

Hyposensitization in asthmatics with mPEG-modified and unmodified house dust mite extract

III. Effect on mite-specific immunological parameters and correlation to changes in mite-sensitivity and symptoms*

H. MOSBECH, R. DJURUP, S. DREBORG,¹ L. KÆRGAARD POULSEN, P. STAHL SKOV & I. STERINGER²

Allergy Unit, Medical Dept. TTA, State University Hospital, Copenhagen, Denmark. ¹Dept. of Paediatrics, University Hospital, Linköping, and ²Pharmacia Diagnostics, Uppsala, Sweden

Forty-six adult asthmatics allergic to *D. pteronyssinus* (Dp) participated in a 2-year study. Thirty-one underwent hyposensitization (HS-group). Fifteen were treated with Dp-extract (Dp-group), and 16 with a similar extract modified by monomethoxypolyethylene glycol with reduced allergenicity (mPEG-Dp-group). Fifteen patients served as controls. Dp-specific antibodies and histamine release from blood basophils were determined and compared with Dp-sensitivity in lungs and skin. In addition, IgG and IgE against the major allergen *Der p* I were followed in a subgroup. Dp-specific IgG, IgG₁, and IgG₄ increased significantly in both HS-treated groups after 1 and 2 years (median: 2.5- to 11.6-fold). IgG₄ was not induced if maintenance dose during the first year was less than 20,000 BU. Median skin sensitivity decreased 4.4- to 8.2-fold after 1 year and 7.4- to 21.4-fold after 2 years. *Der p* I specific IgG response was unrelated to the occurrence or change in IgE with the same specificity. The mPEG-Dp-extract tended to have less effect on skin sensitivity and immunological parameters, differences reaching statistical significance for skin sensitivity only. In the HS-group, the decrease in bronchial sensitivity was significantly correlated to a decrease in IgE ($r = 0.36$), IgG₁/IgG₄ ($r = 0.49$), Dp-specific histamine release ($r = 0.58$), and to an increase in Dp-specific IgG₄ ($r = -0.36$) and IgG₄/IgE ($r = -0.48$). In patients improving clinically, Dp-specific IgG₄/IgE increased, and median Dp-specific IgE was reduced to 80% compared with an increase to 150-160% seen in the unchanged or deteriorated group ($P < 0.05$). Findings indicate an improvement of effect, if the allergen dose is sufficient to reduce specific IgE and/or induce an IgG and especially IgG₄ response.

Key words: bronchial challenge; clinical effect; *Dermatophagoides pteronyssinus*; histamine release; hyposensitization; IgE; IgG subclasses; monomethoxypolyethylene glycol, skin test.

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Although for more than 20 years IgE has been known to be the central pathogenic factor in allergic asthma and rhinitis, and although hyposensitization (HS) has been practised for a

much longer period, it has not yet been possible to explain with certainty the effect of this treatment by immunological or other mechanisms. Several theories have been proposed, one of the

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more substantial ones focuses on a beneficial effect of IgG seen in studies on insect venom and ragweed HS (12, 18). IgG was supposed to act as a "blocking" antibody. Subsequent HS studies have focused on IgG subclasses, but no general agreement has been established as to their effect or predictive value at an early or late phase of therapy (7, 14, 19, 29).

In the present study, patients with asthma and bronchial sensitivity to the house dust mite *Dermatophagoides pteronyssinus* (Dp) were treated for 2 years with Dp-extract or Dp-extract coupled to monomethoxypolyethylene glycol (mPEG). Allergen modification by means of mPEG has been shown to reduce the allergenicity of the extract (26). The clinical outcome and change in Dp-sensitivity in various organs have previously been described (24, 25). The aim of the present investigation was to study whether HS with Dp-extract acted by any of the mechanisms proposed, with special focus on IgG and IgG-subclasses. The spontaneous immunological changes occurring in mite allergics were compared with changes induced by HS with potent Dp- and mPEG-Dp-extracts, and co-variation between these changes and changes in Dp-sensitivity as measured in bronchi and skin and in symptoms/medication were analysed.

MATERIAL AND METHODS

Patients

Fifty-five adult house dust mite allergic asthmatics with symptoms requiring regular symptomatic medication entered the study. Only data from the 47 patients completing the first year and the 46 completing the second year have been included in the analyses. The control group comprised 15 patients, and the HS-group consisted of 15 patients treated with Dp-extract (Dp-group) and 17 patients receiving mPEG-Dp-extract (mPEG-Dp-group). In the latter group, one patient left the study after 1 year.

Diagnostic criteria, description of patients and study design have been reported (24).

