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A controlled dose-response study of immunotherapy with standardized, partially purified extract of house dust mite: Clinical efficacy and side effects

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Background: *Seventy-four asthmatic patients allergic to house dust mite were included in a double-blind, controlled study to establish the optimal maintenance dose of a standardized extract of* Dermatophagoides pteronyssinus *(Der p I) during 24 months of immunotherapy (IT).*
Methods: *The patients were given the following maintenance doses: 19 patients 10,000 standardized quality units (SQ-U) (group 10, 0.7 μg* Der p *I), 20 patients 100,000 SQ-U (group 100, 7 μg* Der p *I), 16 patients 300,000 SQ-U (group 300, 21 μg* Der p *I), and 19 control patients (group 0) had no injections. After 24 months bronchial challenge demonstrated a dose-related increased tolerance to* Der p *I, group 10 (p = 0.003), group 100 (p = 0.0005), group 300 (p = 0.0007), with no change in group 0 (p = 0.6). Patients given IT had a decrease in medication and peak expiratory flow score. In total, 2104 injections were given, and 3.5% were followed by a systemic reaction, defined as a fall 15% or greater in forced expiratory volume in 1 second within 30 minutes. A dose-response relation was demonstrated, with rates of systemic reactions in percent of injections; group 10, 0.56%; group 100, 3.30%; and group 300, 7.10% (p < 0.0001). No anaphylactic reactions occurred, and no late systemic reactions were observed. This study demonstrated a dose dependence of efficacy and side effects of IT in asthmatic patients. We suggest a maintenance dose of 100,000 SQ-U (7 μg* Der p *I) as an appropriate guideline for IT with house dust mite extract. (J ALLERGY CLIN IMMUNOL 1993;91:709-22.)*

Key words: *Dose response, immunotherapy, bronchial asthma, houst dust mite allergy*

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Immunotherapy (IT) has been used in the treatment of allergic diseases since it was introduced by Noon[1] in 1911. Although it is generally accepted as efficient when administered under appropriate circumstances,[2, 3] controversies still exist about optimum doses and regimens of IT.[4] The efficacy has been shown to be related to dose.[5-8] The value of a low maintenance dose has been comparable to placebo,[9] and high doses seem to be necessary to achieve clinical efficacy.[10] Because of this, high-dose regimens of IT

TABLE I. Demographic data

	Group 0	Group 10	Group 100	Group 300
M/F	12/7	9/10	12/8	13/3
Age (yr)	27 (10-45)	28 (12-64)	27 (10-57)	27 (12-57)
Duration of asthma (yr)	6 (1-20)	7 (1-25)	7 (2-49)	6 (1-55)
Baseline FEV_1 % predicted	85 (50-129)	81 (48-100)	83 (56-110)	85 (51-114)
Positive SPT				
Dog/cat	5	8	11	5
Pollen	10	9	6	3
Medication				
Inhaled β_2-agonists	19	19	20	16
Inhaled disodium cromoglycate	0	1	1	0
Inhaled steroids	5	12	5	6
Oral theophylline/β_2-agonists	5	8	8	5

Median values given for age, duration of asthma, and baseline FEV_1; range in parentheses.

Abbreviations used

IT: Immunotherapy
SQ-U: Standardized quality units
FEV_1: Forced expiratory volume in 1 second
PEF: Peak expiratory flow
BCA: Bronchial challenge with allergen extract
BCH: Bronchial challenge with histamine dihydrochloride
CPT: Conjunctival provocation test with allergen extract
PD_{20}: Cumulated dose of inhaled allergen extract producing a 20% fall in FEV_1
PC_{20}: Concentration of inhaled histamine dihydrochloride producing a 20% fall in FEV_1
DP: *Dermatophagoides pteronyssinus*
Der p I: Standardized extract of *DP*
SPT: Skin prick test
LAR: Late asthmatic reaction
SR: Systemic reaction
ELR: Early large local reaction
LLR: Late large local reaction

have been recommended, aiming at the maximum tolerated dose—a dose just below that which produces systemic side effects.[4, 11] The dose-response relationship of IT has been studied with use of IT with ragweed extracts in hay fever,[10] and studies in bronchial asthma were performed before the development of standardized extracts.[5, 6] In combination with the development and use of standardized and more potent extracts, high-dose IT has resulted in a number of side effects.[12-15] This has brought focus on the safety of IT,[16, 17] and the treatment has been restricted in some countries.[16, 18] Consequently, a need exists to determine the optimum maintenance dose in IT,[11] assessing the risk of side effects and clinical efficacy in relation to different dosage regimens. House dust mites are one of the most important allergen sources in allergic asthma,[19] and an international standard for *Dermatophagoides pteronyssinus (DP)* has recently been adopted by the World Health Organization.[20] The access to allergen extracts with known content of major allergens has made comparisons of different IT regimens in asthma possible and has made studies aiming to establish the optimum maintenance dose in IT possible.

The purpose of the present study was to examine the dose dependence of IT. This was done by evaluation of clinical efficacy and side effects in patients with bronchial asthma who are allergic to house dust mites and were given IT with one of three different maintenance doses of a standardized, partially purified extract of *DP*.

