

- phylactoides aux myorelaxants. Etude rétrospective à partir de 103 chocs. *Ann Fr Anesth Reanim* 1985;4:152-7.
15. Fisher MM, Outhred A, Bowey CJ. Can clinical anaphylaxis to anaesthetic drugs be predicted from allergic history? *Br J Anaesth* 1987;59:690-2.
 16. Green GR, Rosembaum A. Report of the penicillin study group—American Academy of Allergy. *J ALLERGY CLIN IMMUNOL* 1971;48:331-43.
 17. Kraft D, Roth A, Misher P, Pichler H, Ebrer H. Specific and total serum IgE measurements in the diagnosis of penicillin allergy: A long term follow-up study. *Clin Allergy* 1977;7:21-8.
 18. Adkinson NF. Risk factors for drug allergy. *J ALLERGY CLIN IMMUNOL* 1984;74:567-72.
 19. Ertlin RA, Hoigné R, Bruppacher R, Muller U, Ballmer U. Individual disposition for adverse drug reactions: the role of atopy and nonatopic allergic diathesis. Bamberg, West Germany: F. Hoffman - La Roche, 1986.
 20. Rajka G, Skog E. On the relation between drug allergy and atopy. *Acta Allergol* 1965;20:387-94.
 21. Smith JW, Johnson JE, Cluff LE. Studies on the epidemiology of adverse drug reactions. II. An evaluation of penicillin allergy. *N Engl J Med* 1966;274:998-1002.
 22. Assem ESK, Vickers MR. Serum IgE and other in vitro tests in drug allergy. *Clin Allergy* 1972;2:325-34.
 23. Müller U, Morell A, Hoigné R. IgE in allergic drug reactions. *Int Arch Allergy Appl Immunol* 1973;44:667-78.

Prospective study on immunologic changes induced by two different *Dermatophagoides pteronyssinus* extracts prepared from whole mite culture and mite bodies

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Twenty-four children with bronchial asthma and hypersensitivity to Dermatophagoides pteronyssinus by skin titration, specific serum IgE, leukocyte histamine release, and bronchial challenge test were selected for immunotherapy with two partially purified mite extracts. On the basis of age and allergen-specific IgE responses, patients were randomly divided for treatment with either an extract prepared from whole mite culture (WMC) or from purified mite bodies (MB). The content of major allergens was quantified for both extracts. The allergen dose administered during immunotherapy was increased up to the highest tolerated dose. The mean cumulative dose of Der p I was 98.9 µg for the WMC-treatment group and 76.7 µg for the MB-treatment group. Small, but statistically significant, decreases in RAST and crossed radioimmuno-electrophoresis responses to major allergens were recorded after 2 years of treatment. A small increase in specific IgG1 and a large persistent increase in specific IgG4 was recorded in both groups. There was a significant decrease in allergen-specific bronchial sensitivity, skin reactivity, and leukocyte sensitivity in both treatment groups. Between both groups, changes in the different parameters were not significantly different. Our data indicate that immunotherapy with both partially purified house dust mite extracts, WMC, and MB leads to a decrease in allergen-specific IgE responses and induce a response in specific IgG4. Both extracts are comparable in modulating leukocyte and skin sensitivity, as well as bronchial reactivity to mite allergens. (J ALLERGY CLIN IMMUNOL 1988;82:360-70.)

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In many parts of the world, house dust mites are known to be the major allergen source in house dust, containing or producing molecules that are able to induce IgE responses in humans and trigger anaphylactic reactions. Extracts, especially from mites of the genus *Dermatophagoides*, have been demonstrated to induce positive skin tests in atopic patients with

Abbreviations used

WMC:	Whole mite culture
MB:	Mite body
CIE:	Crossed immunoelectrophoresis
CRIE:	Crossed radioimmunoelectrophoresis
SI:	Skin index
HEP:	Histamine equivalent prick
SQ:	Units of biologically standardized, partially purified allergen extracts
Ag ₃₀ :	Allergen dose causing 30% histamine release in vitro
PD ₂₀ :	Provocative dose causing a 20% fall in FEV ₁
BPT:	Bronchial provocation test

allergic airway diseases.¹ In recent years, several groups have been able to identify allergenic molecules from mites; those with the highest allergenic activity have been isolated and purified.²⁻⁴

For years the results of controlled studies on the clinical and immunologic efficacy of immunotherapy with *D. pteronyssinus* extracts have been controversial. Placebo-controlled studies have recently been reviewed by Mosbech.⁵ The design of the different studies is hardly comparable. The type of extracts differed considerably, as did the duration of treatment and parameters used for evaluation of clinical results. Although most of the studies demonstrated statistically significant effects of immunotherapy,^{6,7} in general, clinical results were not impressive. Standardization of the mostly poorly characterized extracts was different. Therefore, until recently, there was no information on the cumulative dose of major allergens that were administered during immunotherapy.

With modern protein-chemical separation methods, it is possible to produce preparations that contain exclusively, or predominantly, those components against which most mite-allergic patients develop specific IgE antibodies. The established manufacturing procedure involves an extraction of WMC followed by the removal of low molecular components of a molecular weight <5000 daltons. The resulting preparation has a very high content of major allergens that mainly originate from the feces of the mites.⁸

By means of techniques that use the difference in size and density of the particles in mite cultures, it is possible to purify MBs alone and produce an allergen extract. This extract, containing the major allergens, as well as considerable amounts of somatic antigens, some of which act as allergens in men, has been characterized recently.⁴

The purpose of our investigation was to evaluate the effect of hyposensitization on in vivo and in vitro

parameters of hypersensitivity with two different extracts of *D. pteronyssinus*: WMC and MB.

MATERIAL AND METHODS

Allergen extracts used for diagnosis and immunotherapy

WMC and MB extracts have previously been demonstrated to contain nine and 15 to 18 allergenic molecules, respectively.^{4,8} The content of the major allergens, *Der p* I and *Der p* II^{3,9} was quantitated by rocket immunoelectrophoresis and CIE, respectively,¹⁰ with mono- or oligospecific rabbit antibodies and internal standards with known antigen content. These were denoted as international units of *Der p* I and *Der p* II¹¹ and microgram weight of *Der p* I.³ At a dilution of 1 HEP, WMC contained 12,000 IU/ml (equal to 2.3 µg/ml) of *Der p* I and 10,600 IU/ml of *Der p* II. At 1 HEP, MB contained 6600 IU/ml (equal to 1.3 µg/ml) of *Der p* I and 14,500 IU/ml of *Der p* II. Allergen extracts for immunotherapy were labeled in SQ units. The conversion factor is 1 HEP equals 20,000 SQ units per milliliter.

