mediated allergic reaction (12). In fact, an increase in specific IgG antibody has been encountered in many studies on insect venom immunotherapy (8, 11, 18, 20), the major effect probably being on the IgG₄ subclass antibodies (3). Levels of specific IgG of more than 400 u/ml (21) or 5 µg/ml (7) have been suggested as protective. In the present study significant changes of longer duration in specific IgG were only observed in the group receiving Al(OH)₃ adsorbed extract, although all three groups seemed to be equally protected by the treatment. The evidence for circulating IgG as a factor of major importance in this kind of immunotherapy can thus still be discussed (1, 17).

In the diagnostic phase the correlation between case history and skin test seems to be a little better than between case history and the amount of specific IgE (1, 15). In studies where changes in skin test results following immunotherapy have been observed (19) one has to rule out that these changes are due to the natural history alone. Little, if any, effect of immunotherapy on skin sensitivity is a general finding (17, 18, 23).

At present, sting challenge seems to be the only reliable way of evaluating the effect of venom immunotherapy, and identification of the stinging insect and a full dose sting are only achieved by an intentional challenge. In other studies immunotherapy with insect venom has

been fully effective in nearly all patients (1, 4, 10, 19, 20, 23). None of the 19 patients challenged in our study developed systemic reactions, thus extract and dose regimens encountered seem to be at least as effective as those described in previous studies.

In general, the three investigated extracts were equally effective in terms of protection. Immunologically, there were some minor differences, the most outstanding being the specific IgG response, which was significantly increased for a longer period in the Alutard treated patients only. When evaluating the feasibility of the treatments no large differences in adverse reactions were observed.

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