

Effects of immunotherapy with a purified standardized allergen preparation of *Dermatophagoides farinae* in adults with perennial allergic rhinoconjunctivitis

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Immunotherapy (IT) was performed for 12 months with a purified and standardized preparation of *Dermatophagoides farinae*. Twenty adults with rhinoconjunctivitis sensitive to house-dust mite were given IT, and a similar group of 11 patients served as open controls. A total of 512 injections were given. Twenty-eight episodes of allergic side-effects occurred, 13 general and 15 local, most of them during the initial incremental dose period. No severe reactions started later than 30 min after the injection. One patient suffered anaphylactic shock. In this patient, specific antibodies fell immediately, followed by an increase within 1 week. The therapy group improved significantly in relation to the open controls in patients' subjective evaluation of symptoms ($P < 0.028$) and skin ($P < 0.0001$) and conjunctival ($P < 0.001$) sensitivity. Specific IgE increased in controls ($P < 0.0001$) but not in IT-treated patients during the "mite season"; that is, there was a significant difference in change during the observation period ($P < 0.0001$). There was also a difference in change of specific IgG between the groups during the first 4–5 months and the whole year ($P < 0.0001$), but not from 4–5 months to 12 months. In contrast to changes in antibody titers (IgG, $P = 0.04$), changes in conjunctival ($P < 0.01$) and especially skin sensitivity ($P < 0.005$) correlated well with subjective improvement. This implies that the skin prick test and the conjunctival test can be recommended for follow-up of IT. Furthermore, we recommend that changes in skin sensitivity be expressed as change in allergen concentration either inducing the same wheal response before and after IT as evaluated by parallel-line bioassay, or inducing the same wheal response before and after IT as that induced by histamine tested at the same time. Finally, our findings support the hypothesis of a switch from IgE + to IgG – during successful IT.

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Allergy to house-dust mite is an important cause of perennial rhinitis in adults in northern Europe. Mygind et al. (20) in Copenhagen and Holopainen et al. (12) in Helsinki demonstrated positive skin tests to house-dust mite in 30 and 10% of atopic patients, respectively; in a Swedish study (14), the corresponding figure was 20%.

The therapy of perennial rhinitis includes various preventive measures and pharmacologic treatment with or without immunotherapy (IT). Since mite allergen avoidance is often not effective, there are, in

reality, two alternatives: pharmacotherapy with or without IT.

IT has been part of allergy treatment since its introduction 80 years ago (21), and it has been found effective in patients with pollinosis (22). The documentation of the efficacy in adults with perennial nasal mite allergy was, until recently, less impressive, but, in 1984, Blainey et al. (1) found IT with a tyrosine-absorbed extract of *Dermatophagoides pteronyssinus* (*Der p*) to be safe and clinically effective but without concomitant changes in specific IgE and

IgG, possibly indicating that too low a top dose (400 Noon units) was given.

In 1988, Ewan et al. (10) used a partially purified extract of *Der p* which contained 60 µg of *Der p I* per 100 000 BU (100 000 BU corresponding to 700 000 IU). After 3 months of treatment with top doses ranging from 1000 to 60 000 BU, mean 17 000 BU, they noted both clinical improvement and serologic changes. However, generalized allergic reactions induced by the injections were common. In 1989, Corrado et al. (3) studied a standardized extract of *Der p*, conjugated to alginate and containing top doses up to 450 000 IU, as determined by *Der p I* content. They observed clinical improvement as evaluated by subjective recovery and nasal provocation tests, but only after long-term treatment.

Recently, two other studies have compared the effect of different extracts. Pécoud et al. (25) studied two different extracts of *Der p*, Pharmedex® and Alvac®. Both induced an improvement in clinical scores, a decrease in skin reactivity, an increase in mite-specific IgG, and a marked increase in nasal tolerance. The Pharmedex extract, with top doses of 40 000–80 000 BU, decreased the skin reactivity, raised the specific IgG more, and influenced the level of specific IgE to a higher extent than did Alvac. McHugh et al. (17) used two *Der p* (Pharmedex and Allpyral®) extracts (top doses up to 60 000 BU in the Pharmedex group) and placebo. They found clinical improvement and a serologic (IgE and IgG) response only in the Pharmedex-treated group.