Patients

Twenty-four children between 8 and 15 years of age were selected for the study. They all had a minimum of 2 years of history of asthma and did not suffer from seasonal allergy or any other sensitivity that substantially contributed to their symptoms. Additional allergies to other aeroallergens were excluded by skin puncture test with biologically standardized extracts from grasses, birch, mugwort, cat, dog, horses, *Alternaria*, and *Cladosporium*.

All patients were sensitive to *D. pteronyssinus* by skin puncture test (SI, 1 HEP >1), RAST (class 3 to 4), and BPT (threshold concentration necessary to induce PD₂₀ at an allergen concentration of $\leq 10^{-1}$ HEP).

On the basis of age and their allergen-specific IgE responses, determined by CRIE, patients were randomly divided for treatment with either WMC or MB extracts to obtain two groups of 12 patients with IgE responses comparable in strength and specificity. Patients with a previous hyposensitization treatment or any chronic disease were excluded from the study.

Besides inhalation with disodium cromoglycate, patients were not receiving any other continuous medication throughout the study. Additional medication (β_2 -agonists, theophylline, or corticosteroids) was only administered when it was necessary. One week before each follow-up examination, therapy was stopped, and only β_2 -mimetics were allowed up to 12 hours before testing.

Allergen analysis

To measure the allergen exposure in patients' environment, dust samples from their mattresses were collected in the month of December and analyzed by ELISA for the presence of allergens *Der p* I, *Der m* I, and *Der f* I.¹²

D. pteronyssinus was clearly the dominating species in the Bochum area, West Germany, 16/17 homes containing dust levels >1000 ng of *Der p* I per gram of dust. Levels

of *Der f I* or *Der m I* >1000 ng/gm were found in 5/17 homes. Combined levels of *Der p I*/*Der f I*/*Der m I* ranged from 650 to 174,000 ng/gm of dust. Mean allergen concentration was 11,600 and 10,500 ng/gm of dust in the WMC- and MB-treated groups, respectively, levels not significantly different by the Mann-Whitney U test.

Immunotherapy

Injectations were administered subcutaneously in the middle third part of the volar forearm. The allergen dose was increased by weekly injections up to the highest dose. Local reactions were not allowed to be >8 cm in diameter. After the maintenance dose was reached, injections were administered at monthly intervals.

The maximum tolerated dose differed considerably among patients in both treatment groups, ranging from 10,000 SQ units to 100,000 SQ units in the MB-treated group, and from 5000 SQ units to 100,000 SQ units for the WMC-treated group. The mean cumulative allergen dose during treatment during 24 months was comparable in both groups (mean dose of allergen *Der p I* for WMC, 98.9 µg, and for MB, 776.6 µg).

Effect variables

Immediately before the beginning of immunotherapy, after 6, 12, and 24 months, patients were examined by skin titration, BPTs, and histamine release from washed leukocytes, as well as serum levels of specific IgE, specific IgG subclass antibodies, and CRIE.

Skin titration

Skin tests were done in duplicate with a Dome-Hollister-Stier (Köln, West Germany) puncture test needle. Both MB and WMC extracts were tested at the following concentrations: 0.3, 1, 3, and 10 HEP; control histamine chloride (1:1000) and diluent as a negative control were registered in addition. Wheal sizes were expressed by the mean skin wheal diameter

$$D_M = \frac{D_1 + D_2}{2}$$

(D_1 being the maximum diameter and D_2 , diameter perpendicular to the maximum diameter). SIs were calculated as the mean wheal diameter induced by the allergen extract divided by the mean wheal diameter induced by histamine.

BPT

BPT was only performed when a patient's basal value of FEV₁ was >80% of the predicted¹³ after abstaining from bronchodilators. WMC extracts were inhaled from an electric nebulizer (Pari Inhaler Boy, Pari Werk, Starnberg, West Germany; particle size 0.5 to 5.0 µm; output 0.75 ml/min) for 2 minutes at tidal volumes in a titrated way.

Forced expiratory maneuvers were performed 3 and 10 minutes after inhalation. The best of three attempts was used for analyses. The allergen concentration producing at least PD₂₀ was taken as the threshold concentration. Lung

function was registered by a Vitalograph (Vitalograph Medical Instrumentation, Kansas City, Mo.) spirometer 3 and 10 minutes after the inhalation of each allergen extract dilution.

Blood samples

Blood was obtained from each patient at the time of each follow-up examination immediately before provocation tests. Serum samples were kept frozen at -20°C until they were used for antibody assays.

Histamine release from leukocytes

Preparation of leukocytes, histamine release, and histamine assay was carried out as described previously.¹⁴ Serial dilutions of both WMC and MB extract at concentrations ranging from 10⁻⁸ up to 10⁻¹ µg/ml were used for histamine-release experiments with each patient's leukocytes. In all experiments, reaction tubes were in duplicate. Histamine was assayed by the automated fluorometric technique, as described by Siraganian.¹⁵

CRIE

CRIE was carried out as described previously¹⁴ with 20 µg of reference WMC extract and 20 µg of reference MB extract, applied separately on the same plates and with a mixture of rabbit antibodies to WMC and MB.⁴ IgE binding from patients' sera (0.3 ml) to precipitated antigens was scored after 9 days of autoradiography at -20°C on a log² scale with reference to dilutions of the labeled rabbit anti-IgE precipitated in single radial immunodiffusions with swine antirabbit IgG.¹⁴

RAST

Determination of specific IgE by RAST¹⁶ was carried out with CNBr-activated paper disks coupled with 1 mg/ml of WMC (25 disks per milliliter), patient serum (used diluted 1 plus 1 unless otherwise stated), and ¹²⁵I-labeled rabbit antihuman IgE.¹⁷ Results are presented as retained counts in percent of total added activity.

Solid-phase radioimmunoassays for specific IgG1 and IgG4

Patient serum (100 µl) was affinity purified by reaction for 30 minutes with WMC coupled to CNBr-activated Sepharose (500 µg/25 mg). After three washings in 1 ml of 0.05 mol/L sodium citrate buffer, pH 6.2, containing 1.0 mol/L of sodium chloride with subsequent centrifugations, mite-specific antibodies were recovered by addition of 1 ml of 0.05 mol/L sodium citrate buffer, pH 2.7, containing 1.0 mol/L of sodium chloride, by centrifugation, and by decantation of the supernatant. For stabilization, 250 µl of 1.0 mol/L of sodium hydrogencarbonate and 20 µl of 10% bovine serum albumin were added. RASTs, carried out on 23 purified sera and on the same unpurified sera in corresponding dilution (1:12), correlated well ($r = 0.94$) with a regression slope of 0.73, indicating at least 70% recovery of specific antibody after purification.

