

Clustered Immunotherapy with Yellow Jacket Venom

Evaluation of the Influence of Time Interval on *in Vivo* and *in Vitro* Parameters

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To evaluate difference in clinical efficacy, side effects, *in vivo* and *in vitro* parameters, 25 patients allergic to Yellow Jacket were treated with clustered immunotherapy using either 7 or 14 days interval between clusters. Twenty-one patients completed the 6 months' treatment period and four were withdrawn due to adverse reactions (2 cases of anaphylactic shock). Sixteen patients were challenged by in-hospital sting and the clinical efficacy was complete. Local side effects were observed in the majority of patients, but only rarely limited the course of immunotherapy. Skin sensitivity estimated as the venom concentration eliciting a wheal equal to histamine HCl 0.1 mg/ml using intradermal test was significantly reduced after 6 months of treatment. Specific IgE showed an initial increase, thereafter declining to pretreatment levels. IgG subclasses were determined by a triple antibody assay. Only subclasses 1 and 4 showed response. Subclass 4 showed a steady increase contrary to subclass 1 which decreased after reaching maintenance dose. No unambiguous relation between either the absolute value or the change of IgG₁ and IgG₄ at the time of challenge was observed in the patients who tolerated a sting. Furthermore, the IgG response was not correlated to the cumulative dose of venom administered. No simple regulatory function of IgG subclasses in the skin and IgE response was found, and the occurrence of local side effects did not seem to be determined by IgG antibodies. We conclude that clustered immunotherapy with Yellow Jacket venom is highly effective and that the frequency of side effects is acceptable. Neither the IgE nor the IgG response is significantly different with a 7- and 14-day interval between clusters, indicating that a protective dose of venom might be reached within 1 month.

Key words: hymenoptera allergy; immunotherapy; CRIE; RAST; skin test; specific IgG antibodies; clinical efficacy.

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CLINICAL ASPECTS

Some sort of fast-dose-increase regimen might be life-saving in hymenoptera allergic patients who are at risk in the stinging insect season. Using conventional "one-injection-per-week-immunotherapy" protective venom doses are normally reached after approximately 14 weeks. The same might be achieved within 1 week with a rush immunotherapy, but we consider this regimen so riskful that it should be carried out on in-hospital patients only. Using a clustered dose increase regimen (5 injections per visit) a protective dose might be reached within four visits, and no difference was observed between the two groups with either 7 or 14 days interval between clusters. Sting challenge was performed on 16 patients and the efficacy was 100 %, indicating that full protection might be achieved after 1 month of treatment.

The clinical efficacy of venom immunotherapy, provided a sufficient dose is administered, is documented by several studies (8, 9, 10, 23, 28). Furthermore, patients seem to be protected

against generalized anaphylactic reactions after reaching a maintenance dose of approximately 100 µg venom (9).

Due to the rapidly achieved protection some

sort of urgent dose-increase regimen might be valuable, especially to patients at high risk, i.e. during the stinging insect season. Using conventional weekly injection of a single dose the maintenance dose is reached after 12–14 weeks (8). Alternatively, using a rush regimen, i.e. continuous injections to in-hospital patients, the maintenance dose might be reached in about 1 week. The risk of systemic side effects is, however, considerable (5, 22, 23, 28) and the treatment is even more expensive due to hospitalization of the patient. A modified "rush" schedule, implying clusters of injections with rapid escalation of the dose and further dose-increase after an interval of 2–3 weeks, seems to be of value (8). As for an IgG immune response, intervals of 2 weeks should be optimum (26).

This study is an investigation of allergen

dose, adverse reactions and changes in skin sensitivity, specific IgE and IgG in two groups receiving clustered immunotherapy either once a week or with an interval of 14 days.

MATERIAL AND METHODS

Patients

Twenty-five patients referred to the allergy clinic on the basis of a positive history of insect sting-related anaphylaxis were included in the study. Systemic reactions were classified into 4 grades of severity according to a modification of Muller (22). The specific diagnosis of Yellow Jacket allergy was based on intradermal test and RAST. The patients were randomly allocated to the two treatment groups (7 days and 14 days respectively). Patient data of those completing the trial are evident from Table 1. Patients who had been treated with venom within the last 3 years, or had chronic diseases, were not included in the study. Repeated systemic and type III reactions during immunotherapy caused withdrawal.