Thus, most IT trials in mite-sensitive patients have been performed with *Der p* extracts. Our *Der f* extract was biologically standardized by the same method used for standardization of the above mentioned *Der p* (Pharmedex) extracts. Therefore, doses used in these trials can be compared with those used in our trial. Furthermore, a very close resemblance but not a total identity, has been demonstrated between *D. pteronyssinus* and *D. farinae* by immunochemical analyses and skin prick tests (18). An extract of *Der f* containing all important allergens purified and standardized (4) was commercially available and used to determine whether IT with this potent preparation was tolerated and whether such IT decreased sensitivity to *Der f* in adult patients with perennial rhinitis and allergic to *Der f*.

Material and methods

Design

The patients were divided into two groups, one receiving IT and the other serving as open controls. The latter were patients who hesitated to participate in an IT course. The sensitivity was measured by skin test; conjunctival or serum *Der f*-specific IgE-

antibodies did not differ between the two groups. The study was performed during 1 year from October to October. In the region the "mite season" is during the autumn.

The IT group was first given weekly incremental doses of *Der f* for 4–5 months. Subsequent maintenance doses were given at 6–8-week intervals. At every visit, before the injection, serum was taken for subsequent analysis of antibodies.

Skin prick tests and conjunctival provocation tests were performed before the start, after reaching maintenance dose (4–5 months), and after 12 months of IT, and patients subjectively evaluated their improvement on the same occasions.

The control group was also tested, and blood samples for serologic analysis were drawn on three occasions.

Treatment with antihistamines (H₁-receptor antagonists) was allowed except during the month preceding the occasions of evaluation.

Patients

Thirty-one patients with documented allergic rhinitis to house-dust mites were included. Twenty patients, with a mean age of 28 years and a duration of symptoms of 7 years, were given IT, and 11 patients, with a mean age of 24 years and symptoms for 3 years, served as an open control group.

All patients had a history of perennial rhinoconjunctivitis; positive skin prick test; positive radioallergosorbent test, class 3 and 4; and positive conjunctival provocation test to *D. farinae* with the trial extract, 10 000 BU/ml. Some of the patients were clinically sensitive to other allergens (seven to grass pollen, seven to cat, and five to dog). Symptoms from animals were reduced adequately by allergen avoidance, and seasonal symptoms were reduced by antihistamines, sodium cromoglycate, and topical corticosteroids. None of the patients had clinical asthma and none had previously received IT. Pregnancy or planned pregnancy entailed exclusion. Written, informed consent was obtained before treatment.

Allergen extracts

A highly purified, characterized (4), freeze-dried, and biologically standardized preparation of *D. farinae* (*Der f*) (Pharmedex, Pharmacia Diagnostics AB) was used. This material was delivered freeze-dried in four concentrations (100, 1000, 10 000, and 100 000 BU/ml). The same batch was used throughout the study also for *in vivo* diagnosis, i.e., skin prick test and conjunctival provocation test, and for IT.

Recently, the potency of biologically standardized extracts has been reported to be closely associated

with the major allergen content. Thus, the top dose used in this trial, 100 000 BU, roughly corresponds to 100 µg of major allergen (8).

For skin tests, Glycerol Diluent® (50% glycerol and 0.5% phenol in water) (Pharmacia); for conjunctival provocation tests, Albumin Diluent® (0.03% human albumin and 0.4% phenol in saline) (Pharmacia); and for IT, Depot Diluent® (aluminum hydroxide 0.2%) (Pharmacia) were used for reconstitution and dilution. Vials used for diagnosis were reconstituted on the day of testing and then discarded.

Patients' subjective evaluation

After reaching maintenance dose (4–5 months) and after 12 months, each patient's subjective evaluation of the therapeutic effect was recorded as improved (2), somewhat improved (1), unchanged (0), and worse (–1).

Skin prick test (SPT)

Skin prick tests were performed in duplicate on the volar side of the forearm with three 10-fold concentrations, 1000, 10 000, and 100 000 BU/ml. Histamine HCl, 1 mg/ml, was included as reference substance. Lancets of the Østerballe type (24) were used. After 15 min, the urticarial reactions were marked with a soft, fine-tip ballpoint pen, and a transparent adhesive tape was placed over the wheals. The tape was transferred to a record sheet, and the area of the wheals was measured with a digitizer connected to a computer.