Polyclonal rabbit antibodies, raised against human IgG1 and IgG4 myeloma protein, were obtained from Dakopatts, Copenhagen, and subjected to immunoabsorption and affinity purification, according to the same procedure as previously used for antihuman IgE.¹⁷ A monoclonal mouse antibody (M11013, previously BAM11) to human IgG4 was obtained from Unipath, Bedford, U.K. These reagents were radioiodinated by the chloramine-T procedure.¹⁸ Paper disks coupled with WMC (1 mg/ml) were incubated with 50 µl of purified patient serum overnight at room temperature followed by three washings and overnight incubation with 50 µl of radiolabeled antibody to IgG subclasses (20,000 cpm). All determinations were done in duplicate. Patient serum assayed for IgG1 was further diluted three times. Results are presented as retained counts in percent of added activity.

Statistics

All tests performed were nonparametric. For differences in mean values between responses at time 0 and later, Wilcoxon matched-pair signed-rank test was applied. Mann-Whitney U test was used to calculate differences in mean values between changes in response for WMC-treated versus MB-treated groups. Correlations were tested by the Spearman rank correlation test.

RESULTS

Immunotherapy in general was well tolerated in both treatment groups. The maximum tolerated dose varied within a range of 5000 and 100,000 SQ units. In a few cases, we observed mild systemic reactions (wheezing and rhinitis) at the highest dose, which disappeared after dose reduction. Severe side effects were not observed.

Since it was not practicable to measure clinical efficacy of the treatment by clinical parameters (symptom score cards and peak flow values) because of noncompliance of the children, airway tolerance to allergens was determined only by titrated bronchial challenge test, indicating threshold concentration of the WMC extract inducing an immediate bronchial response of PD₂₀.

Mean bronchial sensitivity before the start of immunotherapy was not significantly different between treatment groups. Individual PC₂₀ values varied over a wide range (WMC, 0.001 HEP up to 1 HEP; MB, 0.001 HEP up to 0.5 HEP) (Fig. 1, A and B). After 6 months, there was a significant ($p < 0.01$) decrease of allergen-specific bronchial reactivity in both treatment groups, which was also demonstrable after 12 and 24 months, excluding seasonal variations caused by possible seasonal differences in allergen exposure. Among 23 patients after 12 months of immunotherapy, 16 patients demonstrated an increase in PC₂₀ values of at least 1 log dose. There were no significant

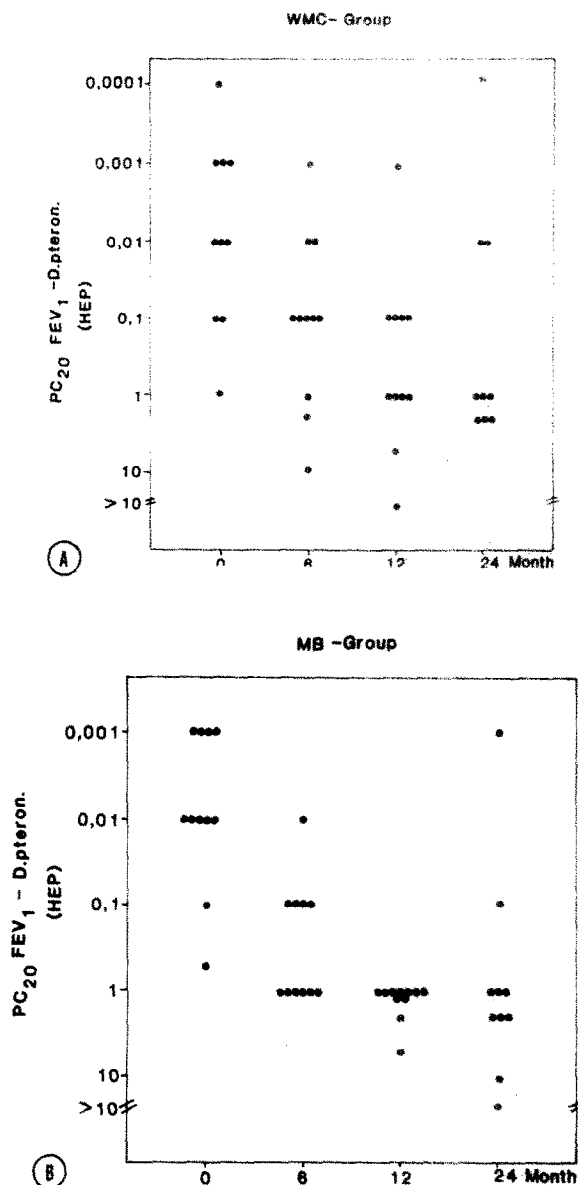


FIG. 1. Threshold concentration of *D. pteronyssinus* extract (WMC) inducing bronchoconstriction with a decrease in FEV₁ of at least 20% before and after 6, 12, and 24 months of immunotherapy. A, In the WMC-treated group. B, In the MB-treated group.

differences between treatment groups in the changes of bronchial reactivity to the WMC allergen extract induced by immunotherapy (Table I).

Mean skin wheal diameters induced by MB and WMC extracts were similar in both treatment groups before therapy. The mean reaction to 1 HEP was comparable to the mean wheal diameter of the histamine-induced control wheal, indicating that our groups of mite-sensitive children were reacting similarly, as did individuals used for biologic standardization of the

TABLE I. Comparison of study parameters between the WMC- and MB-treatment groups before and after treatment (mean values and SEM)

Parameter	Before treatment		6 Mo		12 Mo	
	WMC	MB	WMC	MB	WMC	MB
SI (HEP)						
MB (3)	1.42 ± 0.38	1.63 ± 0.36	1.31 ± 0.13	0.97 ± 0.15*	0.94 ± 0.15	1.13 ± 0.15
MB (10)	1.46 ± 0.25	2.13 ± 0.33	1.88 ± 0.29	1.42 ± 0.26*	1.44 ± 0.23	1.47 ± 0.21
WMC (3)	1.45 ± 0.25	1.79 ± 0.46	1.65 ± 0.31	1.42 ± 0.23	1.17 ± 0.26	1.02 ± 0.21
WMC (10)	1.79 ± 0.33	2.15 ± 0.38	1.96 ± 0.25	1.53 ± 0.28	1.46 ± 0.28	1.06 ± 0.18
BPT-WMC†	-2.27 ± 0.38	-2.12 ± 0.27	-0.97 ± 0.38	-0.54 ± 0.21	-0.45 ± 0.34	-0.09 ± 0.07
LS-MB‡	-4.28 ± 0.40	-4.32 ± 0.54	-3.20 ± 0.25	-3.10 ± 0.13	-3.24 ± 0.23	-3.23 ± 0.36
LS-WMC§	-3.15 ± 0.23	-4.62 ± 0.76	-3.52 ± 0.76	-3.34 ± 0.50	-2.84 ± 2.73	-3.58 ± 0.72

*Significantly larger changes in the MB-treated group ($p < 0.01$).