Sera

Minimum interval from challenge or allergen injections to blood sampling was 1 week. Sera were stored at -18°C . All antibody levels were determined in duplicate, and sera from the same patient were analysed in the same run at the end of the study. Dp-specific IgE and IgG subclasses were measured in the HS-group: 1) after 2 months; 2) half-way through dose-increase phase; and 3) when reaching maintenance dose. In addition, these antibodies plus Dp-specific IgG were measured prior to treatment and at 1 and 2 years. *Der p* I-specific IgE and IgG were determined prior to and after 2 years of treatment.

Allergen extracts

A biologically standardized and purified, unmodified Dp-extract (Pharmalgen[®]*) was prepared from whole mite culture. It contained 60 μg of the major antigen *Der p* I per 100,000 Biological Units (BU) corresponding to 750,000 International Units by RAST inhibition. The mPEG-modified Dp-extract was produced by coupling activated mPEG-succinate to the unmodified Dp-extract (26, 41) (Pharmacia AB, Uppsala, Sweden). The mPEG-Dp-extract was given BU after calibration by protein content. During the whole study the same batch of freeze-dried material was used for treatment, IgG subclass measurement, and challenge tests in bronchi, skin, and blood cells. For measurement of specific IgE and IgG, an identical extract from the same manufacturer was applied.

Clinical assessment

Diary cards reporting daily symptoms and medicine consumption were completed during

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autumn months prior to and during the study. Additionally, at the end of the study patients were asked to complete a questionnaire describing clinical changes during the treatment period. A combined assessment was performed classifying the patients as having improved only if improvement was reported in both diary card and retrospective questionnaire, or in one of them and no change in the other. Deterioration would mean deterioration reported in both assessments, or in one and no change in the other. Patients were classified as unchanged, if this was reported twice, or if both improvement and deterioration were recorded by the same patient (24).

Bronchial challenge

Bronchial challenge with Dp was performed with inhalation of 0.9 ml of allergen solution in increasing concentrations by tidal volume breathing for 5 min with a 10-min interval (23). After the first year, bronchial challenges with Dp were not performed in four patients due to circumstances unrelated to treatment or allergic status.

Skin test

Tests were performed in quadruplicate with four 10-fold dilutions of Dp-extracts from 100 to 100,000 BU/ml using lancets with 1 mm stylus (23). The change in concentration from 1 year to another necessary to elicit wheals of the same size was estimated by parallel-line assay in a (log) wheal size/(log) allergen concentration system (9). Results were omitted in two cases with non-parallel regression lines.

Histamine release assay

Washed blood cells were challenged with Dp- and mPEG-Dp-extract, respectively, in a glass-fibre-based histamine assay system (Lucotest-HR®, Lundbeck Diagnostics A/S, Copenhagen, Denmark) (34). Ten-fold dilutions from 100 to 0.001 BU/ml were used. The concentration

yielding half the maximum histamine release was determined (27). Two patients who became non-reactive to the highest concentration were assigned a sensitivity 10-fold this concentration. Four of the planned 140 blood samples were not analysed, and 14 samples from seven patients were negative to anti-IgE and omitted from calculations of histamine release. Changes could be analysed in 37 and 36 patients after 1 and 2 years, respectively.

Determination of IgE antibodies

Serum IgE against Dp was determined by radioallergosorbent test (11) (Phadebas RAST®, Pharmacia AB, Uppsala, Sweden). Within-assay coefficient of variation was 8.5-10.4%.

Serum IgE against the major allergen *Der p* I was determined by immunoprint technique (2). Sera from 38 randomly selected patients were analysed. Sixteen belonged to the mPEG-Dp-group and 14 to the Dp-group.

Determination of IgG antibodies

Dp-specific IgG in serum was measured by a radioallergosorbent test kit according to the manufacturer's instructions (IgG RAST, Pharmacia AB, Uppsala, Sweden). Within-assay coefficient of variation was 7%.

IgG against major allergen *Der p* I was determined by crossed radioimmuno-electrophoresis (CRIE) (30, 37). The analysis was performed on sera from 12 mPEG-Dp- and 11 Dp-treated patients included in the IgE immunoprint analysis.

Determination of IgG subclass antibodies

IgG subclass RAST was made as a capturing antibody modification of a previously described method (7). Polystyrene tubes were coated with the immunoglobulin fractions of a rabbit anti-serum raised to house dust mite allergen. The Dp-extract was added and incubated with the serum to be tested. The relevant anti-IgG subclass monoclonal antibody was then added and,

finally, the tubes were incubated with iodine-labelled rabbit anti-mouse IgG. The assay had previously been demonstrated to express equipotency of the four subclasses. Thus, allergen-specific IgG_x titres of unknown sera were expressed relative to a standard curve established with known concentrations of IgG₄. Serum from a patient who had undergone HS for several years, was used as standard and arbitrarily assigned to 100 sorbent units (SU)/ml. A pool of normal human sera contained < 20 SU/ml of IgG₂ and < 10 SU/ml of IgG subclasses 1, 3, and 4. When a positive control serum was repeatedly tested on 11 different days, coefficients of variation of 8.3% and 10.5% for IgG₁ and IgG₄ determinations were obtained.

