

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

II. Effect evaluated by challenges with allergen and histamine

H. MOSBECH, S. DREBORG,¹ L. FRØLUND, I. LJUNGSTEDT-PÅHLMAN,¹
U. G. SVENDSEN, M. SØBORG,² E. TAUDORF & B. WEEKE

Allergy Unit 7511, Medical Dept. TTA, State University Hospital, Copenhagen, Denmark, ¹Pharmacia Diagnostics, Uppsala, Sweden, and ² Allergy Clinic, Medical Dept. B, County Hospital, Hillerød, Denmark

In a 2-year study, 46 asthmatics with verified allergy to the house dust mite *D. pteronyssinus* (Dp) were included either as controls (Ctls) or receiving hyposensitization (HS) with unmodified or monomethoxypolyethylene glycol (mPEG) modified Dp-extract. Patients were monitored by annual challenges with histamine in bronchi, and Dp allergen in bronchi, nose and conjunctiva. mPEG-modified extract was not inferior to unmodified Dp-extract; both were to some extent able to improve tolerance to Dp and histamine in bronchi and to Dp in nose and eyes. During the 1st year, the bronchial sensitivity to Dp decreased significantly in the HS groups but not in the Ctls. During the 2nd year, improvement was more pronounced in the Ctl group. The relative increase in Dp or histamine tolerance did not differ significantly between groups after either 1 or 2 years; the only exception was conjunctival sensitivity, which in the Ctl group was unchanged, and a 10-fold increase in tolerance in the HS groups. No direct benefit was seen on late-phase bronchial reactions. In patients with improved pulmonary symptoms a tendency was seen towards reduced sensitivity to histamine and Dp. Variation within groups was extensive.

Key words: bronchial challenge; clinical effect; conjunctival challenge; *Dermatophagoides pteronyssinus*; hyposensitization; monomethoxypolyethylene glycol; nasal challenge; un-specific reactivity.

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In hyposensitization (HS), the occurrence of side effects generally implies reducing the allergen dosage and probably reducing the benefits of this treatment as well. Binding monomethoxypolyethylene glycol (mPEG) to allergens was performed in an attempt to reduce such side effects without eliminating the ability of the extracts to induce (immunological) tolerance during HS (21). In the present study, the effect of HS with an unmodified and a mPEG-modified version of a potent extract prepared from the house dust mite

Dermatophagoides pteronyssinus (Dp) was assessed in asthmatics.

Each patient was followed up for more than 2 years, during which time many factors not related to HS might influence the net results, especially when based on various clinical assessments. To circumvent such factors, patients were additionally challenged in various organs before and during treatment. The present paper describes the results of these challenge tests and compares results with the outcome of clinical assessments previously reported (10).

MATERIAL AND METHODS

Patients

Fifty-five adult house dust mite-allergic asthmatics with symptoms not completely controlled by symptomatic medication entered the study. All showed changes in peak expiratory flow (PEF) of at least 15% of predicted values, either spontaneously or following treatment. Diagnostic criteria and description of patients have been reported (10).

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 BU. The mPEG-modified Dp-extract was produced by coupling activated mPEG-succinate to the unmodified Dp-extract (12, 21). Both extracts were delivered freeze-dried (Pharmacia AB, Uppsala, Sweden). Throughout the study the same batch was used for both treatment and challenge tests. Extracts for test procedures were used within 8 h of reconstitution.

Clinical assessment

Diary cards reporting symptoms and medicine consumption were completed twice daily during a 12-week period starting at the beginning of September, when maximum load of Dp-allergen could be expected. Changes in symptoms and medicine consumption were classified as reduction if at least 10%, no change if less than 10%, or increase if at least 10% (10).

At the end of the study, patients were asked to complete a questionnaire describing changes in symptoms and medicine consumption during the whole treatment period. Changes were re-

ported as improvement, deterioration, or no change.

