

Suppression of the late asthmatic reaction by hyposensitization in asthmatic children allergic to house dust mite (*Dermatophagoides pteronyssinus*)

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Summary

In this study, we wanted to test the hypothesis that hyposensitization (HS) abrogates the late asthmatic reaction (LAR). We therefore selected 15 asthmatic children (subjects) sensitized to house dust mite (HDM), as proven by positive prick tests and/or specific IgE via the RAST. All children demonstrated a positive bronchial provocation test (BPT) to HDM; 14 showed a dual asthmatic reaction and one child showed an isolated LAR. All children were treated with anti-asthmatic drugs and received HS. They were rechallenged after 1 yr of HS while their anti-asthmatic medication was not changed, except for β -agonists which were used only for relief of symptoms. Two BPTs, with a mean interval of 6.4 months, were also performed in eight asthmatic children (controls), who did not receive HS. In the controls, the same reaction pattern was observed during both BPTs. In the subjects, the LAR completely resolved in 5/15 after 1 yr of HS ($P=0.04$). Furthermore, as a group, the subjects showed a less severe LAR after 1 yr of HS (expressed as mean fall of FEV_1) ($P<0.0001$). The PD_{20} of the immediate asthmatic reaction (IAR) was the same as before HS was started, but the IAR was also less severe after 1 yr of HS (expressed as mean fall of FEV_1) ($P=0.028$). From these observations it is concluded that, in asthmatic children, HS may resolve and/or reduce the severity of the LAR. Although the PD_{20} of the IAR is not changed, the severity of the IAR is also reduced after 1 yr of HS.

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Introduction

There is still a lot of debate concerning the role of hyposensitization (HS) in treating allergic asthma. However, recently there has been new interest in HS, mostly due to studies demonstrating the reduction of the late asthmatic reaction (LAR) by HS [1,2]. The aim of this report is to expand these observations. We therefore studied the occurrence of a LAR in 15 asthmatic children, before and after HS was instituted. We also performed two bronchial provocation tests (BPTs) in eight asthmatic children with the same degree of asthma, but who did not receive HS.

Subjects and methods

Subject selection

For this study, 23 asthmatic children were selected and proposed to receive HS. Fifteen children accepted to receive this treatment (subjects), in four subjects HS was refused by the parents and in an additional four HS was delayed for several months. The last two groups constituted the controls.

All were sensitized to house dust mite (*Dermatophagoides pteronyssinus*) (HDM) as proved by positive prick tests and/or specific IgE (RAST). Clinically, all children suffered from perennial asthma. In an attempt to assess the severity of asthma, those who had required admission to the hospital within the last year or who had symptoms most days of the week were regarded as 'severe', those who had weekly symptoms were classified as 'moderate'

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Table 1. Baseline data (at the time of the first BPT) of the 15 subjects and the eight control subjects

		Severity*	Age (yr)	FEV ₁ (%)†	RAST (PRU)‡	Prick test (mm)§	Medication¶
Subject							
1 (BT)	Mo		7.3	83	34	7	Beta
2 (VK)	S		12.3	83	30	6	Beta/Beclo
3 (WK)	S		11.4	74	52	2	Beta/Beclo
4 (SJ)	Mi		8.4	82	17	2	Beta/DSCG
5 (DRG)	S		12.0	89	56	6	Beta/DSCG
6 (BC)	Mi		7.0	99	52	8	Beta/DSCG
7 (LK)	Mo		13.7	94	36	4	Beta/Beclo
8 (CD)	S		9.6	74	20	9	Beta/Beclo Theo
9 (DST)	S		12.3	83	63	4	Beta/Beclo
10 (DBT)	S		11.8	100	14	6	Beta/DSCG
11 (WB)	Mo		12.2	97	40	12	Beta/DSCG
12 (WT)	S		12.8	100	37	5	Beta/DSCG
13 (DBT)	Mo		10.3	86	25	6	Beta
14 (DMK)	Mo		14.2	85	28	8	Beta/Beclo
15 (PG)	S		8.8	87	9	11	Beta/Beclo
Control							
1 (LI)	Mo		7.2	86	37	3	DSCG
2 (WC)	S		10.6	74	52	3	Beta/Beclo
3 (DPP)	S		9.3	84	5	11	Beta/DSCG
4 (BS)	S		11.8	100	61	18	Beta/Beclo
5 (VPS)	S		14.7	87	46	8	Beta/Beclo
6 (VRK)	S		8.5	91	14	3	Beta
7 (LK)	S		9.8	83	18	8	Beta/DSCG
8 (NJ)	S		9.7	76	18	5	Beta