The change in skin sensitivity was estimated by two methods. The first method was to estimate before IT and after 4–5 and 12 months the concentration eliciting a wheal of the same size as that of histamine dihydrochloride 1 mg/ml (5.43 mmol/l); the second method was to avoid influence of histamine reactivity, by estimating the change in skin sensitivity by parallel-line bioassay (5, 11).

Conjunctival provocation test (CPT)

The method described by Möller et al. (19) was used, starting with one drop of extract 100 BU/ml and increasing the dose with half-log steps up to 32 000 BU/ml. Criteria for a positive test were congestion of the conjunctival mucosa and itching.

Serologic analysis

Specific IgE and IgG antibodies were examined in samples taken before each injection and in the control group on three occasions (start, 4–5 months, and 12 months).

Specific IgE was determined in duplicate by Phadebas RAST with fivefold dilutions in order to reckon on the straight portion of the reference curve. The results are given in PRU/ml (Phadebas RAST units/ml).

Specific IgG was determined in duplicate by an IgG RAST method. *Der f* allergen was coupled at a titrated optimal protein concentration to paper disks. The solid phase was incubated with serum diluted 1/200 at room temperature for 3 h, followed by incubation with a radiolabeled polyclonal antihuman IgG rabbit antibody. The results are given in percentage of total activity (% T); that is, the radioactivity taken up by the same disks, incubated with a serum standard with high content of *Der f*-specific IgE antibodies.

Immunotherapy

The injection schedule recommended by the manufacturer (Pharmacia, Sweden) was followed. In essence, the initial dose was 50 BU (0.5 ml 100 BU/ml), the doses were doubled every week, and the planned maintenance dose 100 000 BU was reached by 13 weekly injections and then repeated at intervals of 6–8 weeks.

Local and general side-effects were registered at each visit. After each injection, the patient was under observation in the clinic for 30 min, and the diameter of local reactions (swelling) was measured. The patients registered local side-effects at home, measuring the largest diameter of the infiltrate after 6–8 h or at maximum reaction. General reactions or large local reactions, i.e., more than 10 cm in diameter, were followed by a reduction of the subsequent dose. A prolonged interval between the injections had the same consequence. A maximum tolerated dose was defined as the highest dose not inducing any general reaction or any local swelling > 10 cm diameter.

Statistical analysis

Diameters of wheals/concentrations used in skin tests, concentrations used in conjunctival provocation tests, and IgE values were log-transformed, since data were normally distributed only after log transformation. However, IgG levels were treated without log transformation.

For evaluation of changes in skin sensitivity related to histamine dihydrochloride, regression analysis was used. For estimation of the change in skin sensitivity, parallel-line bioassay was also used (11). Changes within and between groups were estimated by Student's t-test (two-tailed).

Addendum

Because of an observed serious side-effect (anaphylactic shock) in one patient, a separate study was performed on changes in anti-*Der f* antibodies in serum in connection with anaphylaxis. Serum was taken immediately after the episode, 1 week later, and 1 month later. Five other patients, given the same amount of allergen and without anaphylaxis, served as a reference group, and serum was taken at the same intervals.

Results

Top doses

The top doses (Fig. 1) were reached after 18–21 visits. Most of the patients – 13/20 – were given 60 000 BU or more.

Side-effects

Side-effects were noted (Fig. 2) almost exclusively during the incremental dose period when 25 episodes (6.5% of injections) were recorded – 10 general (2.6%) and 15 large local reactions (3.9%) of a total of 385 injections. They were seldom seen at doses lower than 10 000 BU. Among the general reactions, four occurred within 30 min. Of these, three were asthma and urticaria induced by 10 000 BU, and in one patient 60 000 BU provoked anaphylactic shock. Furthermore, six late mild general episodes occurred with either rhinitis, conjunctivitis, urticaria, headache, or fever.