MATERIAL AND METHODS

Patients

Eighty-one patients from our outpatient clinic were recruited for the study: 30 female and 51 male patients, with a mean age of 29 years (range, 10 to 64 years). Fifteen were children less than 15 years of age (Table I). The patients included in the study had the following characteristics: (1) a typical history of bronchial asthma,[21] (2) a positive bronchial challenge test with histamine, (3) a positive skin prick test (SPT) with *Der p* I, (4) a positive reaction to bronchial challenge with *Der p* I, and (5) a positive test of serum-specific IgE to *DP* (Phadebas RAST, Pharmacia, Sweden; ≥class 1). Patients with cardiovascular or immunologic diseases were not included. No patients with a positive SPT to molds or storage mites participated, and patients with

TABLE II. Medication score

Medication	Consumption	No. points
Inhaled β_2-agonists	0-2 puffs	1
	3-6 puffs	2
	>6 puffs	3
Sustained oral formulas	Theophylline 300 mg	1
	Salbutamol 8 mg	1
	Terbutaline 7.5 mg	1
Sodium cromoglycate	≤4 puffs	1
Inhaled steroids	1-399 μg	1
	400-799 μg	2
	800-1199 μg	3
	1200-1599 μg	4
	>1600 μg	5

animal dander allergy were not included if they were exposed to animals. The patients had mild-to-moderate asthma, and none required treatment with systemic corticosteroids. Use of concomitant medication is shown in Table I. None of the patients had ever had IT with house dust mite extracts. Informed consent to participate in the study was given by all patients, and the protocol was approved by the Ethical Committee of Aarhus County.

Extracts

Extracts used were partially purified, standardized extracts of *DP* whole mite culture from which components with molecular weight less than 5000 d were removed.[22, 23] Extracts were standardized relative to an internal standard. The content of the major allergens *Der p* I and *Der p* II was quantified by immunoelectrophoretic methods. Total allergenic activity was measured by RAST inhibition, and the biologic activity of the batch of extract used was determined by SPT as previously described.[24, 25] The potency of the extract was expressed in arbitrary SQ-U (standardized quality units) by the manufacturer (ALK Laboratories, Hørsholm, Denmark). At a concentration of 100,000 SQ-U/ml, the extract contained 23,000 IU/ml of *Der p* I (7 μg/ml) and 34,500 IU/ml of *Der p* II. A depot extract adsorbed to aluminium hydroxide (Alutard SQ; ALK Laboratories) was used for IT. The same batch of extract in different diluents was used throughout the study for diagnostic tests and treatment.

Bronchial challenge tests

For bronchial challenge tests we used a standard Wright (Aerosol Products Ltd., London, U.K.) nebulizer driven with compressed air at 0.5 to 0.7 bar. The nebulizers were calibrated to an output of 0.13 to 0.15 ml/min, and a flow regulator ensured a constant pressure. The mist was inhaled via a mouthpiece at tidal breathing, and the nose was clipped to ensure oral breathing. Forced expiratory volume in 1 second (FEV_1) was measured with a dry bellow spirometer (Vitalograph 20600, Buckingham, U.K.), and peak expiratory flow (PEF) was measured with a mini-Wright (Clement Clarke International, Harlow, U.K.) peak flow meter. All measurements of lung function were repeated three times, and the highest value was used for calculations. Oral bronchodilators were withheld 24 hours before challenge; inhaled β_2-agonists and steroids were withheld for 12 hours. No patients used antihistamines. None of the patients had a respiratory tract infection within 4 weeks before the challenges.

Bronchial challenge with allergen (BCA). An aqueous solution of *DP* extract (Aquagen SQ; ALK Laboratories) was used for bronchial allergen challenge test (BCA). SPT titration with *DP* was performed, and the allergen concentration giving a 2 to 3 mm wheal was used as the starting concentration. FEV_1 and PEF were measured before the challenge was started. Lung function measured 5 minutes after eight inhalations of diluent was used as baseline for the BCA. The first allergen dose was two inhalations of the starting concentration. Throughout the challenge FEV_1 was measured 5 and 10 minutes after inhalation. The challenge was continued with doubling doses (2, 4, 8 inhalations) and 10-fold increments in allergen concentration until a fall 20% or greater in FEV_1 was obtained. Cumulated dose of inhaled allergen extract producing a 20% fall in FEV_1 (PD_{20}) was determined by linear interpolation on the log dose-response curve.[26] Lung function was followed in the laboratory for at least 60 minutes after the last dose of allergen, and in most cases it was seen to normalize. Patients were not given bronchodilators after challenge, and they measured PEF every hour for the next 10 to 12 hours, only using inhaled β_2-agonists when necessary. A late asthmatic reaction (LAR) was defined as a fall in PEF 20% or greater of prechallenge value, measured 2 to 10 hours after challenge, and with a typical gradual deterioration of lung function after spontaneous reversal of the early athmatic reaction.

Bronchial challenge with histamine. Bronchial challenge test with histamine dihydrochoride (BCH) was performed as a modification of the method described by Cockcroft et al.[27] The same type of Wright nebulizer as for BCA was used. The patients inhaled for 2 minutes at tidal breathing. Challenge was started with inhalation of isotonic saline

TABLE III. Schedule for dose increment

Vial no.	Injection no.	Week no.	Volume (ml)
I	1	1	0.1
	2	1	0.2
	3	2	0.4
	4	2	0.8
II	5	3	0.1
	6	4	0.2
	7	5	0.4
	8	6	0.6
	9	7	0.8
III	10	8	0.1
	11	9	0.2
	12	10	0.3
	13	11	0.4
	14	12	0.6
	15	13	0.8
IV	16	14	0.1
	17	15	0.2
	18	16	0.3
	19	17	0.4
	20	18	0.5
	21	19	0.6
	22	20	0.8
	23	21	1.0

solution. FEV_1 measured 90 seconds after this inhalation was used as baseline. Then histamine dihydrochloride was inhaled in doubling concentrations, starting with 0.03 mg/ml to maximum concentration used 32 mg/ml. FEV_1 was measured 30 and 90 seconds after each inhalation, and the challenge was continued with intervals of 5 minutes between inhalations until a fall in FEV_1 20% or greater was obtained. Concentration of inhaled histamine dihydrochloride producing a 20% fall in FEV_1 (PC_{20}) was calculated by linear interpolation on the log dose-response curve.