* S=severe, Mo=moderate and Mi=mild. † Percentage predicted for height. ‡ PRU=Phadebas RAST units. § Millimetres greater than control solution. ¶ Beta = β -agonists (salbutamol or fenoterol); Beclo = beclomethasone dipropionate; DSCG = disodium cromoglycate; and Theo = theophylline (oral, long-acting).

and those with monthly or less symptoms were classified as 'mild'. Details of the subjects and controls are shown in Table 1.

Study design

Before entering the study, all children demonstrated a positive BPT to HDM. The subjects were treated with different combinations of anti-asthmatic drugs (β -agonists, beclomethasone dipropionate, disodium cromoglycate, and theophylline) and received HS with HDM during 1 yr. After 1 yr of HS the subjects were rechallenged. The controls were only treated with different combinations of anti-asthmatic drugs and were rechallenged after a mean period of 6.4 months (range: 2–19 months). During the study period, all subjects were seen

monthly at the out-patient clinic. The results of the two BPTs were compared in each group. In both groups, treatment with disodium cromoglycate, theophylline and beclomethasone was constantly maintained between the two BPTs. Based on symptom score and daily peak-flow measurement, β -agonists were used as needed.

The nurses performing the BPT were not aware whether the children received HS or not. Informed consent was obtained from all subjects and their parents before entering into the study.

Bronchial provocation test

In each child two BPTs were performed using a standardized procedure as described earlier [3]. Briefly, subjects had stopped inhaled corticosteroids, theophyl-

line or disodium cromoglycate 48 hr and β -agonists 24 hr, before the challenge. None of the children used systemic corticosteroids or antihistamines. Bronchial challenges were started between 8 a.m. and 9 a.m. After a physical examination, FEV₁ and vital capacity (VC) were measured.

Challenge was performed if the child's resting FEV₁ and VC exceeded 70% of the predicted normal, and if the child was free of respiratory infection for at least 3 weeks. The subjects inhaled through a mouthpiece with the nose occluded, and solutions were delivered with a Bird Mark 7 respirator (Bird Corporation, Palm Springs, U.S.A.) with nebulizer (particle size 0.5–5 μ m according to the manufacturer) at a mean flow of 0.9 l/sec.

Buffer solution was first nebulized for 1 min as a control. Progressive dilutions (10, 100, 500 and 1000 biological units (BU/ml) of the same commercial allergen extract of HDM (*D. pteronyssinus*, Haarlem Allergen Laboratories, Haarlem, The Netherlands) that was used for skin testing and HS, was inhaled during 1 min, starting at a dilution 100 times less (10 BU/ml) than the skin-test concentration.

Bronchial responses were measured by serial monitoring of FEV₁ and VC immediately, and 10 minutes, after each inhalation.

If the decrease in FEV₁ was <20% (compared with the post-buffer control) after inhalation of 1000 BU/ml, no further challenge was given and the subject was considered as having no immediate asthmatic reaction (IAR). To compare degrees of specific hyperreactivity to HDM between the two BPTs, PD₂₀ was determined. This was based on the first decrease of FEV₁ of >20%, the concentration of allergen causing a 20% decrease in FEV₁ being calculated arithmetically.

After measuring the IAR, the FEV₁ was recorded every hour up to 8 hr, or until a significant LAR occurred. A LAR was defined as a decrease in FEV₁ of at least 20% of the post-buffer value in the observation period starting from 4 hr after the beginning of the challenge. A symptomatic LAR was reversed by nebulized fenoterol, and occasionally oral prednisolone was given.