Due to extensive inconsistency between the two ways of assessing the clinical effect (10), a combined assessment was performed classifying the patients as having improved only if improvement was reported in both diary card and questionnaire, or in either of them with no change reported in the other. Deterioration would mean deterioration reported in both assessments or in one with no change reported in the other, and patients were classified as having no change if this was reported twice, or if both improvement and deterioration were recorded by the same patient.

To study both subjective and objective improvement, the patients were classified according to the combined clinical assessment and to their change in bronchial Dp-tolerance. Relative changes in tolerance of at least ± 10 -fold (1 log-step) were regarded as relevant.

Bronchial challenge with histamine

This was performed to evaluate unspecific bronchial hyperreactivity (11). Histamine dihydrochloride was inhaled in doubling concentrations from 0.03 mg/ml by tidal volume breathing for 2 min with intervals of 5 min. Challenge was continued until a concentration was reached which induced at least a 20% decrease in the forced expiratory volume in the 1st s (FEV₁) when compared with the level obtained after inhalation of isotonic saline. A provocative concentration resulting in a 20% decrease in FEV₁ (PC₂₀) was determined by linear interpolation in a logarithmic/linear dose-response curve. Repeatability studies have shown 95% confidence interval for subjects based on a single determination which was ± 1.72 2-fold concentration differences (8).

If the patient had a positive reaction to the lowest concentration of Dp or histamine applied in a challenge test, the test was repeated at a later stage using a lower starting dose. The interval between challenges of any kind was at least 2 weeks, and no hyposensitization was given within 2 weeks prior to the tests. Apart

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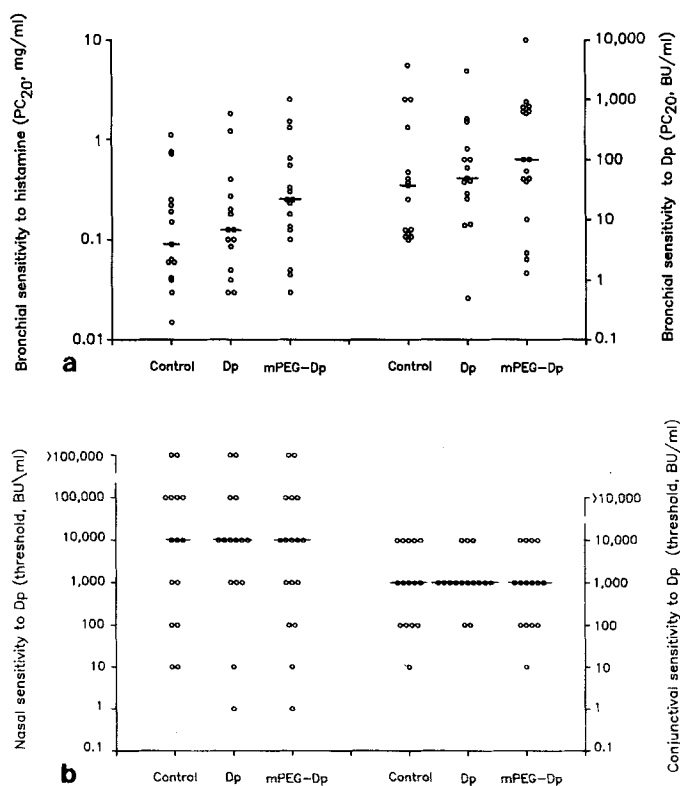


Fig. 1. Pre-treatment sensitivity (a) in bronchi to histamine and Dp (provocative concentration, PC_{20}) and (b) in nose and conjunctiva to Dp (threshold concentration). Median = —.

from 30 patients initially using inhaled corticosteroids, no other steroids or bronchodilators of any kind were allowed prior to challenge as recommended (6). During the following years the dose of inhaled steroid was kept constant prior to challenge.