Conjunctival provocation test (CPT)

A drop of the test solution was applied in the lower conjunctival sac alternating between the two eyes. Aqueous diluent acted as control, and *DP* extract (Aquagen SQ) was administered in concentrations from 100 SQ-U/ml and increased in half-$\log_{10}$ steps with intervals of 10 minutes to a maximum of 320,000 SQ-U/ml. A positive reaction was recorded when both itching and congestion of the conjunctival mucosa occurred.[28]

SPT

Skin prick test was performed in duplicate with *DP* extract in concentrations of 1, 3, 10, 30, and 100 histamine equivalent pricks (HEP) before start of IT and after 12 and 24 months. The results of this will be reported elsewhere. An SPT was also performed with a standard panel of allergens (Soluprick SQ, ALK Laboratories) including birch, timothy, mugwort, hair and dander from horse, dog, and cat, and the three molds *Cladosporium herbarum*, *Aspergillus fumigatus*, and *Alternaria tenuis*. Included were also extracts of the storage mites *Lepidoglyphus destructor*, *Tyrophagus putrescentia*, and *Acarus siro* (Diephuis Laboratories, Groningen, The Netherlands). Histamine dihydrochloride in a concentration of 10 mg/ml served as positive control.

Diary cards

The patients kept a diary for assessment of their asthma during five 4-week periods: during the run-in period before starting IT treatment in spring 1987, during September 1987, April 1988, September 1989, and just before IT was stopped in April 1989. The following were registered daily:

1. Night symptoms noted on a scale from 0 to 4.
2. Asthma symptoms during daytime recorded on a scale from 0 to 3. The symptom score values used in calculations were a percentage of the maximum score obtainable.
3. The intake of antiasthmatic drugs was scored according to Table II. Medication score was expressed as a percentage of the highest score recorded by any patient during the study.
4. PEF was measured morning and evening with a mini-Wright peak flow meter, and the best of three measurements was recorded in the diary. The mean morning and evening PEF during the run-in period was used as baseline, and mean PEF recorded during treatment periods was expressed as a percentage of this. Therefore a higher percent in all parameters reflected a worsening of the patient's asthma.
5. A global score was calculated as the mean of symptom and medication scores and PEF measurements.[29]

Study design

Diagnostic tests including BCA, BCH, CPT, and SPT were performed before IT and after 12 and 24 months of treatment. Clinical assessment by patient diaries were made before IT and every 6 months during IT, including the peak mite seasons in autumn 1987 and autumn 1988. The environmental exposure to house dust mite allergens was determined by analyses of dust samples from the patients' homes before IT and after 12 and 24 months. After initial diagnostic tests the patients were assigned to one of four groups on the basis of age and bronchial sensitivity to *DP*. The first group of patients were to receive a maintenance dose of 10,000 SQ-U (group 10), the second group received a maintenance dose of 100,000 SQ-U (group 100), the third group received a maintenance dose of 300,000 SQ-U (group 300), and the last group acted as a control group (group 0), and received no injections.

TABLE IV. No. of injections, maintenance doses, and cumulated doses

	Treatment group		
	Group 10	**Group 100**	**Group 300**
12 months			
No. injections	31 (30-33)	32 (30-40)	32 (30-42)
Maintenance dose SQ-U	10,000 (10,000-10,000)	100,000 (20,000-100,000)	300,000 (150,000-300,000)
Cumulated dose SQ-U $\times 10^{-3}$	111.93 (85.85-127.63)	1,111.8 (726.25-1,373.3)	3,426.5 (2,694.8-3,739.5)
Cumulated dose *Der p* I IU	25,744	255,714	788,095
Cumulated dose *Der p* II IU	38,616	383,571	1,182,143
24 months			
No. injections	38 (35-39)	39 (36-46)	40 (37-49)
Maintenance dose SQ-U	10,000 (7,000-10,000)	100,000 (50,000-100,000)	300,000 (150,000-300,000)
Cumulated dose SQ-U $\times 10^{-3}$	176.63 141.73-191.63)	1,732.8 (802.25-1,916.3)	5,358.8 (4,027.7-5,839.5)
Cumulated dose *Der p* I IU	40,625	398,544	1,232,525
Cumulated dose *Der p* II IU	60,937	597,816	1,848,786

Median values; range in parentheses.

Immunotherapy

Four consecutively numbered vials of allergen extract were prepared for each patient. In vial no. 1 the allergen concentration was 10 SQ-U/ml, 100 SQ-U/ml, and 300 SQ-U/ml, respectively, for the three groups of patients given IT. The actual allergen concentration was unknown to the patient and the physician giving the injections. Each following vial contained a 10-fold higher allergen concentration. The dose increment schedule is shown in Table III. After maintenance doses were reached, injections were given every 6 to 8 weeks. Indications for dose reductions were the following: a systemic reaction (SR), an early large local reaction (ELR) defined as local swelling at the injection site 5 cm or greater in diameter occurring 30 minutes after injection, or a late large local reaction (LLR) 10 cm or greater in diameter occurring 8 to 24 hours after an injection.

Assessment of local reactions and SRs

At all visits the patients were questioned about ongoing infections, the status of their allergic disease, and reactions to the previous injection. Before each injection the patients' FEV_1 was measured. After allergen injection the patients stayed at the clinic for at least 30 minutes, and signs and symptoms of SRs were registered. After 30 minutes the diameter of local wheal and flare was measured, and FEV_1 was recorded. After allergen injections the patients filled in a diary form for the next 2 days. Questions concerned the occurrence of cough or asthma, symptoms of rhinitis, conjunctivitis, or other symptoms such as unusual tiredness, headache, arthralgia, and flulike symptoms. PEF was measured with a mini-Wright peak flow meter on the evening of the day of injection and on the following 2 days, morning and evening. The patients measured and recorded local wheal and flare at the site of injection 6 to 8 hours after the injection and also on the following two mornings. Consumption of medicine to relieve allergic symptoms was registered. This diary was brought to the clinic on the next visit and used for assessment of late reactions.