During the maintenance-dose period, when 127

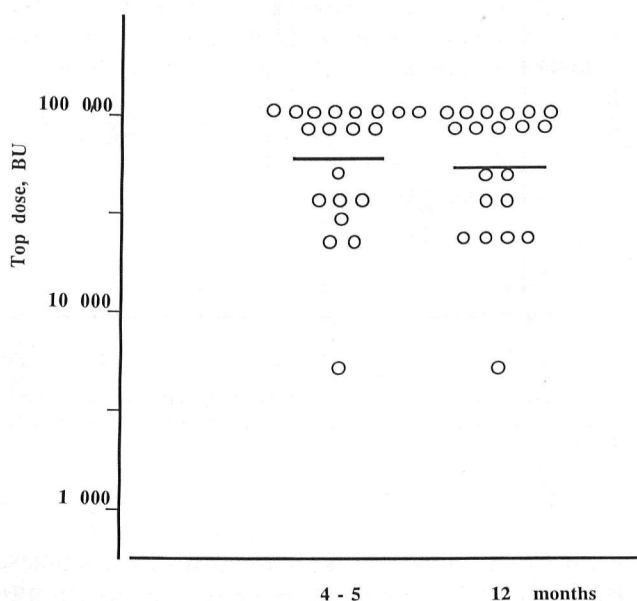


Fig. 1. Median top dose and individual top doses after 4–5 and 12 months' treatment in group given *Der f* immunotherapy.

Effects of immunotherapy

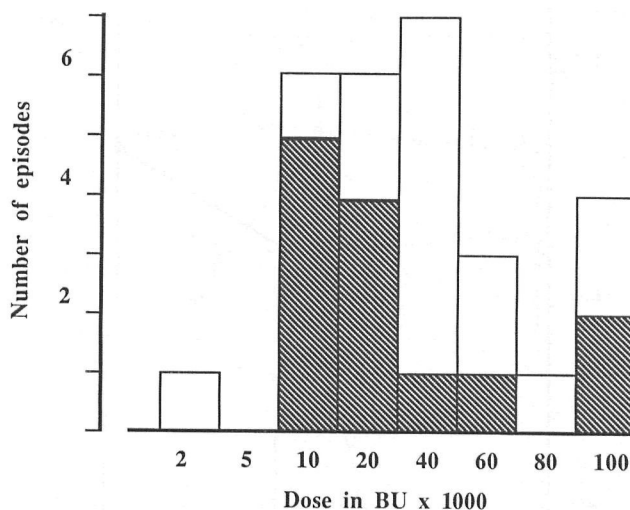


Fig. 2. Number of side-effects and distribution in relation to dose of *Der f* in 512 injections. □ local reactions ($n = 15$); □ general reactions ($n = 13$).

injections were given, there were no large local reactions, but there were three episodes (2.4% of injections) of urticaria which occurred in the same patient given 20 000, 10 000, and 20 000 BU.

Subjective evaluation

Already after 4–5 months, patients on IT considered themselves to be significantly more improved than did controls ($P < 0.028$), and this improvement lasted for the period of study (Fig. 3). The subjective improvement after 1 year correlated to the change in skin sensitivity ($r = 0.66$; $P = 0.0014$), conjunctival sensitivity ($r = 0.57$; $P = 0.0095$), and *Der f*-specific IgG antibodies ($r = 0.46$; $P = 0.04$) during the first 12 months of IT.

Skin sensitivity

There was no detectable difference in skin sensitivity (Fig. 4) between the groups at the start of IT. However, already after 4–5 months ($P < 0.001$), when the maintenance dose had been reached and to a more pronounced degree after 12 months ($P < 0.0001$), the skin sensitivity was significantly lower in the group given *Der f* IT than in the controls. Furthermore, after 4–5 months, the skin sensitivity had decreased 13 times ($P < 0.0001$) in the group receiving *Der f* allergen IT. After 12 months, the skin sensitivity had decreased further (NS) to 18 times ($P < 0.0001$). In contrast, the skin sensitivity of the control group decreased three times after 4–5 months ($P < 0.005$) and only 1.5 times during 12 months (NS) of observation.

When the skin sensitivity was evaluated by estimating the concentration eliciting a wheal of the same