Venom extracts

Yellow Jacket venom used for diagnosis and immunotherapy was obtained from ALK (Copenhagen, Denmark). Raw venom was obtained from Vespa Laboratories Inc. (Spring Mills, P.A., U.S.A.) and consisted of a mixture of *Vespula* species (primarily *Vespula vulgaris* and *Vespula germanica*). Venom used for skin test and immunotherapy in the rising phase was a lyophilised extract (Aquagen®) containing glycine (0.24 mg/ml). High and low molecular weight substances were removed by column fractionation. The extract was reconstituted to 100 µg/ml and diluted in ALK diluent (saline, human serum albumin, phenol). The extract used for maintenance immunotherapy was an aluminium hydroxide-adsorbed venom preparation (Alutard®, ALK). The same batch of venom was used for both skin test and immunotherapy. Venom used for RAST, CRIE and IgG RAST was a non-dialysed crude extract obtained from Vespa Laboratories Inc. The raw

Table 1

Patient data at the time of entrance to study of those completing 6 months immunotherapy

Group	7 days	14 days	
<i>n</i>	12	9	NS
Age	52 28–71	26 17–45	$P < 0.001$
Sex (female/male)	4/8	4/5	NS
Previous large local reaction	5	1	NS
Atopic disposition	2	3	NS
Systemic reaction	I 2 II 7 III 1 IV 2	3 2 3 1	NS
Pre-treatment SSH	0.8 µg 0.07–3.4	1.7 µg 0.2–1.9	NS
Pre-treatment RAST	46 SU/ml 4–290	15 SU/ml 4–382	NS

Median value and range.

NS = not significant.

extract used for *in vitro* tests was identical to that used for the production of Aquagen® and Alutard employed in this study.

Immunotherapy

Immunotherapy was initiated with 0.02 µg venom and an intended top dose of 200 µg (dosage schedule, see Table 2). During the dose-increase regimen injections were given as clusters with five injections every half hour. The dose at each cluster was given either according to the schedule or as maximum tolerated dose, i.e. the dose resulting in local reactions after 30 min, > 5 cm swelling (immediate local reaction) or a systemic reaction. Clusters were given either once a week (7 days group) or every fortnight (14 days group). The maintenance dose was defined as the venom concentration eliciting a delayed (within the first 2 days) local reaction of 8–10 cm four consecutive times without accompanying systemic reaction. Maintenance dose was continued once every second month.

Sting challenge

Sting challenge with Yellow Jacket (*Vespa vulgaris*) was carried out in-hospital with complete anaphylactic equipment including intravenous line. The insects were allowed to sting for 60 s. Entomological examination was done to establish precise identity of the insect.

Skin test

Intradermal skin test was performed on the volar aspect of both forearms in quadruplicate determinations of histamine dihydrochloride 0.1

mg/ml diluent, and venom (0.001; 0.01; 0.1 and 1.0 µg/ml). No skin tests were carried out within 3 days of antihistamine intake and/or application of steroid ointment. The wheal reactions were outlined after approximately 15 min and the contour transferred to a record sheet. The wheal area was calculated in mm² using a digitizer (Hewlett-Packard 9111 A graphic tablet interfaced to a Hewlett-Packard 85). The mean wheal areas of the allergen reactions were plotted as a function of the venom concentration in a log-log system. A linear regression using the least square method was performed (log area = intercept + slope × log concentration). From the regression line the allergen concentration eliciting a wheal equal to the histamine reaction was estimated (skin sensitivity histamine = SSH) (15). Tests with a correlation coefficient < 0.9 were excluded from the study as non-valid. Repeated tests have shown a reproducibility within 0.5 log step. Skin tests were performed at initiation of immunotherapy, at maintenance dose, and after 6 months of treatment.

Patient serum

Venom blood was drawn immediately before skin testing and immunotherapy. Serum was separated and stored in small aliquots at -20°C until analysed. Samples were only frozen and thawed once.