†Bronchial sensitivity to WMC extract (log threshold concentration in HEP).

‡Leukocyte sensitivity to MB extract (log Ag₅₀ in micrograms per milliliter).

§Leukocyte sensitivity to WMC extract (log Ag₅₀ in micrograms per milliliter).

mite extracts. In both treatment groups after 24 months, we observed a significant decrease of wheal diameters induced by both MB and WMC extract ($p < 0.05$ in the MB-treated group for 3 HEP with bronchial extracts, $p < 0.05$ with WMC extract, and $p < 0.01$ with MB extracts in the WMC-treated group for 3 HEP).

Mean SIs calculated from each patient's ratio of wheal diameter induced by allergens and wheal diameter induced by histamine after 24 months, also significantly ($p < 0.05$) decreased during immunotherapy, as illustrated in Fig. 2 for 3 HEP. As with BPT, changes were comparable in both groups.

Leukocyte sensitivity was expressed by the allergen concentration inducing 30% histamine release in the ascending part of the dose-response curves (Ag₃₀). As expected, basophil sensitivity to both MB and WMC extracts varied over a wide range of approximately 4 logs before immunotherapy. The mean sensitivity was comparable in both groups. After 6 months of treatment, basophil sensitivity to both extracts decreased significantly in both treatment groups ($p < 0.01$ for the MB-treated group, challenged with both extracts, and $p < 0.05$ challenged with WMC and $p < 0.01$ challenged with MB for the WMC-treated group). This pattern was sustained after 12 and 24 months (Fig. 3). In the WMC-treated group, leukocyte sensitivity to WMC was decreased in nine of 12 patients after 1 year, among them four patients for >1 log dose. Leukocyte sensitivity to MB increased in 10 of 12 patients, in six patients for >1 log dose. In the MB-treated group, leukocyte sensitivity to WMC increased in 10 of 11 patients (four, >1 log dose) and to MB, 10 of 11 patients (six of 11, >1 log dose).

Neither the changes in basophil sensitivity nor in

allergen-specific skin reactivity correlated with the changes in bronchial reactivity induced by immunotherapy.

All pretreatment sera produced clearly visible IgE binding to at least two antigens in CRIE; most sera reacted with three to five antigens, and one serum reacted with eight antigens. Of nine antigens binding IgE from at least two sera, four were represented with MB extract only. However, the previously defined major allergens, *Der p* I and *Der p* II, and the intermediate allergens, *Der p* 23 and *Der p* Y,⁸ appeared as the most frequently and intensely radiostained antigens.

The number of IgE-binding antigens and the relative intensity of radiostaining among these were generally constant in sera obtained from the same patient during the treatment course. Fluctuations in the total staining intensity (with a minimum value at 12 months) occurred for a few sera. The general trend was one of a slight average decrease at 24 months, compared to pretreatment level. Average CRIE scores for antigens *Der p* I, *Der p* II, and *Der p* Y were reduced by 0.5 to 1 score (Table II). Specific IgE, measured by the percentage binding in RAST, likewise displayed a modest fall. Changes generally reached levels of statistical significance only when data from both treatment groups were combined (Table II).

Changes in CRIE scores to *Der p* I and *Der p* II correlated strongly with each other ($r_s = 0.74$; $p < 0.05$), and at 24 months, both correlated with changes in RAST ($r_s = 0.63$ and 0.63 , respectively; $p < 0.05$) and histamine release ($r_s = 0.60$ and 0.59 , respectively; $p < 0.05$) but not with changes in bronchial or skin reactivity.

The CRIE responses to *Der p* 23 (an MB-associated

24 Mo	
WMC	MB
0.61 ± 0.12	0.72 ± 0.18
1.07 ± 0.24	1.06 ± 0.17
0.74 ± 0.17	0.70 ± 0.18
1.22 ± 0.18	1.14 ± 0.23
-0.79 ± 0.51	-0.08 ± 0.38
-3.31 ± 0.45	-3.29 ± 0.54
-2.44 ± 1.00	-2.73 ± 2.13

allergen³) tended to increase in the WMC-treated group and decrease in the MB-treated group (Table II). However, changes from pretreatment levels were not significantly different between groups at either 12 or 24 months when testing was done by Mann-Whitney U test.

Increase in CRIE score to *Der p* 23 was positively correlated with decrease of allergen-specific bronchial reactivity ($r_s = 0.61$; $p < 0.05$) at 24 months but not with changes in leukocyte sensitivity or skin reactivity. The association between increased response to *Der p* 23 and improved threshold in bronchoprovocation was strongest when only patients in the WMC-treated group were considered ($r_s = 0.85$; $p < 0.05$).

Four sera displayed de novo antigen binding in CRIE at some time during treatment. Reactions were weak to moderate (CRIE score 2 to 4). Three of the four antigens involved were represented by the MB extract alone. One patient, treated with MB extract, gave a transient response at 6 months to an MB antigen. Response to *Der p* 23 was initiated in another MB-treated patient at 24 months. Two patients, treated with WMC, developed IgE antibodies at 12 months to an MB antigen and to *Der p* 23, *Der p* 37, and an MB antigen, respectively.

Average pretreatment levels of specific IgG1 and IgG4 were similar to normal serum (Table II). None of the IgG determinations correlated with pretreatment RAST, and IgG1 did not correlate with any of the IgG4 determinations. Although the actual percentage binding was of very different magnitude in the two IgG4 assays (with polyclonal and monoclonal reagent, respectively), results were strongly correlated ($r = 0.83$; $p < 0.01$).