Preincubation of positive sera with soluble allergen extract inhibited the response in a dose-dependent manner. If either positive serum or monoclonal antibody was omitted from the sandwich, the response was always below 10 SU/ml. The specificity of the monoclonal antibodies has previously been demonstrated. Only the immunosorbents of the relevant IgG-subclass myeloma caused significant binding (8). Values below detection limit (10 SU/ml) were fixed at 5 SU/ml to allow statistical analyses.

Statistics

Absolute and relative concentrations were logarithmically transformed to increase symmetry. Within-group changes over time were analysed by Wilcoxon test for matched pairs and between-group differences were evaluated by Kruskal-Wallis or Mann-Whitney tests. Chi-squared test was used for categorical variables. Correlation between parameters was described by the correlation coefficient (*r*). Data sets containing missing data were excluded, and 5% significance limit was used.

RESULTS

Part I: Comparing therapeutic groups

Pre-treatment assessment

The three groups were comparable with respect to initial Dp-specific IgE, IgG, IgG₂, IgG₃,

IgG₄, histamine release and skin sensitivity. Dp-specific IgG₁ was higher in the mPEG-Dp- and Dp-groups compared with the control group, median values were, however, < 10, 10, and < 10 SU/ml, respectively. Only one patient had measurable anti-Dp IgG₂ and none had anti-Dp IgG₃, anti-Dp IgG₄, or anti-*Der p* I IgG before treatment. Anti-*Der p* I IgE was demonstrated in 16 of 38 patients tested pre-treatment.

Immunological changes during HS

Histamine release: A 10-fold decrease in sensitivity to both Dp and mPEG-Dp allergen was seen in the Dp-treated group after the second year (Table 1). Apart from this, no significant changes in basophil sensitivity were seen in any group.

Skin sensitivity: In mPEG-Dp- and Dp-groups, the skin sensitivity decreased significantly during the first year: 4.4- and 8.2-fold, respectively (Fig. 1, Table 1). The control group was unchanged. After the second year an additional improvement was observed in the HS-treated. The median tolerance was 21.4-fold the pre-treatment value in the Dp-group. This was significantly higher than the 7.4-fold increase in the mPEG-Dp-group and the insignificant change (1.5-fold) in the control group seen after 2 years.

Dp-specific IgE-antibodies: The only significant change observed in Dp-specific IgE was a 1.2-fold increase in the Dp-group after 1 year. However, this was not different from changes observed in the two other groups.

After HS for 2 years, the concentration of *Der p* I specific IgE increased in six of 16 patients in the mPEG-Dp-group compared with one of 14 Dp-treated ($P < 0.05$). A reduction was seen in two patients in each group.

Dp-specific IgG-antibodies: The median increase of 2.5- to 3.6-fold in Dp-specific IgG seen after 1 and 2 years in the Dp- and mPEG-Dp-groups was more pronounced than the insignificant

0.9-fold change in the control group (Table 1). Similarly, Dp-specific IgG₁ increased significantly in both Dp- and mPEG-Dp-groups during the first year (medians: 4.2- to 3.0-fold), and continued to be high after the second year (medians- 3.2- to 2.5-fold) (Fig. 2). No significant changes were seen in IgG₂ or IgG₃ in any group. The most distinct increase was seen in Dp-specific IgG₄ in Dp- and mPEG-Dp-groups (medians: 8.6- and 5.8-fold) (Fig. 3). A further increase after 2 years was observed (medians: 11.6- and 9.3-fold pre-treatment values).

Change in IgG₁, and IgG₄ was significantly more pronounced in the two HS-groups than in the control group ($P < 0.01$), but IgG and IgG subclass changes were not significantly different in the Dp- and mPEG-Dp-groups. Maximum increase in IgG₄ during the first year was positively correlated to maintenance dose in both groups ($r = 0.44$ – 0.45). Only for the mPEG-Dp-treated, was the IgG₁ increase similarly correlated to both maintenance and accumulated doses. No IgG₄ response could be recorded after 1 year in any of the seven patients unable to reach maintenance dose of at least 20,000 BU.