Radio-allergosorbent test

Specific IgE in serum was measured by RAST using paper discs as the solid phase. Yellow Jacket crude extract was coupled to cyanogen bromide-activated discs and incubated overnight at room temperature with undiluted serum and serum diluted 5 and 10 times. After washing, ¹²⁵I-labelled rabbit anti-IgE (Pharmacia, Uppsala, Sweden) was added and incubated overnight at room temperature. The binding of radioactivity was measured and compared with a reference serum from a person with medium sensitivity to Timothy stated to contain 100 SU/ml. Different dilutions of serum were used in order to compare patient samples

Table 2

Allergen dosage during dose-increase regimen

Cluster No.	Allergen dosage (µg)	Cumulative dose (µg)
I	0.02 - 0.1 - 0.2 - 0.6 - 1.0	1.92
II	1.0 - 1.6 - 2 - 4 - 8	16.6
III	8 - 12 - 16 - 20 - 40	96
IV	40 - 80 - 120 - 160 - 200	600

with the standard in the most linear part of the standard curve. All tests were performed in duplicate and samples from the same patients were run in one set up. The reproducibility of double determinations was within 10 %.

Crossed radio-immunoelectrophoresis

Crossed immunoelectrophoresis was performed with 50 µg raw Yellow Jacket extract run in the first dimension for 30 min (10 V/cm). The second dimension electrophoresis was run overnight, (2 V/cm) against rabbit anti-Yellow Jacket (ALK). CRIE was carried out by incubation of plates with patient serum overnight at room temperature followed by washing and incubation overnight with ¹²⁵I-labelled anti-IgE. Autoradiography was conducted for 1 week, 2 weeks and 1 month and the relative amount of specific IgE bound to the various precipitates graded in CRIE-score 0-8 according to increased radiostaining (27).

Specific IgG

Total and subclass specific IgG were estimated as previously described (6). In short, the IgG-RAST was performed as a triple antibody assay. Yellow Jacket raw extract was adsorbed to polystyrene tubes and incubated with diluted serum as the 1st antibody. Human IgG was

detected by monoclonal mouse antibodies to human IgG and IgG subclasses (2nd antibody), and as a tracer ¹²⁵I-labelled rabbit anti-mouse IgG (3rd antibody) was used. Equipotency was obtained by appropriate dilution of the monoclonal antibodies, and the assay calibrated in relation to a reference serum.

Statistics

Only non-parametric statistics were used. Changes of parameters within the groups were evaluated by a Wilcoxon's signed rank test and differences between the groups by a Mann-Whitney rank sum test. Correlation was evaluated using Spearman's Rho.

RESULTS

Pretreatment tests

Immunotherapy with Yellow Jacket was initiated in 25 patients, and 21 completed the study (12 in the 7 days group and 9 in the 14 days group). The degree of pretreatment reaction to Yellow Jacket was estimated by intradermal test and RAST. The median allergen concentration eliciting a wheal equal to histamine (SSH) was 0.8 µg venom in the 7 days group (range 0.07-3.4) and 1.7 µg in the 14 days group (0.2-1.9). (difference not statistically significant). The RAST value was 46 SU/ml (4-290) in the 7 days group and 15 SU/ml (4-382) in the 14 days group (NS) (Table 1). No correlation (Spearman's test) was found between either pretreatment SSH and RAST or between pretreatment SSH or RAST and the previous systemic reaction.

Immunotherapy

Maximum single doses and cumulative dose during dose-increase phase (aqueous extracts) and after 6 months of treatment (alum absorbed extracts) are evident from Table 3. To reach maintenance dose a median number of five visits (4-7) and 19 injections (18-27) were used in the 7 days group and four visits (4-5) and 20

Table 3
Allergen dosage (median and range)

	7 days group	14 days group
Top dose aqueous	140 120-200	160 100-200
Cumulative dose aqueous	485 330-835	515 319-715
Maintenance dose aluminium-adsorbed	200 100-200	200 100-200
Cumulative dose aluminium-adsorbed	875 370-1100	800 400-950
Total dose	1375 830-1711	1315 808-1515

(19-22) injections in the 14 days group (non-significant difference). At the shift from aqueous- to alum-absorbed extract the dose was fractionated in two times half the top dose and then continued as one dose. No side effects were registered at the extract shift. The allergen top dose and the cumulative dose were not correlated to either the pretreatment skin sensitivity or to pretreatment RAST.

Side effects

Systemic side effects were observed in five patients (2 in the 7 days group and 3 in the 14 days group) and local reactions in almost all patients (Table 4). The systemic side effects included two cases of anaphylactic shock at cumulative doses of 50 and 75 μ g respectively, one case of type III reaction (fever, skin rashes, muscle and joint pain) at a cumulative dose of 94 μ g, one patient with a severe exfoliative dermatitis (cumulative dose 18 μ g), and one patient complaining of persistent pruritus, which later on showed to be caused by scabies. Except for one patient with anaphylactic shock, who completed immunotherapy following a slower dose-increase schedule without further systemic reaction, the above mentioned cases were excluded from the study.