Bronchial challenge with Dp

This was carried out with inhalation of 0.9 ml of allergen solution by tidal volume breathing for 5 min with 10-min interval (6, 11). Concentrations were increased 10-fold from 1 BU/ml to a maximum of 10,000 BU/ml. PC_{20} was calculated as described above. Ninety-five per cent confidence interval for this measurement based on a single determination was $\pm 0.83 \times$ one single 10-fold concentration difference (6).

Patients were asked to measure PEF every 2 h for 24 h after the challenge (except during sleep) and to record all anti-asthmatic medication used. Late-phase bronchial reaction was defined as increased symptoms, otherwise not explained, during the first 2–12 h necessitating a significant increase in basic medication (i.e. addition of at least 2×0.2 mg of inhaled Salbutamol). Similarly, a decrease in PEF of $> 15\%$ would indicate a late bronchial reaction.

Nasal challenge

Challenge was performed by nebulization of 0.2 ml of diluent or increasing concentrations of Dp-extract in the nostrils (11). Ten-fold increasing concentrations from 1 BU/ml (or, if positive, from 0.1 BU/ml) to a maximum of

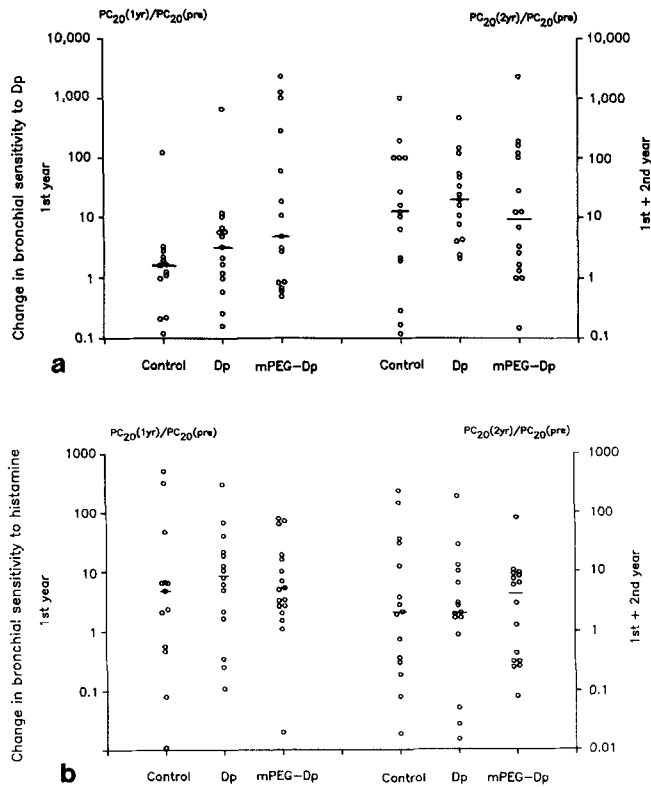


Fig. 2. Relative change in bronchial sensitivity (PC_{20}) to (a) Dp and (b) histamine after 1 and 2 years. Median = —.

100,000 BU/ml were applied. The test was regarded as positive when at least two of the following criteria were fulfilled: 1) decrease in nasal peak flow of at least 20% compared with the initial (post-diluent) value, 2) nasal secretion of at least 0.5 ml/10 min, or 3) five or more sneezes per 10 min.

Conjunctival challenge

Drops of diluent and Dp-extract in increasing concentrations were placed in the lower conjunctival sac of both eyes alternatively at 10-min intervals (11, 14). Ten-fold increasing concentrations from 1 BU/ml to a maximum of 10,000 BU/ml were applied. If redness and itching occurred, the same concentration was instilled in the other eye. The lowest concentra-

tion causing bilateral reactions was regarded as the threshold concentration.

Design

Patients were allocated to either of two treatment groups receiving HS with mPEG-Dp or with unmodified Dp, or to the control (Ctl) group treated with symptomatic medication only. HS was performed for 2 years in a double-blind design (10). Challenges took place in late winter and early spring, when mite exposure was relatively low, and before pollination.