Levels of specific IgG1 were significantly increased after 6 months of treatment. Numerically, the changes were small, however, and the mean level at 24 months was not significantly different from that of normal

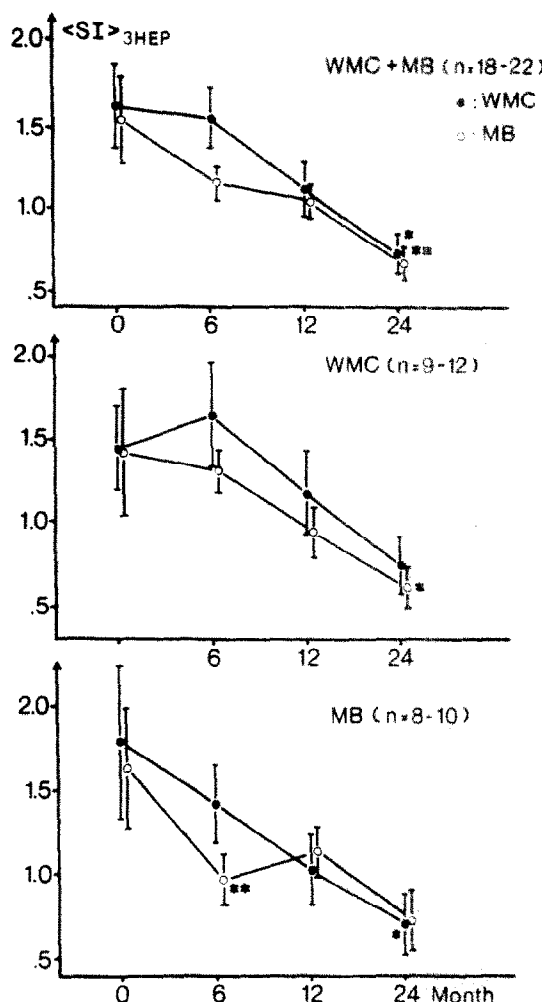


FIG. 2. SIs obtained from skin puncture tests with *D. pteronyssinus* extracts at a concentration of 3 HEP before and during immunotherapy in combined treatment groups and treatment groups WMC and MB. SIs were determined by the ratio of the mean wheal diameters induced by the allergen extract and histamine. The numbers in parentheses indicate the numbers of patients being skin tested.

serum. By contrast, IgG4 levels were increased by a factor of 2.5 to 3 at 24 months, and the mean level was significantly increased relative to normal serum from 6 months forward (Table II; Fig. 4). Changes in IgG1 and IgG4 did not correlate at any time, and none of these parameters correlated with changes in RAST, skin test, histamine release, or bronchial provocation.

DISCUSSION

In children with perennial asthma, mites of the genus *Dermatophagoides*, besides other allergen sources, have been identified as a major trigger of asthmatic episodes. Allergenic proteins from *D. pteronyssinus* induce immediate skin reactions in puncture tests, bronchial obstruction in provocation tests, and

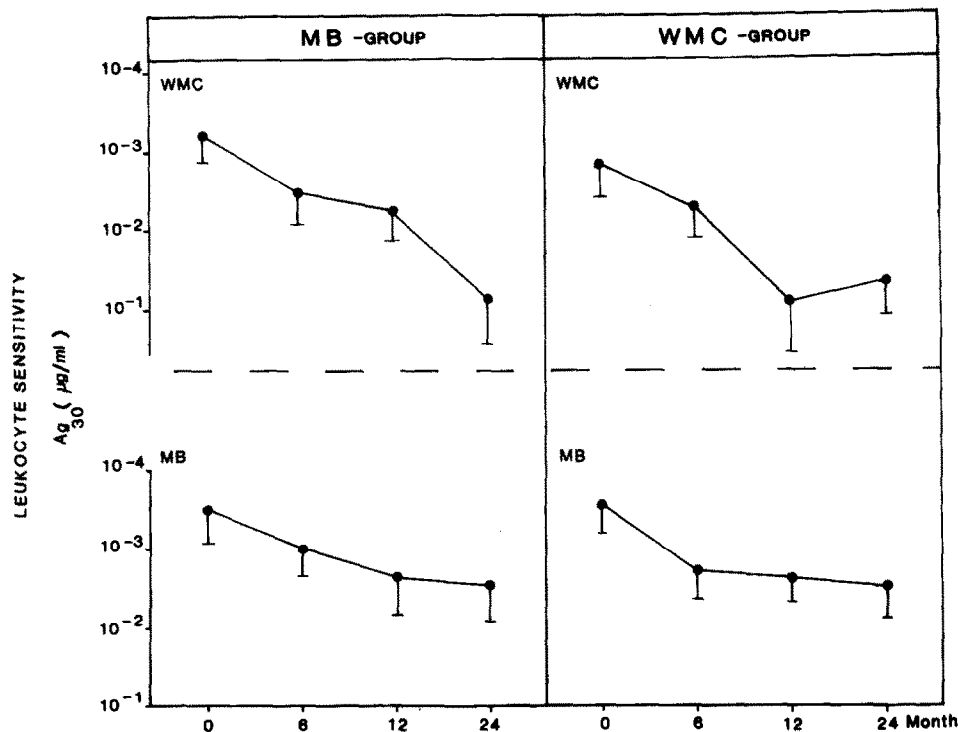


FIG. 3. Mean leukocyte sensitivity to WMC and MB extracts in both treatment groups before and during immunotherapy.

histamine release in vitro on incubation with washed leukocytes. Although the pathogenetic role of mite allergens for the clinical course of asthma is widely accepted, there has, until recently, been controversy on the efficacy of immunotherapy on the clinical course of mite-allergic individuals with asthma.¹⁹⁻²²

Earlier studies used allergen preparations with largely unknown allergen composition and undefined global biologic activity. Recent data, reporting on studies with potent and well-characterized extracts, suggest that both the immediate response to bronchial allergen challenge, as well as the unspecific response to histamine, are influenced by immunotherapy with *D. pteronyssinus* extracts.²³

After a number of mite proteins have been identified as allergens and information exists on patients' spectra of sensitization, as well as the relevance of allergenic proteins for a mite-allergic population,²⁴ the question remains to be answered as to how many allergens should be in an extract to be both most appropriate for treatment of nearly all mite-sensitive patients and also economically acceptable and producible? It has recently been demonstrated for pollen extracts that highly purified extracts, containing only one or two major allergens, do not elicit therapeutic results that are as good as partially purified preparations.