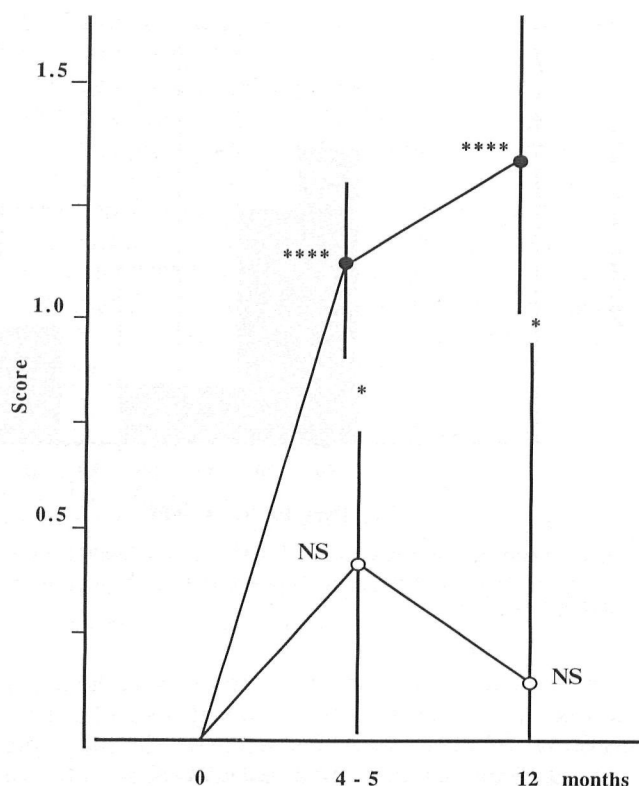


Fig. 3. Subjective evaluation by patients after 4-5 and 12 months' immunotherapy according to scoring system: 2 improved, 1 somewhat improved, 0 unchanged, -1 worse. ● *Derf* immunotherapy group; ○ open controls; | ± 2 SEM; NS = no significant difference; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.

size as that of histamine dihydrochloride 1 mg/ml, the changes were 47 and 54 times in the IT group, and 1.1 and 2.3 times in the control group, after 4-5 and 12 months, respectively. Thus, there was a highly significant difference in change at 4-5 and 12 months ($P < 0.0001$). The larger change in skin sensitivity evaluated by the latter method is a result of larger histamine wheals after IT.

Changes in skin sensitivity correlated to subjective clinical improvement (see above), but only in the active group. Furthermore, changes in skin sensitivity, as evaluated by parallel-line bioassay, correlated to changes in conjunctival sensitivity ($r = 0.53$; $P < 0.01$).

Conjunctival sensitivity

Sensitivity did not differ between the groups at the start of IT, but there was an increasing difference in conjunctival sensitivity between the groups at 4-5 ($P < 0.05$) and 12 months ($P < 0.001$). Conjunctival sensitivity (Fig. 5) changed during therapy within both groups at 4-5 months, about 8.4 times in the IT group, and 2.2 times in the control group ($P < 0.0001$ and 0.01, respectively). The improvement persisted in the active group (13.5 times,

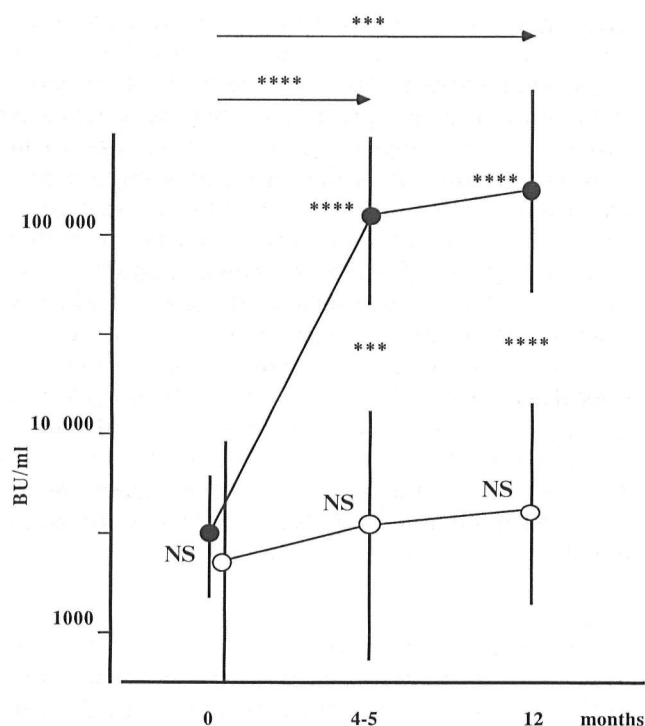


Fig. 4. Skin sensitivity as evaluated by parallel-line bioassay. For calculation of differences in skin sensitivity at 0, 4-5, and 12 months, concentration of allergen inducing wheal of same size as that of histamine dihydrochloride was estimated. Arrows at top of figure indicate significant differences in change between groups. Symbols according to Fig. 3.