Statistics

Data in 2×2 tables were analysed by Fischer's exact test and in 2×3 tables by a similar exact

test (2). Within-group changes along time were analysed by Wilcoxon test for matched pairs and between-group differences were evaluated by Kruskal-Wallis or by Mann-Whitney tests. Five per cent significance limit was used. The effect parameter in the different organs was calculated as the post-treatment provocative or threshold concentration expressed by the pre-treatment concentration, i.e. PC_{20} (1 year)/ PC_{20} (pre). Statistical analyses were made on logarithmic values. Data sets containing missing data were excluded. In the few cases of Dp challenges being negative, the provocative concentration was set to 10 times the maximum concentration applied.

RESULTS

Pre-treatment assessment

Groups were comparable with respect to bronchial, nasal and conjunctival sensitivity to Dp and bronchial sensitivity to histamine (Fig. 1).

Compliance

Eight patients left the study prior to the first follow-up (10) and one of the remaining 47

Table 1

Median concentration of histamine (mg/ml) or Dp extract (BU/ml) necessary to induce reactions in various organs

Challenge	Treatment groups			
	mPEG-Dp	Dp	Hyposensitized (mPEG-Dp + Dp)	Control
Bronchial histamine				
pre	0.23	0.15	0.18	0.12
1 year	0.45	0.25	0.38	0.14
2 years	0.44	0.17	0.24	0.13
Bronchial Dp				
pre	64	61	64	40
1 year	855	355	540	70
2 years	870	780	780	390
Nasal Dp				
pre	10,000	10,000	10,000	10,000
1 year	100,000	32,000	100,000	1,000
2 years	32,000	100,000	100,000	10,000
Nasal Dp (non-rhinitis patients excl.)				
pre	10,000	10,000	10,000	10,000
1 year	320,000	32,000	100,000	1,000
2 years	10,000	100,000	100,000	10,000
Conjunctival Dp				
pre	1,000	1,000	1,000	1,000
1 year	10,000	100,000	10,000	1,000
2 years	100,000	32,000	100,000	1,000
Conjunctival Dp (non-conjunctivitis patients excl.)				
pre	3,200	1,000	1,000	1,000
1 year	10,000	10,000	10,000	1,000
2 years	32,000	10,000	10,000	1,000

I: $0.01 \leq P < 0.05$; II: $P < 0.01$

Table 2

Median therapeutic effect in various organs (provocative concentrations relative to pretreatment values).
 —: $0.01 \leq P < 0.05$ and ===: $P < 0.01$.

Challenge	Treatment groups			
	mPEG-Dp	Dp	Hyposensitized (mPEG-Dp + Dp)	Control
Bronchial histamine				
1 year/pre	2.0	2.5	2.1	2.0
2 years/pre	1.9	1.4	1.5	1.4
Bronchial Dp				
1 year/pre	4.8	3.2	3.9	1.6
2 years/pre	9.1	20.0	12.3	12.2
Nasal Dp				
1 year/pre	10	3.2	10	1
2 years/pre	3.2	10	10	3.2
Nasal Dp (non-rhinitis patients excl.)				
1 year/pre	10	3.2	10	1.0
2 years/pre	1.0	10	10	10
Conjunctival Dp				
1 year/pre	10	10	10	1.0
2 years/pre	32	10	10	1.0
Conjunctival Dp (non- conjunctivitis patients excl.)				
1 year/pre	10	10	10	1.0
2 years/pre	3.2	10	10	1.0

patients left after 1 year (data included). After the 1st year, two bronchial challenges with histamine, four bronchial, four nasal and five conjunctival challenges with Dp were not performed due to circumstances not related to treatment or allergic status. After the second year, bronchial challenges were performed in all patients, and only two nasal and two conjunctival challenges were missed. Two of 42 patients with nasal symptoms had no nasal challenge after 1 year, and one of 22 patients with eye symptoms was not tested in this organ after 2 years. Evaluation of bronchial late-phase reactions at follow-up was possible in 41 patients.