From our data, we conclude that immunotherapy

with the two partially purified house dust mite extracts, WMC and MB, modulates in vitro histamine release from washed leukocytes to mite allergens, as well as skin sensitivity. The coefficient of variants for the results of leukocyte histamine-release experiments performed on different days with 10 to tenfold dilution experiments has been previously demonstrated by us to be in the range of 8% to 35%.²⁶ Therefore, it appears unlikely that a poor reproducibility of basophil sensitivity could explain the observed changes.

Our results do not support the hypothesis that the use of pure MBs as sources for an allergen extract might increase the immunologic efficacy of immunotherapy, *D. pteronyssinus* extract.

Modest reductions in levels of specific IgE were found after the 2-year immunotherapy course of the present study, and the changes recorded could not generally be associated with reduced responsiveness in the airways, or skin, or in basophil leukocytes. This is similar to the situation in most hyposensitization trials with aqueous or tyrosine-absorbed *D. pteronyssinus* extracts in which small and insignificant changes were found after 3 to 12 months of treatment.^{6, 7, 27, 28} However, in one study,²⁹ a large proportion of the patients demonstrated significant rises in specific IgE after 3 months, with the magnitude of the increase being negatively associated with protective effect.

TABLE II. Arithmetic mean IgE and IgG antibody levels during the treatment course

	0 Mo	6 Mo	12 Mo	24 Mo	Normal serum
Rast					
WMC	13.1	12.8	12.5	11.9*	
MB	12.4	12.2	12.0	11.6	2.4
All	12.7	12.5	12.3	11.8*	
CRIE <i>Der p</i> I					
WMC	4.92	4.25	5.18	4.67	
MB	5.41	5.17	4.63	4.38	<1
All	5.15	4.74	4.95	4.50†	
CRIE <i>Der p</i> II					
WMC	4.67	4.13	4.73	4.00	
MB	4.59	4.56	3.56*	3.94	<1
All	4.63	4.36	4.24	3.97*	
CRIE <i>Der p</i> 23					
WMC	2.17	2.38	2.55	2.50	
MB	1.91	1.78	1.38	1.50	<1
All	2.04	2.06	2.05	1.97	
CRIE <i>Der p</i> Y					
WMC	3.08	2.50	2.82	1.83	
MB	3.00	2.72	1.75	1.75	<1
All	3.04	2.62*	2.37†	1.79*	
IgG1					
All	13.8	17.7‡	19.2‡	18.0‡	16.0
IgG4p‡					
All	21.2	43.8‡	52.8‡	54.5‡	15.8
IgG4m§					
All	4.4	9.4‡	11.8‡	12.4‡	4.1

*Significantly different ($p < 0.05$) from pretreatment value by the Wilcoxon matched-pair signed-rank test.†Significantly different ($p < 0.01$) from pretreatment value by the Wilcoxon matched-pair signed-rank test.

‡Assay with polyclonal antibodies.

§Assay with monoclonal antibodies.

The only in vitro parameter in the present study associated with a change in bronchial sensitivity was the CRIE score to antigen *Der p* 23. We note (1) that an increased response to this MB-associated allergen correlated strongly with decreased bronchial sensitivity in the WMC-treated group in which average *Der p* 23 score tended to increase (Table II), (2) that no corresponding association was found between response to *Der p* 23 and histamine release or skin test, and (3) that IgE to this allergen appeared de novo during the treatment course in both the MB- and the WMC-treated group. The changes in response to *Der p* 23 are noticeable but difficult to explain satisfactorily. One possibility is that sensitization to *Der p* 23, uninfluenced by the therapeutic injections, continues to take place as a result of the natural exposure to mite antigens.

Previous studies of the IgG responses to hyposensitization have presented variable results. In two stud-

ies,^{6, 28} small and insignificant rises were reported. One study⁷ reported a transient increase in IgG to F₄P₁ (equal to *Der p* I) at 2 and 6 months, whereas Chapman et al.²⁷ found frequent and large increases in IgG to *Der p* I after 12 months of hyposensitization. In none of these studies was an association between IgG antibody determinations and protective effect of treatment observed.

In the present study, specific antibody responses in two IgG subclasses were determined. A small rise in IgG1 and a large, consistent rise in IgG4 to WMC extract was observed. These findings are in accordance with the subclass-restricted nature of the IgG antibody response observed after prolonged immunization.³⁰ No correlation was found between the increase of the bronchial provocation threshold at 24 months and the IgG1 or IgG4 antibody rises at 6, 12, and 24 months, or the ratio between the IgG1 and the IgG4 rise at 6 months. Thus, our data do not lend

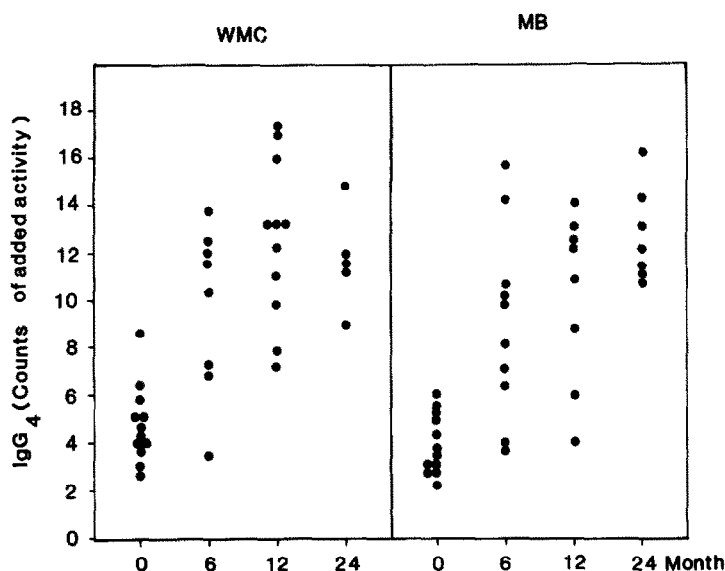


FIG. 4. *D. pteronyssinus* (WMC)-specific IgG4 antibodies before and during immunotherapy with WMC and MB extracts.

direct support to the good prognostic value of an early IgG1 rise and the poor prognostic value of an early IgG4 rise, as recently indicated for grass-pollen and vespid-venom hyposensitization.^{31, 32}

Still, the question remains whether our parameters used for assessment of efficacy are appropriate to predict therapeutic benefit on the long-term clinical course of bronchial asthma.

A number of in vitro parameters have been found to be modulated by immunotherapy, but there is still no evidence that any of the humoral or cellular parameters really correlated with the clinical course of the disease. For both MB- and WMC-treatment groups, basophil sensitivity to mite allergens was significantly reduced, but as in other studies, we were unable to correlate cellular changes to threshold concentrations leading to bronchial obstruction in provocation tests.