Table 4

Side effects during dose-increase regimen

Group	Local side effects per patient		P
	Immediate (> 5 cm)	Delayed (> 8 cm)	
7 days	0 0-3	3 0-4	NS
14 days	1 0-2	2 0-5	NS

Median and range.

NS = not significant.

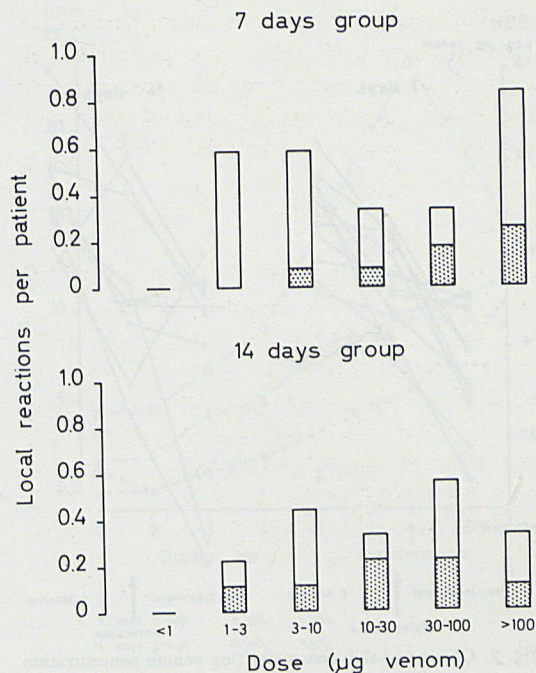


Fig. 1. Local side effects per patient during dose increase regimen. The local reactions are divided into immediate (> 5 cm within 30 min of injection) (hatched columns) and delayed (> 8 cm within 1st day of injection) (open columns). Local side effects are related to the actual dose (half log intervals). No significant difference was found between the two groups.

Local side effects divided into immediate (within 30 min of injection) > 5 cm and delayed (within the first day) > 8 cm were observed in the two groups without significant difference (Table 4). No local side effects were observed at allergen dose < 1 μ g. In the allergen dose range 1-200 μ g both immediate and delayed local reactions occurred almost uniformly distributed with only a faint tendency for more frequent reactions at the higher doses. No difference between the groups was found (Fig. 1). Neither systemic nor local reactions were correlated to pretreatment skin sensitivity, RAST or previous systemic reaction, nor to changes of RAST and IgG during treatment. Subcutaneous noduli caused by the alum content of the slow release extract were observed in approximately 70 % of the patients. Most noduli were observed at the first injection of alum-absorbed venom.

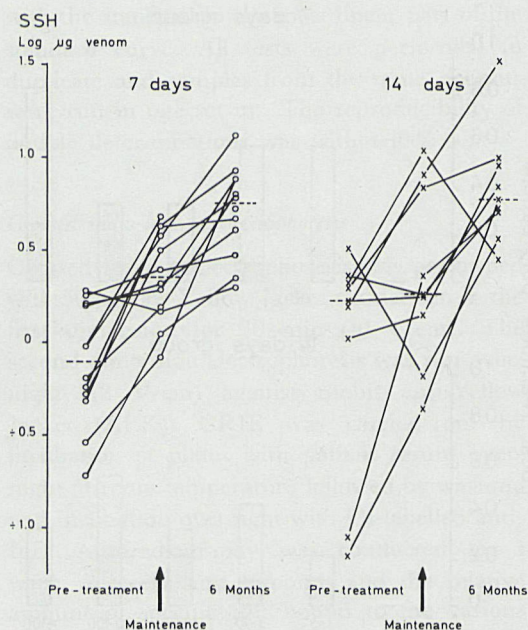


Fig. 2. Change in skin sensitivity (log venom concentration eliciting a wheal equal to histamine HCl 0.1 mg/ml) of the two groups tested before initiation of immunotherapy, at maintenance dose, and after 6 months treatment. The stippled line represents median value. The two groups are not statistically different.

Clinical efficacy

Sting challenge was performed on 16 patients after 4–12 months of treatment. Sting challenge was initiated before IgG assays were performed. A few patients did not want to participate in sting challenge, and in a few others this was considered contra-indicated due to competitive cardiac diseases. All patients tolerated the sting challenge without developing systemic reactions, indicating a clinical efficacy of 100% (95% confidence interval 79–100%). Only a few experienced a local reaction larger than 10 cm. Contrary to normal experience the stinger of the Yellow Jackets remained in the skin in more than half of the challenges.