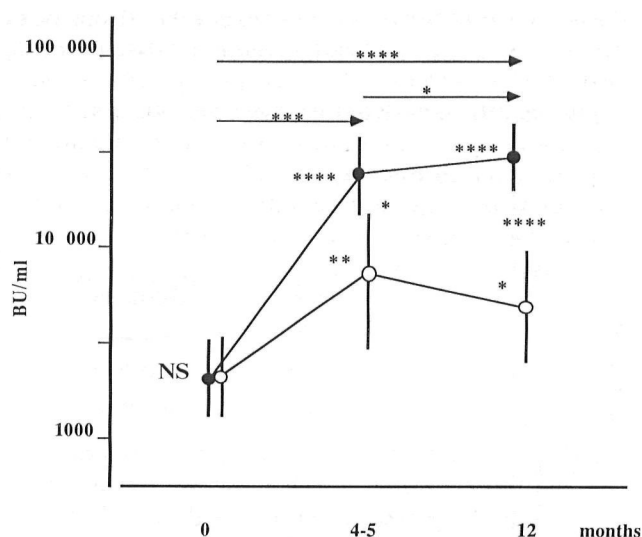


Fig. 5. Conjunctival sensitivity as measured by threshold concentration in conjunctival provocation tests. Symbols according to Fig. 3.

$P < 0.0001$) and partly also in the controls (1.8 times, $P < 0.05$) for 12 months. There was a significant difference in change after 4-5 months ($P < 0.001$) that increased between 4-5 and 12 months ($P < 0.05$).

The difference in change between the start of treatment and 12 months afterwards was more pronounced ($P < 0.0001$).

Changes in conjunctival sensitivity correlated to subjective clinical improvement and to changes in skin sensitivity (see above) but only in the active group.

Circulating IgE anti-*Der f* antibodies

The level of *Der f*-specific IgE antibodies (Fig. 6) did not differ between the groups during the entire study period. During the first 4–5 months, the level of IgE anti-*Der f* antibodies increased slightly in both groups (NS). Between 4–5 and 12 months, the level decreased in the treated ($P < 0.05$) but increased in the untreated patients ($P < 0.0001$), who together accounted for a significant difference in change ($P = 0.001$). The difference of change in circulating antmite IgE during the entire study period was also significant ($P < 0.05$). Changes in *Der f*-specific IgE did not correlate to any other variable studied.

Circulating IgG anti-*Der f* antibodies

During the entire study period, the total anti-*Der f* IgG levels (Fig. 7) remained unchanged in the control group; that is, 190, 191, and 202% T at 0, 4–5, and 12 months, respectively. In contrast, in patients receiving *Der f* IT, IgG levels increased significantly during the first 4–5 months of study ($P < 0.0001$) and then remained largely unchanged (173, 257, 243% T at 0, 4–5, and 12 months, respectively). Thus, there was a significant difference between the groups in change of IgG antibodies during the first 4–5 and 12 months of IT, respectively ($P < 0.001$). The levels of IgG anti-*Der f* antibodies differed significantly between the groups only at 4–5 months ($P < 0.006$).

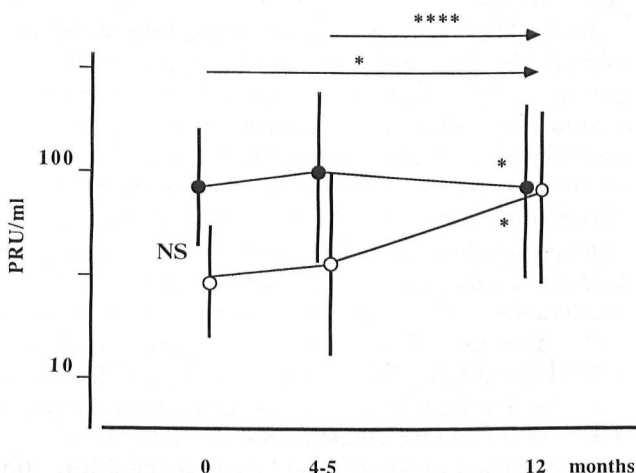


Fig. 6. *Der f*-specific IgE antibodies as measured by RAST. Symbols according to Fig. 3.