The number of patients producing IgG against *Der p* I following HS was similar in the groups: six of 12 and seven of 11 in the mPEG-

Dp- and Dp-groups. Production of this IgG was not correlated to increase in or pre-existence of IgE with the same specificity.

Part II: Comparing clinical and immunological changes during HS

The basic mechanism of action for HS with Dp- and mPEG-Dp-extracts is supposed to be the same. Data from the Dp- and mPEG-Dp-groups were therefore pooled in the analysis of the correlation between immunological and clinical changes during HS.

Simple changes in Dp-specific immunological parameters: Changes in Dp-sensitivity correlated to changes in antibody levels (Table 2). The correlations were generally equal to or more pronounced than correlations including absolute antibody levels instead of changes in concentrations. Similarly, inclusion of maximum increase in antibody levels based on the five samples drawn within the first year did not improve correlations.

In four of 14 patients presenting *Der p* I specific IgE pre-treatment, the concentration decreased. All reported clinical improvement,

Table 1

Dp-specific sensitivity and antibody concentrations relative to pre-treatment levels (medians). Significant change from pre-treatment level within group: ○: $0.01 \leq P < 0.05$; ●: $P < 0.01$. Change within group significantly different from change in control group; ▲: $P < 0.01$. mPEG-Dp differing from Dp-group only in change in skin sensitivity (2 years/pre): $P < 0.05$

Change in Dp-specific		Treatment groups			
		mPEG-Dp	Dp	Hyposensitized (mPEG-Dp + Dp)	Control
Histamine release	1 yr/pre	1	1	1	1
	2 yrs/pre	1	10○	1○	1
Skin sensitivity	1 yr/pre	4.4●▲	8.2●▲	5.5●▲	1.0
	2 yrs/pre	7.4●▲	21.4●▲	14.9●▲	1.5
IgE	1 yr/pre	1.2	1.2○	1.2	1.1
	2 yrs/pre	0.8	1.3	0.9	0.9
IgG	1 yr/pre	2.5●▲	2.8●▲	2.6●▲	0.9
	2 yrs/pre	3.6●▲	3.0●▲	3.6●▲	0.9
IgG ₁	1 yr/pre	3.0●▲	4.2●▲	3.3●▲	1.0
	2 yrs/pre	2.5●▲	3.2●▲	2.6●▲	1.0
IgG ₄	1 yr/pre	5.8●▲	8.6●▲	6.3●▲	1.0
	2 yrs/pre	9.3●▲	11.6●▲	11.0●▲	1.0

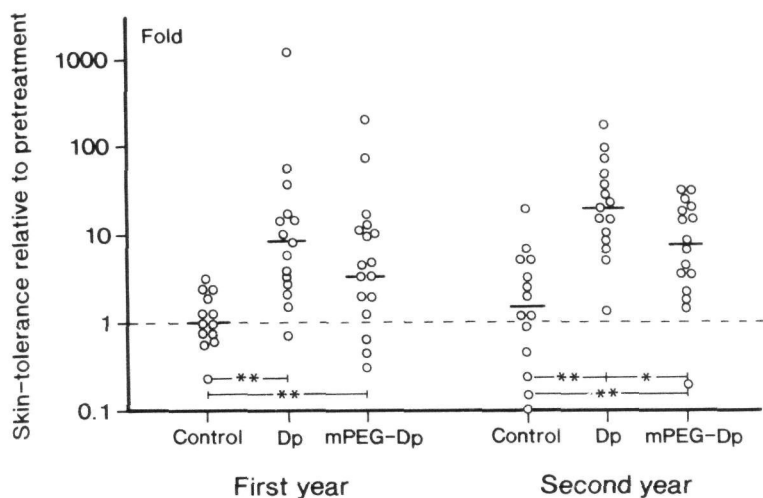


Fig. 1. Increase in Dp-concentration necessary after 1 and 2 years to induce skin prick reaction identical to pre-treatment size (median: —) ($*0.01 \leq P < 0.05$; $**P < 0.01$).

and improvement in bronchial Dp-sensitivity was significantly more pronounced compared with that in other patients. In contrast, development of *Der p* I specific IgG was not correlated to changes in Dp-sensitivity or in clinical state.

After 1 year, decreased sensitivity as measured in the histamine release assay was correlated to decrease in IgE, and increased skin tolerance was accompanied by increase in IgG₄.

After 2 years, improvement in bronchial sensitivity was additionally accompanied by lower histamine releasability (Fig. 4), decrease in IgE and increase in IgG₄. IgG increase correlated to decrease in histamine releasability.