Stafanger³³ recently reported that in mite-sensitive children with asthma, neither skin test nor basophil sensitivity offered 100% sensitivity and specificity in predicting the outcome of BPTs.

The reproducibility of a PD₂₀ allergen dose in children at intervals of months or years, to our knowledge, has not been investigated. We therefore cannot exclude the observed changes to be due to a natural course of the disease. For adults, it has recently been demonstrated that PD₂₀ allergen doses at 2-week intervals are highly reproducible.³⁴

Bronchial provocation tests, although they are helpful for diagnosis as well as documentation of therapeutic results, are still highly artificial procedures that do not simulate natural allergen exposure of patients

with asthma. Platts-Mills et al.³⁵ recently demonstrated that natural exposure to allergenic molecules from mites predominantly occurs via dust particles of a size >5 μ m, which only reach the larger bronchi. In contrast, nebulizers used for inhalation produce aerosol particles of 0.5 to 5 μ m in diameter that are inhaled into the periphery of the bronchial tree.

Even if the provocation procedure is standardized and if the availability of well-characterized extracts allows reproducible conditions, they may not allow conclusions about clinical efficacy, since there are no data in the literature relating the results of allergen-specific bronchial reactivity with a long-term clinical evolution of bronchial asthma.

It has been demonstrated that the disappearance of late reactions after BPTs after immunotherapy with *D. pteronyssinus* extracts is related with clinical improvement.³⁶ Late reactions in our study, however, were observed in a small minority of patients only; therefore, they were not suitable as a study parameter during immunotherapy.

An immediate response to an allergen challenge test is most probably influenced by both the degree of sensitivity of mucosal mast cells as well as nonspecific hyperreactivity of the bronchial tree.³⁷ Therefore, changes in bronchial allergen threshold concentration leading to immediate bronchoconstriction might be due to specific and nonspecific mechanisms. Furthermore, in children the degree of bronchial hyperreactivity changes with age.³⁸ Since we did not perform titrated histamine provocation tests, we are unable to discriminate nonspecific from specific effects. However, Formgren et al.²³ recently presented evidence for

nonspecific bronchial hyperreactivity to be influenced by immunotherapy with mite extracts. Sundin et al.³⁹ reported changes in bronchial sensitivity to both allergen and histamine during immunotherapy with a standardized and partially purified cat-dander extract. According to Cockcroft,⁴⁰ unspecific hyperreactivity obviously is not a constant factor in the course of an asthmatic disease, but rather a variable, being influenced by the degree of allergenic inflammation of the bronchial mucosa, which is mediated by products of immunologically triggered mast cells. Immunologic therapeutic procedures influencing the sensitivity of mediator-screening cells therefore should be expected to lead to changes in both nonspecific and specific bronchial hyperreactivity.

REFERENCES

- Murray AB. Sensitization to house dust mites in different climatic areas. *J ALLERGY CLIN IMMUNOL* 1985;76:108.
- Tovey ER, Chapmann MD, Platts-Mills TAE. Mite faeces are a major source of house dust allergens. *Nature* 1981;289:592.
- Lind P. Purification and partial characterization of two major allergens from the house dust mite *Dermatophagoides pteronyssinus*. *J ALLERGY CLIN IMMUNOL* 1985;76:753.
- Lind P, Løwenstein H. Identification of allergens in *Dermatophagoides pteronyssinus* mite body extract by crossed radioimmuno-electrophoresis with two different rabbit antibody pools. *Scand J Immunol* 1983;17:263.
- Mosbech H. House dust mite allergy. *Allergy* 1985;40:81.
- Blainley AD, Phillips MJ, Ollier S, Davies RJ. Hyposensitization with a tyrosine-adsorbed extract of *Dermatophagoides pteronyssinus* in adults with perennial rhinitis: a controlled clinical trial. *Allergy* 1984;39:521.
- Pauli G, Bessot JC, Bigot H, Delaume G, Hordle DA, Hirth C, Thilig R. Clinical and immunological evaluation of tyrosine-adsorbed *Dermatophagoides pteronyssinus* extract: a double-blind placebo-controlled trial. *J ALLERGY CLIN IMMUNOL* 1984;74:524.
- Lind P, Weeke B, Løwenstein H. A reference allergen preparation of the house dust mite, *D. pteronyssinus*, produced from whole mite culture—a part of the DAS 76 study. Comparisons with allergen preparations from other raw materials. *Allergy* 1984;39:259.
- Marsh DG, Goodfriend L, King TP, Løwenstein H, Platts-Mills TAE. Allergen nomenclature. *Bull* 1986;WHO 64:767.
- Løwenstein H. Quantitative Immuno-electrophoretic methods as a tool for the analysis and isolation of allergens. *Prog Allergy* 1978;25:1.
- Ford A, Seargroatt V, Platts-Mills TAE, Løwenstein H. A collaborative study on the first international standard of *Dermatophagoides pteronyssinus* (house dust mite) extract. *J ALLERGY CLIN IMMUNOL* 1985;75:676.
- Lind P. Enzyme-linked immunosorbent assay for determination of major excrement allergens of house dust mite species *D. pteronyssinus*, *D. farinae*, and *D. microceras*. *Allergy* 1986;41:442.
- Polgar G, Promadhat V. Pulmonary function testing in children: techniques and standards. Philadelphia, London, Toronto: WB Saunders, 1971.
- Wahn U, Peters T, Siraganian RP. Studies on the allergenic significance and structure of rat serum albumin. *J Immunol* 1980;6:2544-9.
- Siraganian RP. An automated continuous flow system for the extraction and fluorometric analysis of histamine. *Anal Biochem* 1974;57:393.
- Wide L, Bennich H, Johansson SGO. Diagnosis of allergy by an *in vitro* test for allergen antibodies. *Lancet* 1967;2:1105.
- Løwenstein H, Weeke B. Purification of human IgE and rabbit antihuman IgE. *Scand J Immunol* 1975;4(suppl):3, 459.
- Greenwood FC, Hunter WM, Glover JS. The preparation of ¹³¹I-labelled human growth hormone of high specific radioactivity. *Biochem J* 1963;89:114.
- Fink JN. Immunotherapy of asthma. *J ALLERGY CLIN IMMUNOL* 1985;76:402.
- Patterson R, Grammer LC, Shangnessy MA. Immunotherapy: parameters of assessment. *J ALLERGY CLIN IMMUNOL* 1985;76:394.
- Grant IWB. Does immunotherapy have a role in the treatment of asthma? *Clin Allergy* 1986;16:7.
- Mosbech H, Weeke B. Does immunotherapy have a role in the treatment of asthma? *Clin Allergy* 1986;16:11.
- Formgren H, Dreborg S, Kober A, Lanner A, Olofsson E. A controlled study of immunotherapy with Pharmedgen *D. farinae* mite extract. *Ann Allergy* 1985;55:312.
- Le Mao J, Danden JP, Rabillon J, Lux M, David B. Comparison of antigenic and allergenic composition of two partially purified extracts from *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* mite cultures. *J ALLERGY CLIN IMMUNOL* 1983;71:588.
- Østerballe O. Immunotherapy with grass pollen major allergens: clinical results from a prospective 3-year double-blind study. *Allergy* 1982;37:379.
- Maasch HJ, Fischer B, Wahl R, Wahn U. Comparison of histamine release assay and RAST inhibition as tools for allergen extract standardization. *Int Arch Allergy Appl Immunol* 1984;73:314-20.
- Chapman MD, Platts-Mills TAE, Gabriel M, Ng HK, Allan WGL, Hill LE, Nunn AJ. Antibody response following prolonged hyposensitization with *Dermatophagoides pteronyssinus* extract. *Int Arch Allergy Appl Immunol* 1980;61:431.
- Turner MW, Yalcin I, Soothill JF, Price JF, Warner JO, Hey EN, Chapman MD, Platts-Mills TAE. In vitro investigation in asthmatic children undergoing hyposensitization with tyrosine-adsorbed *Dermatophagoides pteronyssinus* antigen. *Clin Allergy* 1984;14:221.
- D'Souza MF, Pepys J, Wells ID, Tai E, Palmer F, Overell BG, McGrath IT, Megson M. Hyposensitization with *Dermatophagoides pteronyssinus* in house dust allergy: a controlled study of clinical and immunological effects. *Clin Allergy* 1973;3:177.
- Aalberse RC, van der Gaag R, van Leuwen J. Serologic aspects of IgG4 antibodies. I. Prolonged immunization results in an IgG4-restricted response. *J Immunol* 1983;130:722.
- Djurup R, Østerballe O. IgG subclass antibody response in grass pollen-allergic patients undergoing specific immunotherapy: prognostic value of serum IgG subclass antibody levels early in immunotherapy. *Allergy* 1984;39:433.
- Djurup R, Malling H-J, Sondergaard I, Weeke B. The IgE and IgG subclass antibody response in patients allergic to yellow jacket venom undergoing different regimens of venom immunotherapy. *J ALLERGY CLIN IMMUNOL* 1985;76:46.
- Stafanger G. Specific diagnosis of exogenous bronchial asthma in children. *Allergy* 1986;41:110.
- Frølund L, Madsen F, Svendsen UG, Nielsen NH, Weeke B. Reproducibility of responsiveness to a standardized bronchial