Bronchial sensitivity

The Dp concentration necessary to induce bronchial reaction increased in all groups during the study period (Fig. 2a, Tables 1 and 2). After 1 year, this improvement was statistically significant in the two HS groups but not in the Ctl group. Median increase in tolerated concentration was 3.2–4.8-fold and 1.6-fold, respectively. After 2 years a significant improvement was seen in all groups with an increase in median PC₂₀ from 64 to 780 BU/ml in the HS group, and from 40 to 390 BU/ml in the Ctls,

differences between groups being non-significant.

With regard to bronchial sensitivity to histamine, a similar significant improvement was seen in the HS groups but not in the Ctl group during the 1st year (Fig. 2b, Tables 1 and 2). During the 2nd year, several patients deteriorated, and at the end of the study, median PC₂₀ was not significantly different from pre-treatment value in any group. After 1 year, median histamine PC₂₀ was 2–2.5 times the initial value in the groups, but after 2 years this concentration had decreased to 1.4–1.9 times the initial value. No statistically significant differences in improvement rate between groups were seen.

Late reactions

In two of six Ctl patients late reaction did not reoccur after 2 years compared with nine of 20 patients hyposensitized (Table 3). Similarly, three of six patients in the Ctl group developed a late reaction during this period compared with only one of nine in the HS group, although the provocative allergen dose was increased in all groups.

Nasal sensitivity

A significant (10-fold) increase in nasal tolerance was seen in the HS group during the 1st year, and this improvement was still present after the 2nd year (Tables 1 and 2). In the Ctl group no significant change could be measured. This therapeutic effect was seen in the total group of asthmatics and in the subgroup report-

ing additional nasal symptoms. In the mPEG Dp group the increase in tolerance was significant only after 1 year and in the Dp group only after 2 years. No statistically significant difference between groups in improvement rate was seen except for the mPEG Dp group who tolerated significantly more Dp-extract after 1 year compared with the Ctl group (Table 2).

Conjunctival sensitivity

In the combined HS group, a 10-fold increase in conjunctival Dp tolerance was recorded after 1 year, and was still present after the 2nd year. The Ctl group did not change during this period, the difference between the Ctl and both the mPEG-Dp and the Dp groups being significant for the total material at 1 and 2 years.

mPEG-Dp versus Dp treatment

When comparing the change in tolerance induced after 1 and 2 years, no significant differences in any of the organs tested could be found between the two HS groups (Table 2).

Clinical assessment and bronchial challenges

The majority of patients in all therapeutic groups improved when classified according to a combination of their response to bronchial challenge with histamine or allergen and clinical improvement (pulmonary symptom scores + medication and retrospective judgement) (Table 4), and no significant differences were seen between groups.

Table 3
Occurrence of late-phase reactions after bronchial challenge with Dp

Treatment groups	No late reaction at inclusion				Late reaction at inclusion			
	After 1 year		After 2 years		After 1 year		After 2 years	
	Absent	Present	Absent	Present	Absent	Present	Absent	Present
mPEG-Dp	3	0	3	0	5	6	5	6
Dp	5	1	5	1	5	4	4	5
Control	5	1	3	3	2	4	2	4

Comparison of different effect parameters

Median increase in Dp-tolerance in bronchi was 4- to 6-fold higher in the clinically improved group, compared with the deteriorated group (Table 5). The similar difference in histamine sensitivity was 10-fold. Variation was pronounced, and no significant differences were found when all three response groups were compared. Combination of information from diary cards and retrospective assessments did not increase the agreement. Changes in nose and eye symptoms were not accompanied by parallel changes in sensitivity to Dp in these organs.