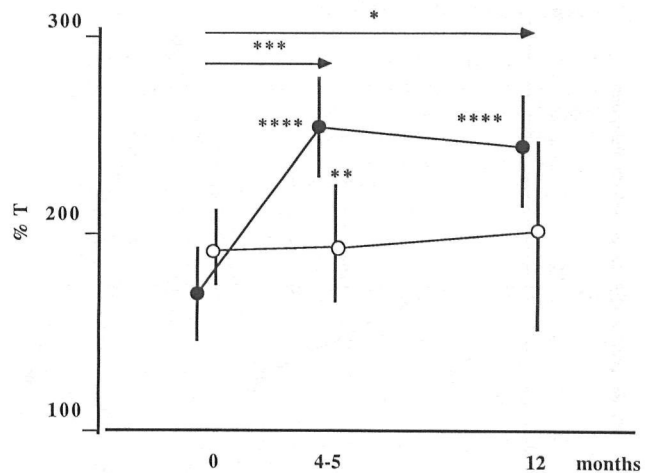


Fig. 7. *Der f*-specific IgG antibodies as measured by RAST. Symbols according to Fig. 3.

Changes in *Der f*-specific IgG did not correlate to any other variable studied.

Anaphylaxis

The patient with anaphylactic shock was treated with epinephrine (0.2 mg), corticosteroids (100 mg), and calcium chloride (0.5 g) intravenously. He recovered rapidly, and some hours after the incident he left the clinic in good health. The amounts of antibodies during the shock and 1 and 4 weeks later are given in Figs. 8 and 9. The antibody levels before the injection in percentage of pretreatment levels are shown. One hour after the injection, specific IgE and especially IgG antibodies were reduced, followed within 1 week by a pronounced increase of antibody titers. Three weeks later, the levels of both antibodies had reached lower levels than before the injection.

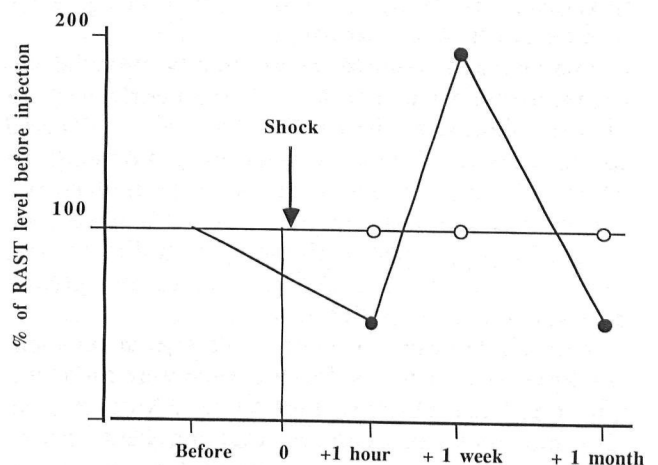


Fig. 8. *Der f*-specific IgE antibodies in percentage before and 1 h, 1 week, and 1 month after anaphylactic shock. Corresponding amounts in reference group are included. ● patient with anaphylactic shock; ○ reference group.

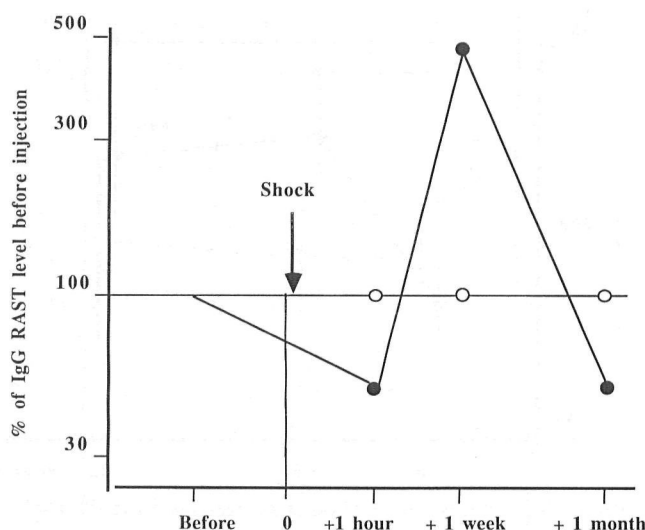


Fig. 9. *Der f*-specific IgG antibodies in percentage before and after anaphylactic shock. Symbols according to Fig. 8.

causing anaphylaxis. No similar changes (Figs. 8 and 9) were seen after 1 and 4 weeks in five control patients (reference group) who were given the same dose (60 000 BU) but who did not suffer anaphylaxis.