- allergen provocation, R., compared to FEV₁ as measurement of response to provocation. *Clin Allergy* 1987;17:217.
35. Platts-Mills TAE, Heymann PW, Longbottom JL, Wilkins SR. Airborne allergens associated with asthma: particle sizes carrying dust mite and rat allergens measured with a cascade impactor. *J ALLERGY CLIN IMMUNOL* 1986;77:850.
 36. Warner JO, Price JF, Soothill JF, Hey RN. Controlled trial of hyposensitization to *Dermatophagoides pteronyssinus* in children with asthma. *Lancet* 1978;2:912.
 37. Stuckey MS. Histamine sensitivity influences reactivity to allergens. *J ALLERGY CLIN IMMUNOL* 1985;76:373.
 38. Riedel F, von der Hardt H. Bronchial sensitivity to inhaled histamine in healthy, nonatopic children. *Pediatr Pulmonol* 1986;2:15.
 39. Sundin B, Lilja G, Graff-Lonnevig V, Hedlin G, Heilborn H, Norrlind K, Peglow K-O, Løwenstein H. Immunotherapy with partially purified and standardized animal dander extracts. *J ALLERGY CLIN IMMUNOL* 1986;77:478.
 40. Cockcroft DW. Mechanisms of perennial allergic asthma. *Lancet* 1983;2:253.

The Hymenoptera venom study I, 1979-1982: Demographics and history-sting data

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The Hymenoptera venom study, a study based on case histories, skin test results and adverse reactions, immunotherapy and adverse reactions, and treatment efficacy, for 3236 Hymenoptera-allergic subjects, was begun in 1979 after the Food and Drug Administration approval of Hymenoptera venoms. Eighty-four Fellows and Members of the American Academy of Allergy and Immunology participated in the study. All subjects had a history of an adverse reaction to one or more Hymenoptera insects. The mean age was 30½ years (range 1 to 83 years). Male subjects accounted for 61.5% and female subjects, 38.5%; 3.1% were beekeepers and 32.3% were atopic. Demographic data were similar for subgroups. There was an average of 2.7 history stings per subject. At least one systemic reaction (SR) was reported by 2866 subjects (89%); 2219 (69%) experienced an SR after their most recent sting before entry into the study, and 70% had experienced only a single SR. Moderate to severe SRs were equally likely after stings of yellow jacket, white-faced hornet, and yellow hornet (65%), honeybee (67%), or wasp (70%), although historical SRs were reported more often after stings of yellow jacket, white-faced hornet, or yellow hornet (30%) than after honeybee (19%) or wasp (14%) stings. No association was noted between the number of stings per episode and severity of the SR. Fifty-one percent of SRs were reported as occurring within 10 minutes of sting; however, the onset of a moderate to severe SR sometimes occurred at 301 or more minutes after a sting episode. Of 2219 subjects with an SR after their most recent sting before entry into the study, 68% received epinephrine for treatment. (J ALLERGY CLIN IMMUNOL 1988;82:370-81.)

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When venom extracts became commercially available late in 1979, an HVS was initiated by questionnaire by the American Academy of Allergy and Immunology Committee on Insects. The study was based on the case histories and VST results of 3236 subjects who had reacted adversely, either with an SR or LR, to stings by Hymenoptera insects. Information was submitted by 84 Fellows and Members of the Academy, and follow-up data were available for most subjects.

The goals of the Therapeutic Safety and Efficacy Protocol were (1) to ascertain the frequency and severity of in-field SSR (with signs and/or symptoms as listed below) in subjects with a history of previously