DISCUSSION

The present study had a longer duration and a more extensive selection and monitoring of patients than most other published controlled studies on HS of asthmatics with Dp-extract. In addition, fairly high allergen doses were aimed at, and a new allergen modification, presumed to be less allergenic, was included. As reported previously (10), patients in the Dp-treated group showed a considerable *clinical* improvement after 6 months; however, after another year additional benefits in the mPEG-Dp and Ctl groups made the net improvement in the three groups nearly equal. Results from challenge tests presented here showed a similar significant decrease during the 1st year in allergen-specific sensitivity in bronchi, nose and conjunctiva in the HS-treated group but not in the Ctl group. The allergen-specific changes

during this period were accompanied by parallel changes in the unspecific bronchial sensitivity. Except for this unspecific bronchial reactivity which returned to pre-treatment levels, the improvement was generally maintained in the HS group during the 2nd year of treatment. During the latter period an isolated reduction in bronchial Dp-sensitivity occurred in the Ctl group which could not be explained by change in Dp-exposure. The 6-weekly visits mainly to the same doctor made it possible to adjust medication and give thorough medical advice. This may, in both HS and Ctl patients, have improved the medical compliance and to some extent the clinical status, impeding the detection of the effects of HS.

Although a beneficial clinical effect was shown when comparing changes *within* groups, due to the generally large variation within groups only a few significant differences were seen *between* groups with regard to change in shock organ sensitivity. The most evident difference between Ctl and HS groups was seen in conjunctival improvement. A similar isolated conjunctival improvement was seen after HS in dog-allergic asthmatics (19).

Results may indicate that conjunctival sensitivity compared to sensitivity in bronchi and nose is less influenced by unspecific factors not related to HS.

Most other studies on HS in mite asthmatics controlled by challenge tests have shown a beneficial effect (1, 3, 4, 15, 20). Generally, the effect and the therapeutic difference between the actively treated and the controls were more pronounced than in the present study. The diversities might be due to 1) the allergen ex-

Table 4

Patients classified according to clinical assessment and change in bronchial sensitivity to Dp-allergen or histamine at end of treatment

Treatment groups	Bronchial allergen sensitivity + clinical assessment			Bronchial histamine sensitivity + clinical assessment		
	Improvement	No change	Deterioration	Improvement	No change	Deterioration
mPEG-Dp	13	1	2	12	2	2
Dp	11	3	1	11	1	3
Control	9	5	1	8	3	4

tracts and dose regimens, 2) the patients, and 3) the monitoring procedures.

The amount of allergen administered was high, judged from the number of large local reactions and systemic side effects in both HS groups (to be reported), and so the results could not be ascribed to too low doses of allergen as in the only other unsuccessful bronchial challenge controlled Dp-study reported (9).

Patient characteristics might be a major reason for the outcome of the present study. Age, severity of asthma and dominance of unspecific in relation to allergen-specific reactivity in the patients might be of special importance. In two of the successful studies (1, 20), children have been treated. In general, they have a shorter duration of symptoms, have less unspecific reactivity and may to some extent grow out of their asthma (7). In the present study, basic lung function varied considerably between individuals. However, improvement in bronchial reactivity has been obtained in patients with both normal (4) and low basic lung function ($FEV_1 < 80\%$) (3).

The monitoring might account for important therapeutic differences between studies. If the present study had been terminated after 1 year, before the improvement in the Ctl group, the

evaluation of HS would have proved to be more favourable. Measurement of effect shortly after the dose-increase phase, as in a recent potent extract study (4), might have produced even more positive results. Such results were in fact observed in a 3-month study on rhinitis using the same unmodified Dp-extract as in the present study (5). However, since patients generally receive HS for years it seems relevant, as here, to perform evaluation (at identical season) after 1 or 2 years.