Skin test sensitivity

No significant difference was found between changes in skin sensitivity (SSH) in the two groups. The median decrease from pretreatment to maintenance dose was 0.46 log step (7 days

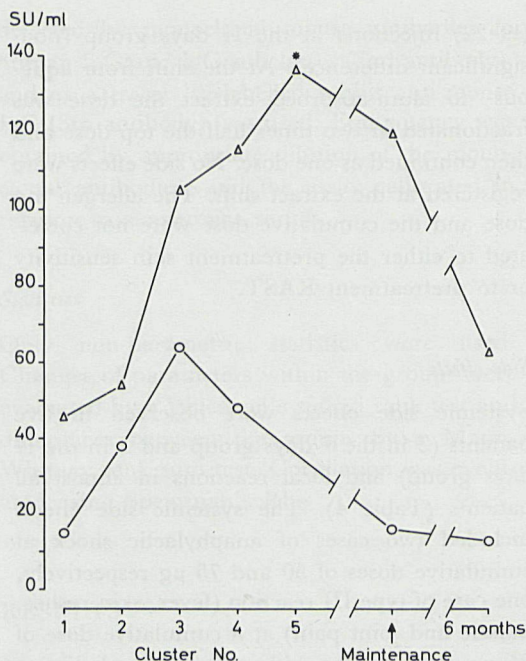


Fig. 3. Changes in RAST value as a function of time. The median value is plotted at each cluster, at maintenance dose, and after 6 months. Difference between the 7 days (Δ) and the 14 days group ($\circ$) are only significant ($P < 0.01$) at cluster No. 4. *Represents only 7 patients who received 5 clusters during the dose-increase phase.

group) and 0.04 log step (14 days group). The total decrease in skin sensitivity was 0.86 log step (7 days groups) and 0.55 log step (14 days group) ($P < 0.01$) (Fig. 2). Further, the figures show that only very few patients become more sensitive at the maintenance dose. In no case was an increased sensitivity to Yellow Jacket found after 6 months of treatment. Change in skin sensitivity was not correlated to the cumulative venom dose either at maintenance or after 6 months. A significant correlation (Spearman's $Rho = -0.75$, $P < 0.01$) was found between pretreatment skin sensitivity and changes in skin sensitivity as a cause of treatment. The highly sensitive patients showed the most pronounced changes.

Circulating specific IgE (RAST)

Changes in RAST values are evident from Fig. 3. The median increase and decrease are lower in the 14 days group, but only at cluster number

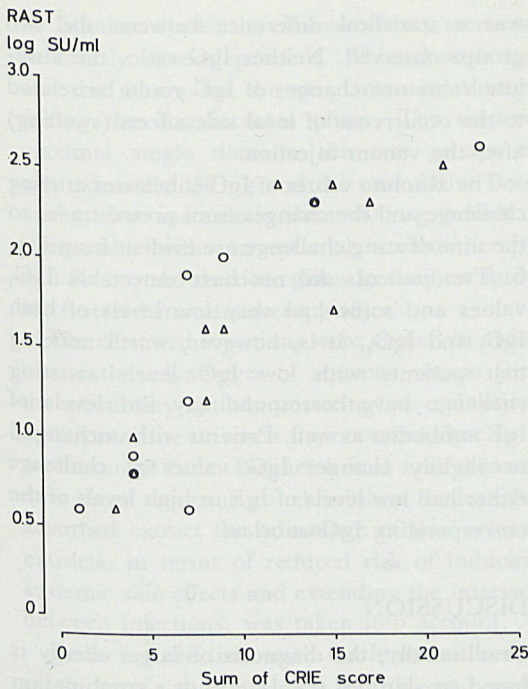


Fig. 4. Correlation between pretreatment RAST (log SU/ml) and the sum of CRIE score (degree of radiostaining of individual antigens after 7 days exposure). Δ = 7 days group and $\circ$ = 14 days group. The correlation is significant (Spearman's Rho 0.91, $P < 0.001$).

4 is the difference significant ($P < 0.01$). In the 14 days group the IgE response has returned to pretreatment values at the maintenance dose. In the 7 days group this is not quite achieved after 6 months of treatment. Changes in RAST values at maintenance dose and after 6 months were not correlated to the cumulative allergen dose given to the patients.