Discussion

There have been demands for purified and standardized allergen preparations for IT and for documentation of both clinical and immunologic effects of such therapy. This study fulfills those demands. In a group of patients with rhinoconjunctivitis sensitive to house-dust mites (*D. farinae*), IT with a well-defined preparation of *Der f* (4) was successful. Thus, there was subjective improvement of nasal symptoms, correlated to reduced skin and conjunctival sensitivity; that is, the desirable consequences of effective IT were shown.

The changes were observed already after the initial incremental dose period. Such an early effect of IT was observed also by Urbanek et al. (27) and Bousquet et al. (2) in grass pollinosis. We found the effect to be even greater after the maintenance-dose period. The methods for SPT and CPT have been adopted from earlier methodologic studies (5, 19); they are easily used, sensitive, and without greater discomfort for the patients.

We included estimation of the change in skin sensitivity both with and without considering the histamine reference. This was done because Stuckey et al. (26) and others have shown that the sensitivity to histamine is related to the number and size of skin test responses and to total IgG. Thus, it could be expected that the histamine response would decrease during IT. Our findings indicate that the histamine

wheals increased during IT rather than decreased; that is, a result opposite to the findings by Stuckey et al. (26). On the basis of these findings, we recommend that both types of evaluation be used when results are reported from IT studies.

We evaluated the change in concentration eliciting the same reaction before and after IT by parallel-line bioassay (6, 7, 11) and the change in concentration eliciting the same reaction as histamine 1 mg/ml before and after IT by regression analysis. These methods better describe changes in skin reactivity than methods based on changes in wheal size, as used by most other authors. Since both these methods describe the change in skin sensitivity in terms of change in concentration, we recommend that either of them be used to determine changes in skin reactivity in trials evaluating changes in skin sensitivity induced by IT or antihistaminic drugs.

The antibody response included changes in specific IgE and IgG seen in earlier studies with other allergens (22); that is, for IgE, an initial increase followed by a decrease and after that a reduced increase during the "season", and, for IgG, a slow increase to a plateau level. In the absence of a clear relation between clinical improvement and serologic changes, we continued the serologic investigation, including monitoring of allergen-specific IgG and IgG-subclass distribution (9).

Changes in anti-*Der f* IgE were not correlated to any clinical variable. The level increased during the initial part of the study when symptoms such as skin and eye sensitivity decreased. This agrees with previous findings by Urbanek et al. (27) and Ewan et al. (10). These results, in combination with the findings by Malling & Stahl Skov (16) that allergen-specific IgE increases in the circulation and on basophils but the releasability of basophils decreases during IT, support the hypothesis of Lichtenstein (13) that the mechanism behind successful IT is a switch from IgE+ to IgE-.

In this study, 28 episodes of side-effects were registered after 512 injections. All but three general reactions occurred during the initial incremental dose period. There were four serious reactions which appeared within 30 min, i.e., asthma in one patient and urticaria on two occasions and one anaphylactic shock. Of course, the shock event had the greatest clinical significance. After immediate institution of therapeutic measures, the patient recovered without immediate or late sequelae. If not properly treated, some episodes of asthma and urticaria might develop into shock. On the whole, allergic side-effects were moderate, but the high potency of modern extracts requires strict monitoring of IT (15).

A special analysis of changes in specific IgE and IgG in the patient with anaphylactic shock showed a marked initial drop, followed by a pronounced

increase of the antibodies 1 week later. The only comment in the literature on the influence of anaphylaxis during IT on specific antibodies is the remark by Norman (23) that patients who show anaphylactic reaction have high titers of specific IgE antibodies but do not have low levels of IgG antibodies. However, the drop in specific antibodies just after an anaphylactic reaction and the subsequent increase have not been described before. The drop in detectable anti-*Der f* antibodies was probably due to formation of immune complexes.

We confirm earlier findings by Pécoud et al. (25) and McHugh et al. (17). Both these studies used *Der p* extracts. The highly potent Pharmalgen extracts used in these studies had biologic activity like that of our *Der f* extract. Like these authors, we observed clinical improvement and decreased skin and shock organ sensitivity. The changes in mite-specific IgE and IgG antibodies were also similar in all three trials. The agreement among these recent IT trials performed in adults with allergic rhinitis leads to our conclusion that mite IT is effective and can reduce symptoms in mite-sensitive patients with rhinitis. Furthermore, sampling of serum before each injection allowed us to register marked changes in IgE and IgG antibody titers after anaphylaxis, indicating probable initial complexing of antibodies, followed by a marked increase in these antibodies.

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