Most other publications on Dp-HS do not contain data on test reproducibility, and, especially in studies with few participants, low reproducibility might lead to erroneous conclusions. In the present study, reproducibility data for bronchial and conjunctival challenge tests applied are acceptable (6, 8). For bronchial challenge, analysis was made on a group of patients with basic lung function similar to that observed in the present study. However, clinically, changes in sensitivity in patients with low lung functions might not be comparable to similar changes in patients with normal lung functions. Furthermore, in other studies, house dust mite exposure was not monitored during the treatment phase. In the present study major systematic errors due to environmental (Dp)

Table 5

Changes in bronchial sensitivity (median and range) according to clinical response and type of assessment at end of treatment. All treatment groups included

Assessment	Change in bronchial symptoms + medication	N	Relative increase in concentrations necessary to induce bronchial response to:	
			Dp	Histamine
Questionnaire	improvement	23	54 (0.2-1000)	2.0 (0.3-185)
	no change	14	11 (1.7-100)	4.4 (0.01-233)
	deterioration	6	8.5 (0.1-100) (NS)	0.2 (0.03-36) (NS)
Diary card	improvement	18	35 (0.2-463)	2.9 (0.02-185)
	no change	18	14 (0.1-1000)	2.4 (2.4-233)
	deterioration	9	10 (0.2-102) (NS)	0.3 (0.01-30) (NS)
Questionnaire + diary card	improvement	23	47 (0.2-1000)	2.8 (0.3-185)
	no change	11	16 (0.1-102)	6.4 (0.1-233)
	deterioration	9	10 (1-16) (NS)	0.3 (0.01-30) (NS)

changes were, however, excluded by measuring changes in concentration of Dp in mattress dust once a year (10).

Ongoing bronchial hyperreactivity, as measured by bronchial sensitivity to histamine, could be caused by late-phase reactions, and some investigators have demonstrated a beneficial effect of HS on late-phase reactions to bronchial Dp challenge in children (20). In the present and another (4) adult study, no direct benefit on these reactions was observed, but in our study there was an indication of a protective effect of HS against the development of late-phase reactions. The reactions seem to vary from year to year even in the Ctl group. This might partly be due to the way challenge tests were performed, with the amount of allergen administered being solely determined by the *immediate*-phase Dp-tolerance. A decrease in PC₂₀ together with disappearance of late-phase reaction would not necessarily mean an improvement in unspecific hyperreactivity or late-phase reaction, since the inhaled allergen dose might be reduced at the test.

Results from studies of the effect on unspecific bronchial reactivity are disagreeing. In an infant study, mite HS seemed to increase this hyperreactivity (13), whereas treatment for cat allergen could induce some improvement in one study (17), but not in others (16, 18). In the present study, an initial improvement in unspecific bronchial reactivity was demonstrated in the HS group, but after another year this effect had disappeared, and the clinical implications of these changes could be questioned.

Mechanisms behind the effect of HS are still unknown, and it might be hypothesised that the effect is mediated through a complex of cellular and humoral factors not necessarily measurable by unspecific or allergen-specific early- and late-phase bronchial reactions. A clinical evaluation might be necessary to recognize the effect in some patients. However, in the present study no significant differences between groups were observed by an assessment combining changes in bronchial reactivity with clinical effect in the individuals.

Although net results from clinical assessments and challenge tests seem to be identical by

group comparison, changes in symptoms and medication did not in the individual patients parallel changes in Dp-reactivity in nose and eyes. Only in patients with respiratory improvement was there a tendency towards reduced sensitivity to histamine and Dp. Such discrepancies conform with the findings of other studies (1, 15) and are not surprising, since clinical assessments are influenced by psychological and other environmental factors unrelated to HS. However, they stress the importance of using both challenge tests and clinical assessments when evaluating the total effect of HS in the individual patient and in clinical trials.

In conclusion, the mPEG-modified extract did not seem inferior to the unmodified Dp-extract. Both could improve tolerance to Dp in the bronchi, nose and conjunctiva. However, a high proportion of patients in the Ctl group improved with regard to bronchial sensitivity to Dp, especially during the second year of the study, possibly due to better compliance. Variation within groups was extensive, and further analyses will show whether it is possible to identify pre-treatment parameters for selection of Dp-allergic patients benefiting from HS.

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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