IgE-CRIE

Based on IgE-CRIE seven allergens were found in the Yellow Jacket extract. There was a tendency for the sum of CRIE-score for individual patients to be slightly, but insignificantly higher after 6 months of treatment compared with the pretreatment value. Furthermore, no difference in changes between the individual allergens during treatment was found. The sum of CRIE-score was significantly correlated (Spearman's Rho 0.91 and 0.86 respectively) ($P < 0.01$) to

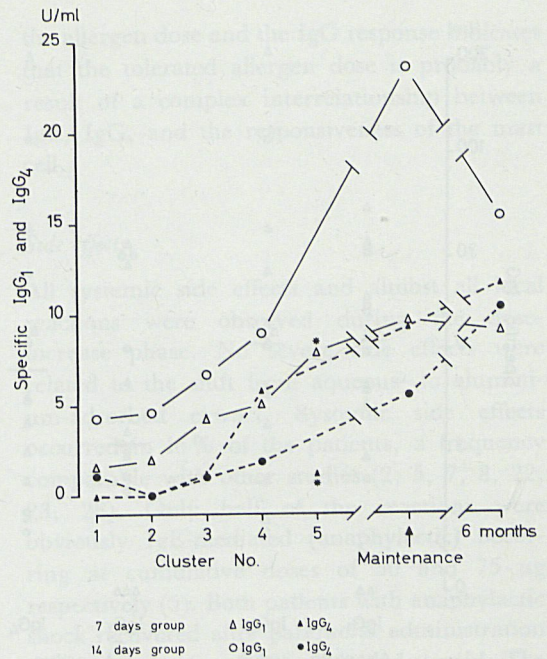


Fig. 5. Changes in IgG subclass as a function of time. The median values of the 7 days group (Δ IgG₁ and $\blacktriangle$ IgG₄) and the 14 days group ($\circ$ IgG₁ and $\bullet$ IgG₄) are plotted at each cluster, at maintenance dose, and after 6 months. The difference between the two groups was not significant. * Represents only 7 patients.

both the pretreatment and the 6 months' value of specific IgE (Fig. 4). No correlation was found to the skin test sensitivity. The main purpose of CRIE was to survey for induction of new IgE antibodies directed against individual allergens present in the treatment extract to which the patient did not react previously. This was not the case in any patient.

Specific IgG

The only IgG response observed was in subclasses 1 and 4. Very low levels of specific IgG₁ and IgG₄ were observed before initiation of immunotherapy. The IgG₁ titer increased until maintenance dose and declined at 6 months, but not to pretreatment levels (Fig. 5). The median value at maintenance dose in the 14 days group was more than double that of the 7 days group, but the difference was not significant. IgG₄ increased during the entire study period

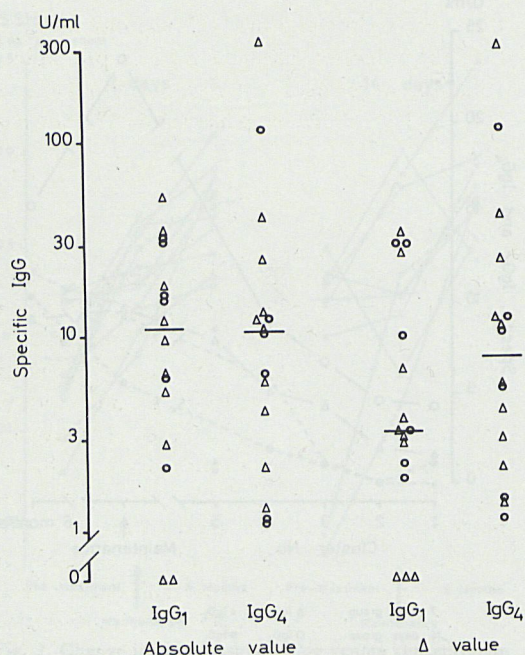


Fig. 6. Absolute values of IgG₁ and IgG₄ and changes from pretreatment levels to the time of sting challenge. Δ = 7 days group and $\circ$ = 14 days group.

without statistical difference between the groups.