Statistics

Nonparametric statistical methods were used for calculations. For differences between groups Kruskal-Wallis one-way analysis of variance (ANOVA) and Mann-Whitney U test were used, and for changes within the groups Friedman two-way ANOVA and Wilcoxon matched-pairs signed ranks test were applied. For parameters where two groups differed significantly at time of run-in, differences in changes during the study were tested. For correlation analyses the Pearson product-moment correlation coefficient or the Kendall rank-order correlation coefficient test was used. The chi-square test of independence was applied for testing the influence of one variable on another. Statistical analyses were performed with the SPSS/PC+ package.[30]

RESULTS

Patients

Seven patients left the study after the run-in period and assignment, leaving 19 patients in group 0, 19 patients in group 10, 20 patients in group 100, and

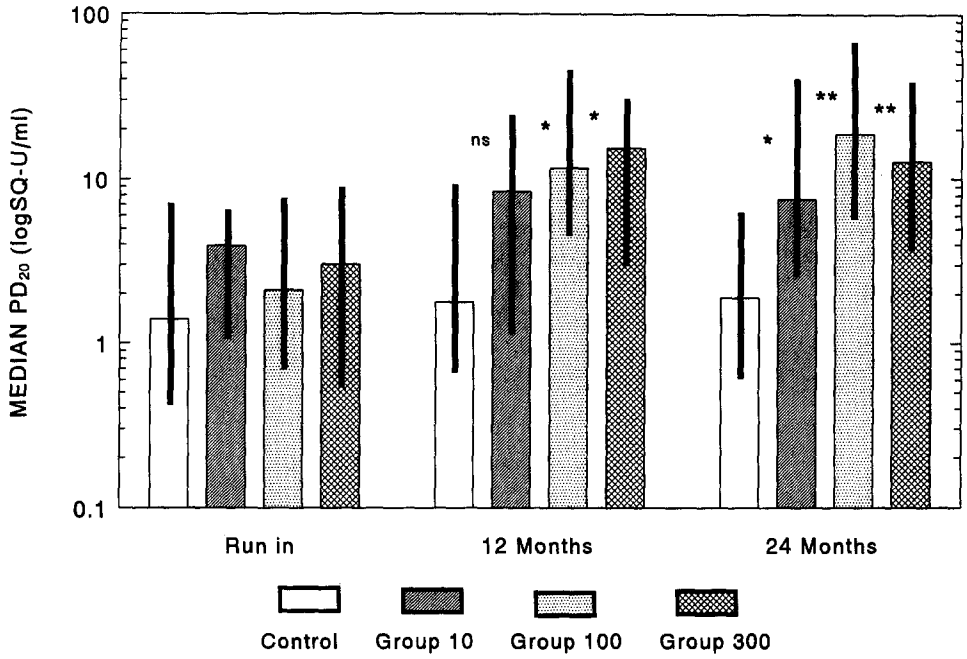

FIG. 1. Changes in median PD_{20} to *DP* extract. Range shown by *bars* indicating 25th and 75th percentiles (*ns;* No significant difference compared with control group, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$).

TABLE V. Results of bronchial challenge with *DP* allergen, histamine, and CPT

	Group 0	Group 10	Group 100	Group 300
PD_{20} BCA SQ-U $\times 10^{-5}$				
Run-in period	1.40	3.93	2.12	3.04
	(0.21-24.5)	(0.11-24.0)	(0.17-19.8)	(0.22-24.0)
12 mo	1.80 NS	8.48**	11.7***	15.4***
	(0.28-29.0)	(0.10-56.7)	(0.24-200)	(0.36-71.3)
24 mo	1.91 NS	7.6**	18.8***	12.8***
	(0.18-29.9)	(0.30-200)	(1.12-200)	(0.53-200)
PC^2_0 BCH mg/ml				
Run-in period	1.14	0.28	0.68	0.32
	(0.06-3.37)	(0.20-3.90)	(0.06-7.07)	(0.10-8.86)
12 mo	0.95 NS	0.63***	1.21**	1.43*
	(0.06-32.0)	(0.14-32.0)	(0.25-32.0)	(0.07-16.9)
24 mo	1.84**	0.66**	1.28**	1.97**
	(0.12-17.1)	(0.13-32.0)	(0.31-19.8)	(0.11-17.0)
CPT threshold SQ-U $\times 10^{-3}$				
Run-in period	10.0	10.0	10.0	3.20
	(1.0-1,000)	(0.32-1,000)	(1.00-32.0)	(1.0-32.0)
12 mo	10.0 NS	100***	100***	100***
	(3.2-100)	(10.0-1,000)	(10.0-1,000)	(10.0-1,000)
24 mo	10.0 NS	320***	320***	100***
	(3.2-100)	(10.0-1,000)	(10.0-1,000)	(10.0-1,000)

Median values; range in parentheses.
NS, Not significantly different from pretreatment value.
$*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

16 patients in group 300. Another six patients dropped out after diagnostic tests had been performed after 12 months. Reasons for dropout were unrelated to the study (pregnancy 1 patient, emigration 2 patients, lack of time 3 patients). At 24 months 18 patients in group 0, 19 patients in group 10, 16 patients in group 100, and 15 patients in group 300 had completed the study.

Dosage

Maintenance doses were very near the intended, with medians of 10,000 SQ-U, 100,000 SQ-U, and 300,000 SQ-U, respectively. Only little variation was found within the groups. This is reflected also in the cumulated doses, demonstrating the homogeneity within groups regarding these parameters (Table IV).