Clinical improvement after 2 years was accompanied by small reductions in IgE (median

level: 80%) compared with the clinically unchanged or deteriorated groups (median levels: 150 and 160%) ($P < 0.05$) (Fig. 5). No significant correlation was seen between clinical improvement and changes in IgG, IgG₄ (Fig. 6), other IgG subclasses, histamine releasability or skin sensitivity.

Change in Dp-specific antibody ratios: An improvement in sensitivity was accompanied by a significant increase in IgG₁/IgE and IgG₄/IgE, and a decrease in IgG₁/IgG₄ ratios (Table 2). The clinical improvement after 2 years was similarly accompanied by a significant increase in IgG₁/IgE, and in IgG₄/IgE ratios.

Antibody ratios (absolute values) after 1 and 2 years were generally less closely correlated to changes in symptoms and Dp-sensitivity than was the ratio between changes.

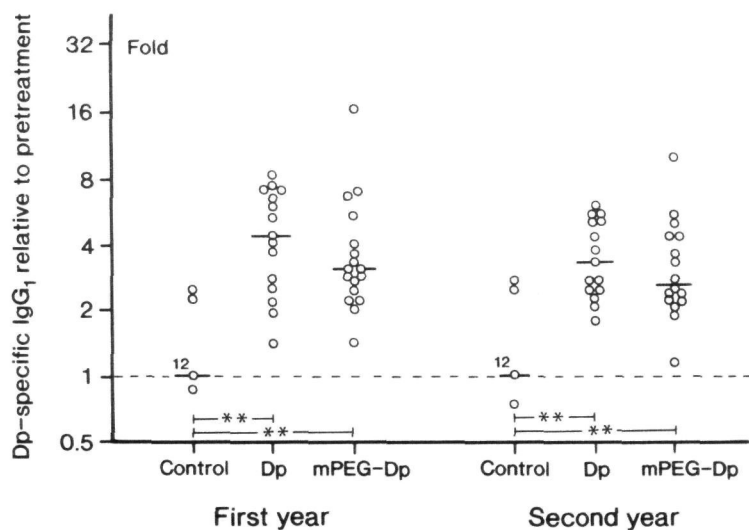


Fig. 2. Change in Dp-specific IgG₁ relative to pretreatment after 1 and 2 years (median: —) ($**P < 0.01$).

DISCUSSION

In the present study, the monitoring of clinical and immunological changes induced by modified and unmodified Dp-extract was intensive and standardized. This has enabled a meticulous analysis of the co-variation between immunological and clinical parameters in the HS-treated mite-allergic asthmatics. It has been possible to demonstrate a correlation between the induction of antibodies and changes in clinical sensitivity. Such findings do not prove a causal relationship but indicate that an allergen dose sufficient to give an intensive immunological response tends to have a beneficial clinical effect as well. The large number of statistical analyses increases the risk of errors of inference. However, our conclusions seem not to be vitiated by type I errors, since nearly all correlations with IgE and IgG₄, significant and insignificant as well, pointed in the same direction (Table 2).

The immunological responses observed have been interpreted as changes in antibody concentration. However, they could as well reflect shifts in antibody affinity or combined changes in concentration and affinity.

Antibodies in the control group

In the group not undergoing HS, the immunological changes were limited during the 2-year study, and although a reduction in specific IgE and IgG has been indicated in a cross-sectional study (32), an extended study period would probably be necessary to detect this. Compared with the present mite allergic patients, mould allergics seem to have more specific IgG of all subclasses, especially IgG₁ and IgG₂, before treatment (19), but whether this may account for differences in clinical response, remains speculative.

Modified vs. unmodified extract

The applied mPEG-Dp-extract has previously been shown to have a 10-fold reduced allergenicity in the skin compared with the unmodi-

fied extract (26), but also to be slightly less effective in HS as judged from clinical scorings (24) and change in skin sensitivity. In parallel, the immunological response induced in the mPEG-group was less pronounced than in the patients treated with the unmodified extract – although the two groups received equal amounts of Dp-extract in terms of raw material. The 10-fold reduction in allergenicity was accompanied by an only 2- to 3-fold less therapeutic effect as measured by reduction in skin sensitivity.

In animal experiments, pre-treatment with mPEG-modified allergens almost totally prevented the IgE response to subsequently administered unmodified allergens (33). Even in animals with ongoing IgE response, treatment with mPEG-modified allergens reduced this IgE response moderately without reduction in the IgG response (1). However, in human studies comparing the immunological effect of treatment with mPEG-modified and unmodified extracts, differences have been modest. In an early study on ragweed hay fever, modified allergen tended to give an increased IgG response, but IgE was not reduced (15). In a study on honey bee venom, immunological responses were identical in the treatment groups (28). Ideally, a comparison should be made between clinically equipotent or maximum tolerated doses of modified and unmodified allergens. Although the mPEG-Dp-extract applied in the present study was not superior to the unmodified Dp using similar doses, it is possible that a more pronounced mPEG modification might allow an increase in allergen dose thereby stimulating the IgG production and maybe increasing the clinical benefit as well.