Hyposensitization protocol

Hyposensitization was performed according to a standardized protocol [4]. In short, HS was started using a semi-rush procedure in which the children were given five injections at 30 min intervals with increasing amounts (0.1, 0.3, 0.5, 0.8, 1.0 ml) of the same aqueous extract of *D. pteronyssinus* (10 BU/ml) as used for the BPT. One week later the same amounts were injected with a tenfold higher concentration (100 BU/ml). Subsequently, twice weekly injections were given with increasing amounts (0.1, 0.2, 0.3, 0.4, 0.6, 0.7, 0.9, 1.0 ml) of allergen extract

(1000 BU/ml). This treatment took 1 month to reach the final concentration of 1.0 ml of 1000 BU/ml. Therapy was continued by injecting this dose, once after 1 week, 2 weeks, and 3 weeks, and finally every 4 weeks.

Statistical analysis

Means were compared by the appropriate Student *t*-test (for paired and non-paired data), by the Wilcoxon signed-rank test and by the test of Kruskal and Wallis. Differences between frequencies were evaluated by Fischer's exact test. Differences were considered significant at the 0.05 level.

Results

The subjects and the controls were comparable according to age, severity of asthma, sensitization to HDM (positivity of RAST, prick test and PD₂₀) and basal FEV₁ (Table 1). There was no difference in the use of anti-asthmatic drugs between the two groups.

The reaction pattern to the first BPT was also comparable between subjects and controls (Table 2). The controls demonstrated the same reaction pattern to the two BPTs (Table 2). The severity of their LAR (expressed as mean fall of FEV₁) and the PD₂₀ were also the same during the two challenge procedures (Table 3). In the subjects, the PD₂₀ of the first BPT was similar to the PD₂₀ of the second BPT (Table 3), and the observed difference of the median value was not statistically significant (Wilcoxon signed-rank test).

The frequency of the IAR was the same in both BPTs, but the LAR occurred less frequently during the second BPT: all subjects showed a LAR before HS, but after 1 yr of HS the LAR was resolved in 5/15 ($P=0.04$) (Table 2). Furthermore, the LAR was less severe during the second BPT: the mean fall of the FEV₁ during the LAR of the first BPT was $42 \pm 14\%$, while during the second BPT the mean fall of FEV₁ during the LAR was $22 \pm 11\%$ ($P<0.0001$) (Table 2). In all subjects, except one (subject 10), the LAR was less severe after 1 yr of HS.

The IAR was also less severe during the second BPT: the mean fall of FEV₁ during the first BPT was $33 \pm 14\%$, while during the second BPT the mean fall was $25 \pm 6\%$ ($P=0.028$).

Comparing the reaction pattern between the two BPTs in the 15 subjects, there was a significant increase in FEV₁ during the second BPT (percentage of predicted) at 3, 10, 240 and 360 min (Fig. 1).

Clinical evolution

In all children the respiratory symptoms were well

Table 2. Reaction patterns and severity of the LAR (expressed in maximal fall of FEV₁ during the LAR)

	First BPT		Second BPT	
	Reaction pattern	LAR (percentage fall of FEV ₁)	Reaction pattern	LAR (percentage fall of FEV ₁)
Subject				
1	IAR+LAR	-50	IAR+LAR	-26
2	IAR+LAR	-20	IAR	-5
3	IAR+LAR	-39	IAR+LAR	-21
4	IAR+LAR	-65	LAR	-28
5	LAR	-23	Negative	-18
6	IAR+LAR	-58	IAR+LAR	-24
7	IAR+LAR	-47	IAR+LAR	-25
8	IAR+LAR	-55	IAR+LAR	-26
9	IAR+LAR	-55	IARs+LAR	-20
10	IAR+LAR	-28	IAR+LAR	-49
11	IAR+LAR	-46	IAR	-10
12	IAR+LAR	-41	IAR	-12
13	IAR+LAR	-47	IAR+LAR	-22
14	IAR+LAR	-24	IAR+LAR	-27
15	IAR+LAR	-29	IAR	-11
Mean		-41.8		-22.2
SD		14.1		10.9
Controls				
1	IAR+LAR	-32	IAR+LAR	-37 (19)*
2	IAR+LAR	-40	IAR+LAR	-39 (9)
3	IAR+LAR	-64	IAR+LAR	-55 (3)
4	IAR+LAR	-20	IAR+LAR	-27 (9)
5	IAR+LAR	-63	IAR+LAR	-32 (3)
6	IAR+LAR	-24	IAR+LAR	-32 (4)
7	IAR+LAR	-37	IAR+LAR	-48 (2)
8	IAR+LAR	-64	IAR+LAR	-48 (2)
Mean		-43.0		-39.7%
SD		-18.3		-9.7%