BCA

A statistically significant increase in median PD_{20} was found in all groups given IT after 12 months, whereas no change was found in group 0, and after 24 months a further increase was found in groups given IT, whereas no significant increase in PD_{20} compared with pretreatment was found in group 0 (Table V). No significant differences in the median PD_{20} were found between groups before treatment. After 12 months a significant difference was seen between group 0 and group 100 and between group 0 and group 300, whereas the difference between group 0 and group 10 was not significant. After 24 months significantly higher PD_{20} *DP* was found in all groups of patients given IT compared with controls (Fig. 1). A BCA was not performed after 12 months in two patients in group 0, in one patient in group 100, and after 24 months in one patient in group 0. After treatment a negative BCA (i.e., no reaction after inhaling a cumulated dose of 10^7 SQ-U) was found in 7 of 104 challenges in patients given IT, and in such cases an arbitrary PD_{20} of 2×10^7 SQ-U was used in the statistical calculations.

LAR

Information about whether an LAR occurred after BCA was obtained in 68 of 74 patients at the run-in period, in 67 of 74 patients after 12 months, and in 62 of 68 patients completing the whole 24 months of study. The number of patients with LARs during the study is shown in Table VI. No significant changes in the occurrence of LAR were found after 24 months.

BCH

A significant decrease in histamine sensitivity was found in all groups given IT after 12 months, whereas histamine sensitivity remained unchanged in the control group. After 24 months a further decrease in histamine sensitivity was found in patients given IT as well as in the control group (Table V). When comparing PC_{20} histamine during the study, no significant differences between groups were found at any time (Fig. 2). After treatment a negative BCH (i.e., no drop in FEV_1 20% or greater at a histamine concentration of 32 mg/ml) occurred in 5 of 142 challenges, and in these cases an arbitrary value of 32 mg/ml was used for PC_{20} histamine in statistical calculations.

CPT

A significant increase in the median concentration of *DP* eliciting a conjunctival reaction was demonstrated in the three groups of patients given IT after 12 months, with a further increase in threshold concentration after 24 months. No significant change in threshold concentration was seen in the control group (Table V). Before IT the patients in group 300 had a significantly higher conjunctival sensitivity to *DP* than the patients in the other groups. A comparison between the four groups of patients of the changes in threshold concentration demonstrated highly significant differences between patients given IT and the control group after 12 and 24 months ($p < 0.001$) (Fig. 3). After treatment a negative conjunctival challenge test (i.e., no reaction to an allergen concentration of 320,000 SQ-U) was found in 33 of 138 challenges in patients given IT, and the threshold concentration in these cases used for statistical calculations was an arbitrary value of 10^6 SQ-U.

Dust samples

No significant differences in the exposure to house dust mite allergens between the groups were found during the study, and no significant changes in exposure were found in any group after 24 months.

Diary cards

A complete diary card during the run-in period was filled in by 79% of patients in group 0, 79% in group 10, 90% in group 100, and by 100% in group 300. After 24 months corresponding figures were 58% of patients in group 0, 74% in group 10, 63% in group 100, and 87% in group 300. Although house dust time allergen is perennial, there is a fluctuation, with a peak season in early autumn.[31] Results from diary cards were compared between run-in period, 12 and 24 months separately and between the two mite seasons alone. When compared with the run-in period, the results from 12 and 24 months showed a decrease in global score in the period 0 to 12 months in group

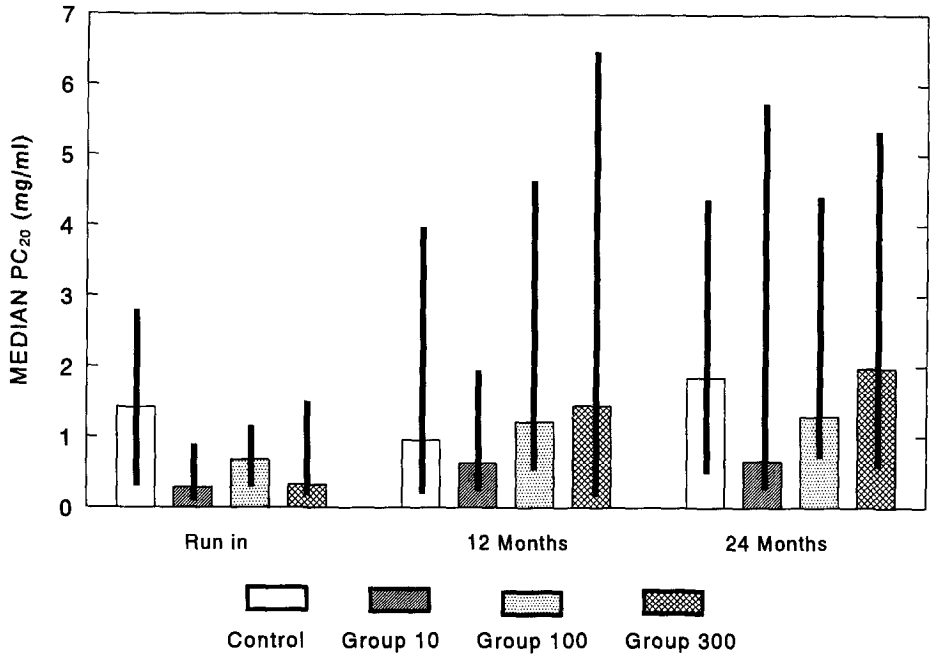

FIG. 2. Changes in median PC_{20} histamine. Range shown by *bars* indicating 25th and 75th percentiles.