Antibody concentration or change in concentration

In several HS-studies, therapeutic groups have been compared during treatment with respect to actual concentration of antibody. If groups are not fully comparable pre-treatment, this might lead to erroneous conclusions. Therefore it seems more relevant to compare the relative changes within and between treatment groups.

In the present study, changes in IgG, IgE, and antibody ratios were in fact better correlated to changes in Dp-sensitivity than were the absolute antibody concentrations during and after treatment. Calculating ratios including arbitrarily chosen values below detection limit seems, therefore, permissible.

IgE

For various allergens, sufficient doses have been accompanied by transient increase in specific IgE in the early phase of HS with a subsequent decrease towards pre-treatment levels (4, 5, 7, 13, 14, 20). In the present study a small early increase in Dp-specific IgE was seen in the Dp-group only, probably due to the more pronounced allergenic stimulus in these patients. On the other hand, decrease in *Der p* I specific and/or Dp-specific IgE later during maintenance phase was correlated to reduced sensitivity of blood basophils and bronchi and to clinical improvement. This indicates a certain role of IgE – not only via postulated qualitative (17) but also via quantitative changes in patients undergoing HS. Other factors might, however, be of greater importance. Similar changes have not been found in mite-allergic patients otherwise selected, i.e. with rhinitis (4, 29), mild asthma (3) or in infants without major improvement of their immediate reactions (36). Low potency allergen extracts used in some of

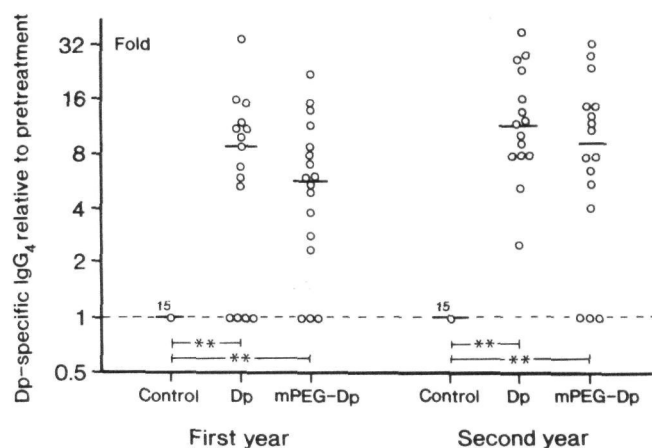


Fig. 3. Change in Dp-specific IgG₄ relative to pre-treatment after 1 and 2 years (median: —) (***P* < 0.01).

these studies might also contribute to the diverging results.

IgG

The idea of IgG antibodies blocking the allergic reaction has been favoured by several controlled studies reporting increasing specific IgG early in HS with mite (3, 4, 6, 13, 31) and with other allergens (14, 15, 18, 38, 40). In the present study the increase was most pronounced in the IgG₄ subclass. This finding is supported by a similar increase following HS with mite (29) or cat and dog allergens (14). In a study on *Cladosporium* HS, however, the IgG₁ subclass response was more marked (19), possibly due to

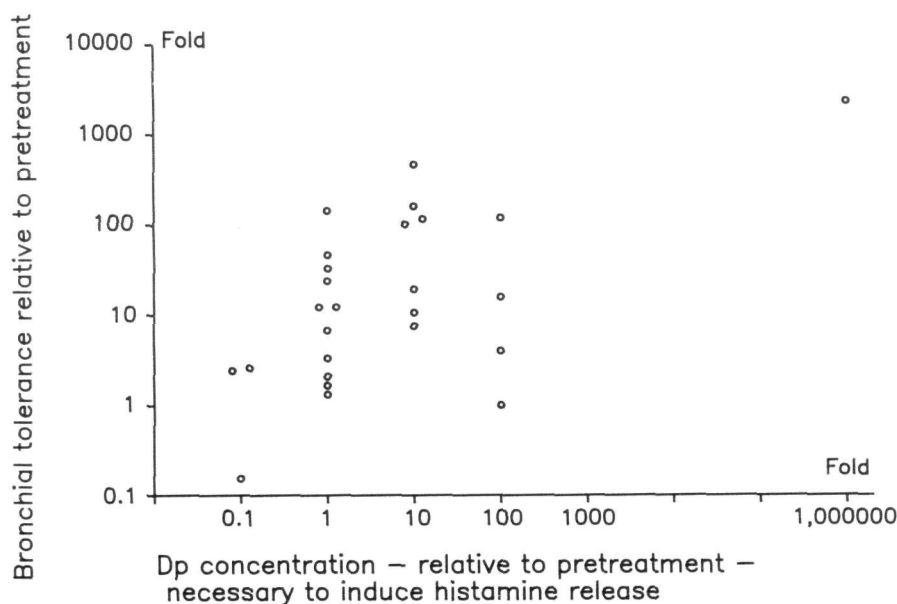


Fig. 4. Change in Dp-specific bronchial sensitivity and Dp-specific histamine releasability after 2 years (*r* = 0.58, *P* < 0.01).