Changes in IgG₁ and IgG₄ were not correlated to the total allergen dose after 6 months (Rho 0.267 and 0.158 respectively). The changes in skin sensitivity after 6 months of treatment were not correlated to changes of either IgG₁ (Rho -0.078) or to IgG₄ (Rho -0.138). There appears to be some sort of relationship between specific IgE and IgG. The changes in RAST value after 6 months of treatment were significantly correlated to IgG₁ response (Rho 0.617, $P < 0.01$) and to a lesser degree to IgG₄ (Rho 0.489, $P < 0.05$). This is in contrast to the relation between RAST changes and the IgG₄/IgG₁ response (Rho 0.041 and -0.001 at maintenance dose and after 6 months, respectively).

The IgG₄/IgG₁ ratio increased from approximately 0.1–1.0 during the 6 months treatment. The IgE/IgG₁ ratio decreased about 5 times in the same period. The IgE/IgG₄ ratio increased during the first injection clusters and declined to pretreatment levels at 6 months. In no case

was a statistical difference between the two groups observed. Neither IgG ratio, the absolute value nor changes of IgG could be related to the occurrence of local side effects (swelling) after the venom injection.

The absolute values of IgG subclasses at sting challenge and the changes from pretreatment to the time of sting challenge are evident from Fig. 6. Two patients did not have detectable IgG₁ values and some had very low levels of both IgG₁ and IgG₄. It is, however, worth noticing that patients with low IgG levels at sting challenge have correspondingly low levels of IgE antibodies as well. Patients with unchanged or slightly changes IgG values at challenge either had low levels of IgE or high levels of the corresponding IgG subclass.

DISCUSSION

Traditionally, the diagnosis of insect allergy is based on skin test (13, 14, 29) or a combination of skin test and RAST (2, 20). In the present study all patients were both skin test and RAST positive, but contrary to reports by others (1, 5, 20) no correlation was found between skin test and RAST. Furthermore, the referring systemic reaction was correlated to neither pretreatment skin sensitivity nor to RAST. The interpretation of this is that neither skin test nor RAST can be used to predict patients who are at risk of repeated systemic reactions as candidates for immunotherapy (23, 24). The pretreatment skin and RAST sensitivity was obtained from 3 months to 2 years after the systemic reaction and is a function of both the decline in sensitivity from the time of the systemic reaction until the test, and the reactivation of the IgE immune response caused by the sting resulting in the systemic reaction.

Immunotherapy

Several studies have documented the clinical efficacy of pure venom immunotherapy compared with whole body extracts (14, 16). Furthermore, a maintenance dose of 50 μ g results in a 79 % clinical efficacy which can be raised to 96–100 % by increasing the dose to 100 μ g (8,

9). In other words, the clinical efficacy seems to be clearly dose dependent. This observation was the reason for the very intensive dosage during the dose-increase phase. The median maximal single dose in the two groups is approximately 150 μg and the cumulative dose during dose increase is about 500 μg , which is comparable with conventional immunotherapy and far beyond the dose given in the first study of clustered immunotherapy (8).

The clustered injections were chosen in order to use the advantages of aqueous venom (i.e. the possibility of administering several injections per visit) to reach maintenance dose in a minimum of time. By the shift at maintenance dose from aqueous to aluminium hydroxide-adsorbed extract the advantage of slow release extracts, in terms of reduced risk of inducing systemic side effects and extending the intervals between injections, was taken into account.

Reducing the time used for immunotherapy might be of value, not only from a cost benefit point of view, but also - of even more importance with venom allergy - in order to obtain rapid protection for patients who are at risk of life-threatening stings during the insect season. The present study has shown that, using a clustered regimen, a dose which is normally regarded sufficient to result in about 100 % clinical efficacy (8, 9) might be reached in 4 weeks. Compared with conventional immunotherapy (8) this is considerably time-saving. Using a rush regimen the protective dose can be reached within a minimum of time (5, 17, 22, 23, 25, 28), but from our own experiments the frequency of adverse reactions is too high, making it unfit for routine immunotherapy and, furthermore, we find the risk of such magnitude that this treatment should only be performed on in-hospital patients (5, 23, 28).

The maintenance dose was defined as the maximum tolerated dose, and, contrary to what might be expected, neither the top dose nor the cumulative dose was correlated to pretreatment skin sensitivity or RAST. From this it may be concluded that factors other than the pretreatment IgE reactivity determined the tolerance of venom administered during immunotherapy (26). Furthermore, the lack of relation between

the allergen dose and the IgG response indicates that the tolerated allergen dose is probably a result of a complex interrelationship between IgE, IgG, and the responsiveness of the mast cell.