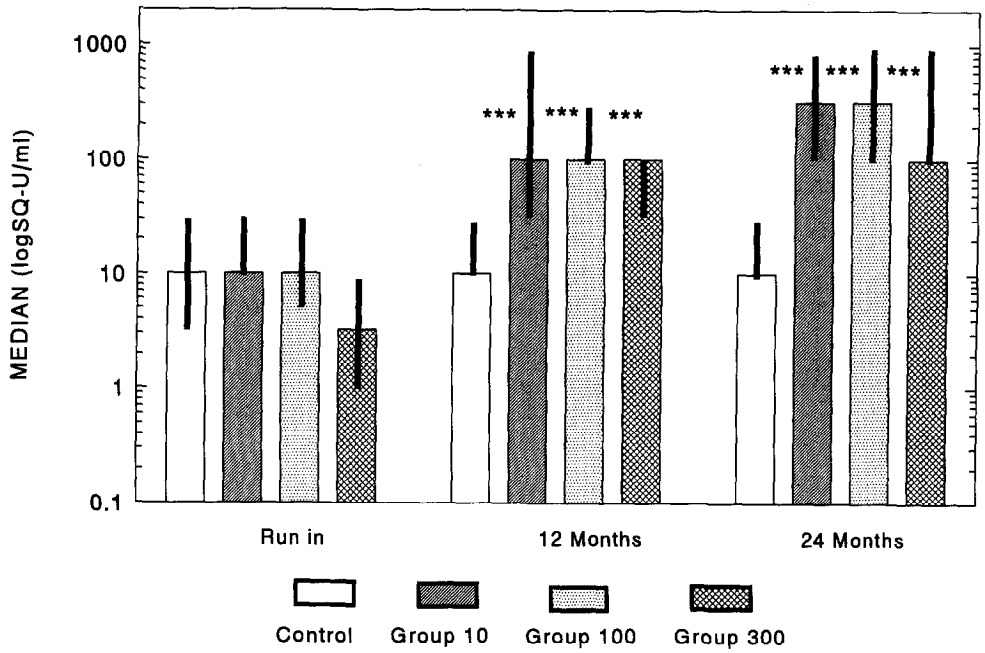

FIG. 3. Changes in median conjunctival threshold concentration of *DP* extract. Range shown by *bars* indicating 25th and 75th percentiles (***$p < 0.001$; difference compared with control group).

100 ($p = 0.01$) and group 300 ($p = 0.03$), and in the period 0 to 24 months in group 10 ($p = 0.07$). During the run-in period a night symptom score of 0 was reported by 33% to 44% of patients, and a daytime symptom score of 0 was reported by 13% to 20% of patients given IT, which made it very difficult to demonstrate any improvement in these parameters. We therefore examined changes in (medication + PEF) score as a separate parameter. The level of significance for changes in this parameter is illustrated in Table

TABLE VI. No. of patients with LAR compared with total number of patients registering PEF rate after bronchial challenge with *DP*

Treatment group	Before	12 mo	24 mo
Group 0	12/17	14/17	10/17
Group 10	12/19	5/19	6/19
Group 100	8/17	7/17	5/13
Group 300	11/15	7/14	7/14

VII. When results from the two mite seasons were compared, no statistically significant differences between the groups were demonstrated in any of the parameters examined.

SRs

No late SRs were reported in any patient. Immediate SRs occurred within 30 minutes after allergen injection and included rhinitis, conjunctivitis, and asthma. No anaphylactic or generalized cutaneous reactions occurred. Asthmatic symptoms reported by the patients were registered as an SR, and a fall in FEV_1 15% or greater after 30 minutes compared with preinjection was also considered an SR, regardless of the presence of asthmatic symptoms. A total of 2104 allergen injections were given. An SR occurred after 74 injections in 19 patients, corresponding to 3.5% of injections. Excluding falls in lung function without asthma symptoms, an SR was registered after 1% of injections. The distribution of SRs in the three groups of patients is illustrated in Table VIII. Most SRs occurred during maintenance therapy, primarily because of the larger number of SRs in group 300 during maintenance. Increasing maintenance doses were significantly associated with increase in the number of patients having an SR ($p = 0.01$) and the number of injections followed by an SR ($p < 0.0001$) (chi-square test). The number of patients with 1 to 4 SRs was three in group 10, four in group 100, and seven in group 300. No patients in group 10 had more than five SRs, whereas two patients in group 100 and three patients in group 300 had 6 to 13 SRs. A drop in FEV_1 of 15% or greater was found on 67 occasions, and in 45 instances it was found without patients complaining of asthmatic symptoms. A correlation was found between a higher frequency of symptoms and a greater drop in FEV_1 ($p < 0.0001$) (Table IX), but even a fall of 35% in FEV_1 occurred without patient complaints of asthma. Symptoms of asthma occurred in no patients in group 10, eight times in three patients in group 100, and 14 times in five patients in group 300. In all cases asthmatic symptoms were associated with a fall in FEV_1 15% or greater. Rhinoconjunctivitis occurred once in group 10, twice in one patient in group 100, and twice in each of three patients in group 300. The increased frequency of SRs was significantly related to higher doses of allergen ($p < 0.0001$).

TABLE VII. Significance level of changes comparing values of medication + PEF score with pretreatment

Treatment group	Time	
	0-12 mo	0-24 mo
Group 0	0.46	0.88
Group 10	0.009	0.002
Group 100	0.006	0.11
Group 300	0.07	0.04

To predict which patients were at risk of having an SR, a comparison of pretreatment parameters was made between patients with and without an SR. Patients who had an SR were significantly more sensitive to bronchial challenge with *DP* ($p = 0.02$) and histamine ($p = 0.03$), whereas skin sensitivity was not significantly different ($p = 0.08$). Bronchial sensitivity to *DP* correlated significantly with the magnitude of reduction in FEV_1 when SRs occurred. A high skin sensitivity was significantly associated with the allergen dose eliciting an SR ($p = 0.004$).