the different chemical properties of this extract or to differences in immunizing regimens. In that study, as in the present, the change in IgG₂ and IgG₃ after 1 year seems to be of less importance.

The clinical sensitivity to mite extracts varies greatly in HS-treated asthmatics, the side effects occurring at both low and high allergen doses (22). A similar distribution might have been expected in the allergen dose necessary to induce IgG production. It is therefore remarkable that a rather high threshold dose seemed necessary for IgG₄ production. Similar thresholds have not previously been reported for other extracts but might be useful in the future as a characteristic of the extracts in addition to BU and quantitation of major allergens.

Histamine release from blood basophils

In the present study, the histamine release assay showed only modest reduction in sensitivity and only in the group receiving the highest allergen load. This is in accordance with the insignificant changes observed by different techniques in three other mite studies (6, 16, 31).

Skin test

In general, it has been easy to demonstrate an effect of HS on skin sensitivity to mite (3, 10) and other allergens (9, 14, 20, 35, 38, 39). The present study is no exception. The additional

benefits seen during the second year while mainly on maintenance dose, might suggest that the optimum treatment period should be even longer. The skin test seems only sparsely influenced by factors other than HS (or additional medication) – in contrast to challenge tests in other organs, in which less equivocal effects have been recorded in the same patients (9, 25).

Correlation to challenge

Although the effect of HS varies in different organs in the same patients (25), results from allergen challenges would more specifically reflect the allergen sensitivity than would scores and clinical evaluation, especially in non-pollen allergics. Increase in specific IgG has been shown to correlate to improvement in nasal sensitivity to Dp (6). Studies of bronchial sensitivity in patients treated with animal dander (14, 38) disagree. Likewise, treatment with *Cladosporium* in a smaller group of patients failed to induce correlating changes in bronchial sensitivity and in IgE (20). In the present study, after either 1 or 2 years or both, improvement in bronchial, skin and blood cell sensitivity was correlated to increase in IgG₄ and decrease in IgE. Change in Dp-specific IgG from other subclasses showed less correlation, if any, to change in Dp-sensitivity, and calculated ratios (IgG₁/IgG₄ and IgG₄/IgE) did not in general increase correlations. A possible immunological

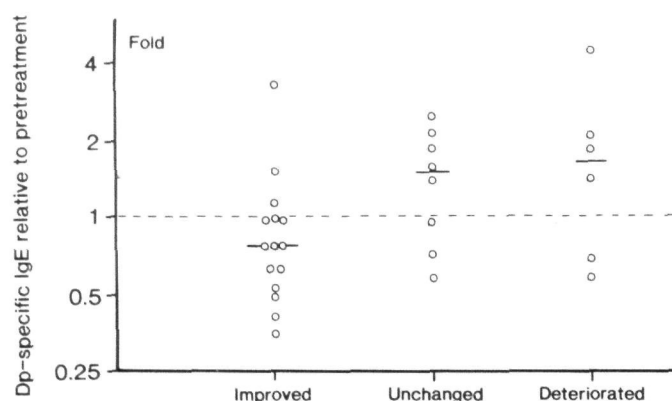


Fig. 5. Change in Dp-specific IgE according to clinical effect of treatment after 2 years (median: —) ($P < 0.05$).

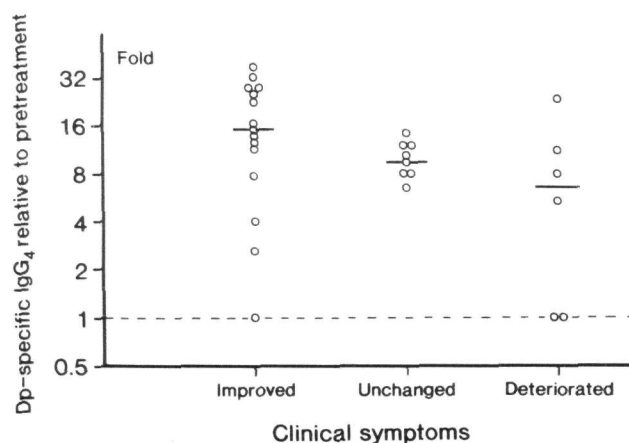


Fig. 6. Change in Dp-specific IgG₄ according to clinical effect of treatment after 2 years (median: —) ($P = 0.08$).