Side effects

All systemic side effects and almost all local reactions were observed during the dose-increase phase. No severe side effects were related to the shift from aqueous- to aluminium-adsorbed extract. Systemic side effects occurred in 16 % of the patients, a frequency comparable with other studies (2, 5, 7, 8, 22, 23, 28). Only half of the reactions were obviously IgE-mediated (anaphylactic) occurring at cumulative doses of 50 and 75 μg respectively (5). Both patients with anaphylactic shock recovered after parenteral administration of epinephrine, antihistamines and steroid. The type III reaction and the dermatitis subsided within 1 month, after discontinuation of immunotherapy.

Local side effects were experienced by the majority of the patients. Forty percent developed one or more immediate reactions and 80 % delayed reactions. Local side effects were accompanied by considerable discomfort, but only very few caused swelling of the entire extremity. Pain and itching were relieved by topical administration of local anaesthetics. The frequency of local reactions is higher than found in some studies (8, 19, 22, 23), but comparable to others (7, 18, 21, 25, 28). Due to dose reduction in the case of a large local reaction, the predictive value in the prevention of systemic reaction cannot be evaluated. Contrary to other reports (8) we did not find that IgG antibodies had any reducing effect on local reactions. As previously reported (8) local reactions tend to diminish at maintenance dose.

Clinical efficacy

The clinical efficacy of venom immunotherapy can only be evaluated by deliberate sting challenge. No *in vivo* or *in vitro* parameter is able to predict the clinical outcome of immunother-

apy (9, 11, 25), which is in accordance with the results presented. We found the same high degree of protection as published in other studies (2, 8, 9, 10, 11, 23, 25). However, it should be noted that immunotherapy was performed with a mixture of *Vespula* species and the sting challenge with only one. Due to an almost complete allergen cross reactivity between various species of Yellow Jacket the use of several species for immunotherapy should result in the same degree of protection with stings by other species.

Contrary to the reports from the Baltimore group (11) we did not find any relation between either the absolute value or the changes in IgG antibodies and the clinical outcome. However, from the data of Golden et al. (11) it is obvious that the relation between levels of specific IgG and "failures" and "success" at sting challenge is only statistical - "success" as a group had higher levels of IgG compared with "failures" - but regarding the individual patients no absolute level indicated absolute protection. The lack of relation between sting challenge and IgG is reported by others (1, 25, 28). In the present study we found a 100 % clinical efficacy independent of changes in IgE, IgG₁, IgG₄, and IgE/IgG ratio. The lack of relation between *in vivo* and *in vitro* parameters and degree of protection does not seem to be a consequence of the immunotherapy regimen as, firstly, no difference between the 7 days group and the 14 days group was found, and secondly, the parameters in our study are in accordance with those previously reported. The skin sensitivity was significantly reduced after 6 months of treatment, although in no cases did it become negative (12, 22). Other authors report a decline in skin sensitivity and some do not find any consistent changes (2):

Immune response

The IgE response in our study is similar to that previously reported (7, 8, 16, 22, 23, 25, 28), i.e. an initial increase and then either plateauing or decreasing values. The IgE response in the 7 days group was more pronounced compared with the 14 days group, but only signifi-

cant at cluster number 4. After 6 months of treatment the median value was at the same level as the pretreatment value. The IgE response in the 7 days group does not support the view that elderly persons have a reduced IgE immune response compared with youngsters.

The claimed regulatory role of IgG antibodies in the IgE response is either not relevant or rather complex. The IgG₄ subclass response in the two groups is similar and only the IgG₁ response is different. The 14 days group has the most pronounced IgG₁ response and at the same time the least IgE response. On the other hand, the magnitude of the IgE increase is positively correlated to the IgG₁ response, a finding that does not support a short-term down-regulatory function of IgG₁ on the IgE response. Lack of correlation between IgG and IgE is reported by others (3, 4, 23).

Conclusion

The present study indicates that venom immunotherapy, using a clustered dose increase regimen, is highly effective and not complicated by a higher frequency of adverse reactions than is found in conventional immunotherapy. The advantage of a clustered design in terms of time-saving is of value: it provides rapid protection for patients at risk and reduces patient costs by requiring fewer consultations for immunotherapy. No relevant differences in clinical efficacy or *in vivo* and *in vitro* parameters between the 7 days and the 14 days groups were found, indicating that time intervals between clusters of 1 or 2 weeks are of equal validity.

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