Large local reactions

An LLR, defined as a local wheal 5 cm or greater in diameter measured 8 to 24 hours after allergen injection, occurred in 292 instances in 27 patients, corresponding to 13.9% of injections. There was a statistically significant increase in the number of injections followed by an LLR with increasing maintenance dose ($p < 0.0001$). The difference in the number of patients having LLRs was significantly lower in group 10 than in group 300 ($p = 0.004$). The increase in frequency of LLRs was significantly related to injection of higher doses of allergen ($p < 0.0001$). High pretreatment skin sensitivity was significantly associated with LLRs ($p = 0.01$), whereas associations with bronchial sensitivity to *DP*

TABLE VIII. No. and distribution of injections and SRs during dose increment and maintenance phase

Treatment group	Total		Dose increment		Maintenance	
	No. inj	No. SRs	No. inj	No. SRs	No. inj	No. SRs
Group 10	714	4 (0.56%)	463	3 (0.65%)	251	1 (0.40%)
Group 100	754	25 (3.30%)	525	13 (2.48%)	229	12 (5.24%)
Group 300	636	45 (7.10%)	402	10 (2.49%)	234	35 (15.0%)

and histamine were insignificant. Occurrence of LLRs did not predict SRs, because the association between LLRs and SRs was not significant. Dose reduction as a result of LLR took place after 16% of LLRs.

An ELR, defined as a local swelling 5 cm or greater 30 minutes after allergen injection, was found after 1.7% of injections. These 35 reactions were distributed among six of the patients. The number of injections followed by an ELR increased significantly with increasing maintenance dose ($p < 0.0001$).

DISCUSSION

The present study demonstrated efficacy of IT in asthmatic patients when a standardized *DP* extract is used. All groups of patients given IT tolerated significantly higher amounts of *DP* allergen at bronchial challenge after 12 and 24 months. The efficacy of IT was related to dose. Median PD_{20} *DP* after treatment in group 10 was two times higher than before treatment, in group 100 five to nine times higher, and in group 300 four to five times higher. The clinical assessment by diary cards was hampered by poor compliance most pronounced among the children and the controls. This is in accordance with a previous study in children, where compliance was too low to use diary cards for assessment of efficacy.[23] Another problem was the high number of patients reporting few symptoms at the run-in period. One reason for this could be that diaries initially were kept in the early spring, where the exposure to house dust mites is low. Furthermore, the graduation of symptoms is determined by individual thresholds and may not correlate with other parameters registered.[32] Analysis of objective parameters alone showed significant improvement only in patients given IT. Most studies performed concerning the efficacy of IT in house dust mite allergy in asthma show a beneficial effect of the treatment. In 14 studies published from 1971 to 1989, a significant effect was demonstrated in nine,[23, 33-40] whereas no significant effect was found in five.[30, 41-44] Standardized extracts and maximum tolerated dose as evaluated by local reactions and SRs were used only in four studies,[23, 39, 40, 44] and inadequate doses of allergen might partly explain lack of effect in four studies.[30, 41-43] However, doses not judged to be maximum tolerated were used in six studies where IT was found to be effective.[33-38] This is in accordance with our findings, that a maintenance dose of 10,000 SQ-U was sufficient to produce and maintain a significant increase in PD_{20} allergen. It was not our purpose to give such extremely low doses as used in therapy described by Rinkel.[9] This is why the lowest dose of 10,000 SQ-U was chosen to be high enough to expect effect in some patients but sufficiently low enough to avoid substantial side effects. The object of the study was a comparison of groups of patients receiving different maintenance doses. It was imperative that the maintenance dose in all patients be as near the intended dose as possible. We had to balance this against our wish to give the highest possible maintenance dose. To maintain the characteristics of a depot extract, and to avoid high concentrations of free allergen, the highest concentration of extract in the present study was chosen to be 300,000 SQ-U/ml, whereas at least 98% of the allergens were adsorbed to aluminium hydroxide. It was not technically possible in a blinded way to produce an aluminium precipitated extract of higher potency because the extract should not contain unacceptable high amounts of nonadsorbed allergen, which could result in more side effects, limiting dose increment. Because a main issue under study was the influence of the allergen maintenance dose on the type and incidence of side effects, the study was designed to minimize the influence of other factors that might affect the result. The dose increment was a weekly, stepwise schedule to minimize side effects, to enable a uniform schedule for all patients, and to have a maximum number of patients reaching the intended maintenance dose. It is likely that some patients could have tolerated a higher maintenance dose and could possibly have benefited from this. Our median top dose of 21 μg *Der p* I is comparable to the median maintenance dose of 24 μg *Der p* I reached in the study of Mosbech et al.,[45] a dose that was the maximum tolerated in that

TABLE IX. No. of cases with fall in FEV_1 ≥15%

Fall in FEV_1 (%)	Treatment group		
	Group 10 No. cases	Group 100 No. cases	Group 300 No. cases
15-20	2 (0)	8 (1)	20 (1)
21-25	1 (0)	6 (1)	10 (4)
26-30	0 (0)	7 (3)	4 (4)
31-40	0 (0)	1 (1)	4 (3)
40-50	0 (0)	2 (2)	2 (2)

No. in parentheses indicates number of cases where patients reported asthmatic symptoms.

study. Very few studies have been published of standardized extract with known content of allergen *Der p* I aiming at maximum tolerated dose. The cumulative doses of *Der p* I administered during 24 months in the present study for group 100 were comparable to the doses in the study by Wahn et al.[23] (78 to 99 μg), and for group 300 in the study by Mosbech et al.[45] (525 μg).