Table 2

Correlation between change in antibodies or in antibody ratios and change in Dp-sensitivity in hyposensitized patients.
Coefficients calculated from log (conc. sample/conc. pretreatment sample)

Increase in Dp-specific	Increase during 1 year in			Increase during 2 years in		
	Bronchial sensitivity	Histamine releasability	Skin sensitivity	Bronchial sensitivity	Histamine releasability	Skin sensitivity
IgE	0.25	0.38*	-0.21	0.36*	0.52**	-0.13
IgG	-0.15	-0.27	-0.13	-0.10	-0.36*	-0.06
IgG ₁	-0.14	0.16	-0.20	0.09	0.10	-0.03
IgG ₄	-0.14	-0.18	-0.53**	-0.36*	-0.29	-0.47**
IgG ₁ /IgE	-0.34*	-0.25	0.02	-0.27	-0.44*	0.10
IgG ₄ /IgE	-0.25	-0.37*	-0.35*	-0.48**	-0.49**	-0.29
IgG ₁ /IgG ₄	0.08	0.29	0.49**	0.37*	0.29	0.42**
Histamine releasability	0.25	-	-	0.58**	-	-
Skin sensitivity	-0.20	-0.21	-	0.04	-0.24	-

*: $0.01 \leq P < 0.05$; **: $P < 0.01$.

mechanism is therefore not just dependent on an antibody ratio, as might be expected for antibodies with a simple blocking effect.

In mite-allergic asthmatics, a correlation has previously been demonstrated between basophil and bronchial Dp-sensitivity (23). Here, changes in these parameters were found to correlate as well.

Although statistically significant, most correlation coefficients (r) did not exceed 50%. In general, a maximum of 25% (r^2) of the variation in sensitivity could therefore be explained or predicted from changes in immunoglobulins. This might indicate that factors stimulating IgG₄ and decreasing IgE could in parallel improve allergen sensitivity as an epiphenomenon. A more causal relationship might be possible as well.

The Dp-extracts were complex mixtures containing also proteins against which few patients, if any, have produced IgE. When injected, these proteins induce production of IgG and, in some cases, IgE (21). These antibodies probably play no therapeutic role in HS, but would be measured as Dp-specific by assays using whole Dp-extract. This might explain why the correlations to changes in Dp-sensitivity or clinical improvement were not more pronounced. Measurements of antibodies against relevant single allergens might be a way to circumvent this problem. However, in the present study induction of *Der p* I specific IgG was not corre-

lated to change in Dp-sensitivity. The explanation might be that, although a major allergen, *Der p* I is not of major importance to all Dp-sensitive patients.

Correlation to clinical changes

In the present study, a clinical improvement was accompanied by increase in IgG₁/IgE or in IgG₄/IgE, and decrease in IgE confirming the findings of a short-term study in which development of specific IgG was accompanied by clinical improvement, especially if parallel increase in specific IgE was only modest (6). No such correlation was demonstrated in another study, probably because there were fewer patients (4). Since IgG₁, IgG₄, and IgE responses did not occur simultaneously, ratios based on maximum increase within the first year were calculated. Correlations were, however, approximately equal to those obtained at 1 year, and could not confirm findings from a clustered treatment with *Cladosporium*, in which increase in IgG₁ – and to a minor extent in IgG₄ – before maintenance phase of HS was correlated to less favourable clinical responses (19).

Conclusion

HS with Dp and mPEG-Dp was able to induce an IgG response, primarily within the IgG₄

subclass, in almost all patients. mPEG-Dp-extract tended to be less effective, but the 10-fold difference in skin test allergenicity of the two extracts was not reflected in a similar reduction in efficacy on the skin test after 1 or 2 years of HS. This might indicate a favourable effect of mPEG-modification, especially if the allergen dose could be further increased. Correlations between changes in clinical sensitivity and in specific antibodies rule out a general sensitizing effect of IgG₄ in relation to HS in mite-allergic asthmatics. In contrast, increase in IgG₄ with or without decrease in IgE may exert a beneficial effect on the clinical and paraclinical state in the majority of patients; this may support the old hypothesis of blocking antibodies.

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Address:

Holger Mosbech, M.D.
Allergy Unit 7511
Medical Dept. TTA
State University Hospital
Tagensvej 20
DK-2200 Copenhagen N
Denmark

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