Although a significant reduction in nonspecific hyperresponsiveness was demonstrated in groups of patients given IT, this was also found in the control group after 24 months, and the effect was not dependent on dose. Other studies in asthma of the influence of IT on nonspecific bronchial hyperresponsiveness have produced unequivocal results. In house dust mite asthma an increase in nonspecific bronchial responsiveness after IT has been found in some studies,[46, 47] whereas another demonstrated no change.[44] A decrease in histamine sensitivity has been reported in studies of cat asthma,[24, 48] where keeping of furred pets at home was not allowed. House dust mite allergens are perennial and ubiquitous, and a higher and continuous allergen exposure may be part of the explanation of the difficulties in demonstrating a decrease in nonspecific hyperresponsiveness after IT with mite extracts. A significant reduction in the number of LARs after bronchial challenge has been reported in a study of IT in children,[38] and it has been suggested that IT should be more efficient in patients with LAR. More than 60% of our patients had an LAR at the run-in period, and the reduction in the number of LARs after IT was not significant, which is in accordance with other studies in adults.[39, 44] The impressive effect of IT on conjunctival sensitivity to allergen is in accordance with other studies, but the relevance of this in asthmatic patients is not known, because significant reduction in conjunctival sensitivity has been found in asthmatic patients without significant reduction in bronchial sensitivity.[44, 49]

We have demonstrated a dose dependence of SRs and local reactions during IT in asthmatic patients, using a standardized depot extract of *DP*. IT was safe, with a low SR rate and no serious SRs. To discriminate small but significant differences in the rates of adverse events, we registered a nonsymptomatic, but significant fall in lung function after allergen injection as an SR. The importance of the dose increment schedule has been demonstrated by Bousquet et al.[50] and Hejjaoui et al.[51] who found rates of SRs ranging from 4% of injections during a rush protocol compared with 0.6% of injections during a semirush protocol in asthmatic patients treated with a standardized aqueous extract of *DP*. With the use of a *DP* high-dose regimen in asthmatic patients Mosbech et al.[52] found SRs after 9% of injections during the phase of dose increase and 5% during maintenance therapy.[52] The rate of SRs found in our study for comparable doses (300,000 SQ-U) was 2.5% of injections during dose increment and 15% of injections during maintenance.[52] It should be stressed that 60% of the SRs in our patients were demonstrated by measurements of FEV_1 before and after every injection, and not only by asthmatic symptoms registered by the attending physician or the patients. Serious SRs during IT are rare,[53] but they seem to be more common in asthmatic patients compared with patients with hay fever.[54] In a retrospective study of fatalities during IT, the cause of death in 14 of 16 patients with asthma was respiratory.[54] The use of simple tests of lung function in the assessment of clinical status in asthmatic patients given IT has been recommended.[2, 4] Our finding of a relative high incidence of asymptomatic, significant falls in lung function after allergen injection was unexpected but in accordance with the finding that significant airflow limitation may not be detected without pulmonary function tests.[55] The importance of asymptomatic bronchoconstriction induced by allergen injection is not known. No serious reactions occurred in the present study, and thus our results do not support a hypothesis suggesting that an early fall in FEV_1 will

predict a risk of more serious reactions.[54] On the other hand, we demonstrated a higher bronchial sensitivity to allergen and histamine in patients in whom respiratory reactions developed, which is in accordance with Østerballe,[56] who found that patients having major SRs during IT for grass pollen hay fever could be preselected on the basis of high pretreatment nasal sensitivity to grass pollen. Most SRs including asymptomatic fall in FEV_1 led to a dose reduction. A general application of this procedure could make IT in asthmatic patients more safe in the decision of an individual maintenance dose. Use of only clinical symptoms of asthma to define the maximum tolerated dose detects a more severe SR and may include a potential risk.[15]

Local reactions are expected to occur during IT. In clinical practice they are used to guide dose increment or maintenance doses.[4] In the present study, 16% of LLRs were 10 cm or greater in diameter and caused dose reduction. In no patients did local reactions without SRs limit maintenance dose. We found a dose dependence of LLRs, which occurred in 75% of patients in group 300 compared with 25% of patients in group 10. Our finding that LLRs did not predict a later SR is in accordance with other studies.[56, 57]

Our patients did not report any late SRs, which is in accordance with the studies by Bousquet et al.[50, 51] In contrast, Mosbech et al.[52] reported delayed mild-to-moderate asthma or rhinitis after 3% to 4% of injections, and urticaria after 0.6% to 0.9% of injections. This difference may partly be explained by a difference in the patients' threshold of reporting symptoms, because our patients were not asked to grade their symptoms. The late asthmatic symptoms found by Mosbech et al.[52] were all characterized as mild to moderate. Greenberg et al.[58] found that late SRs constituted 40% of all SRs, and occurred after 0.16% of injections.[59] In these studies no late reaction involving respiratory distress occurred later than 60 minutes after injection, the most common manifestation of late SRs was urticaria, which occurred in none of our patients. Extracts used by Greenberg et al.[58] were not standardized, and most reactions occurred after injection of pollen extracts.

In the present long-term, double-blind study of IT in asthmatic patients with a standardized extract of *DP*, we demonstrated a dose-dependent efficacy in reducing bronchial sensitivity to this allergen and also a dose-related incidence of untoward reactions. A high-dose regimen was shown to be most efficient, with no difference in efficacy of a maintenance dose of 100,000 SQ-U (7 μg *Der p* I) compared with 300,000 SQ-U (21 μg *Der p* I). The incidence of side effects, however, was significantly higher among patients treated with a maintenance dose of 300,000 SQ-U. It must be emphasized that patients should always be treated individually, but the data in the present study point to a maintenance dose of 100,000 SQ-U (7 μg *Der p* I) as an appropriate guideline.

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