* Interval between the two BPTs in the controls (months).

controlled by anti-asthmatic drugs (Table 1). Drug treatment was kept constant, except for β -agonists. Based on daily symptom score and peak-flow measurement, treatment with β -agonists could not be decreased in the controls, while eight subjects were able to stop their β -agonists. Five subjects were able to decrease their daily dosage of β -agonists and in only two subjects β -agonists could not be decreased. A correlation between clinical improvement (as expressed in daily use of β -agonists) and reduction or persistence of the LAR could not be detected. However, of the five subjects who lost their LAR after 1 yr, four were able to stop their β -agonists completely and one was able to decrease his daily dosage. In the subjects showing persistence of the LAR after 1 yr of HS, four were able to stop their β -agonists, four could

decrease their daily dosage and two needed to take the same dosage of β -agonists as they had before HS was started.

The subject (subject 10) who was found to have a more severe LAR after 1 yr of HS, was able to stop his β -agonists during HS.

Discussion

In this study the efficacy of HS with HDM was evaluated by comparing the bronchial reaction pattern to HDM inhalation, before and after HS was instituted. Although this study was not completely double-blind, the nurses performing the BPTs were not aware of the therapies being administered. The controls (without HS) showed

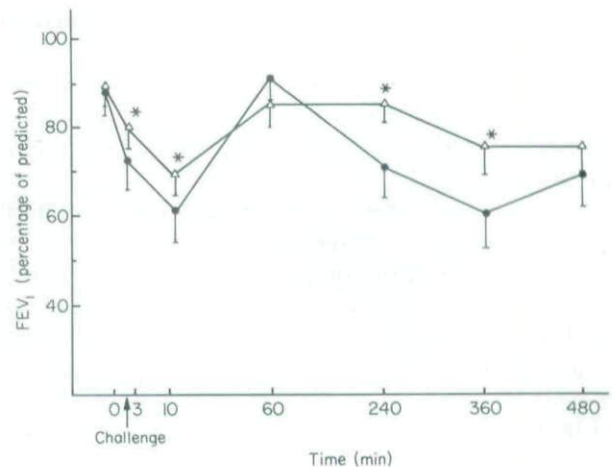
Table 3. PD₂₀ of the IAR and severity of the IAR (expressed in maximal fall of the FEV₁ during the IAR)

	First BPT		Second BPT	
	PD ₂₀ (BU)	IAR (percentage fall of FEV ₁)	PD ₂₀ (BU)	IAR (percentage fall of FEV ₁)
Subjects				
1	5	-46	83	-24
2	270	-37	416	-24
3	91	-22	333	-30
4	555	-36	noIAR*	-13
5	noIAR	-10	noIAR*	-19
6	556	-43	105	-23
7	400	-25	870	-23
8	294	-34	344	-29
9	125	-30	606	-29
10	952	-21	434	-23
11	285	-37	90	-22
12	278	-36	417	-24
13	571	-35	91	-22
14	91	-22	455	-22
15	285	-70	488	-41
Mean	340	-33.6	364	-24.5
SD	252	-13.8	232	-6.2
Median	285		416	
Controls				
1	142	-52	538	-37
2	588	-34	90	-22
3	71	-28	100	-20
4	384	-26	60	-33
5	69	-29	250	-20
6	8	-21	7	-22
7	83	-28	85	-51
8	238	-42	435	-23
Mean	198	-32.5	196	-28.5
SD	198	10.0	194	11.0
Median	113		95	

* noIAR: after inhalation of increasing doses of HDM, up to 1000 BU, the FEV₁ did not decrease > 20%. The PD₂₀ of the IAR could not be calculated in these subjects.

the same reaction pattern during the two BPTs, although in all controls respiratory symptoms were well controlled with the regular use of disodium cromoglycate (three subjects) or beclomethasone dipropionate (three subjects). The interval between the two BPTs in the controls was not the same as in the subjects, but this seems to be of less importance, since the same reaction pattern was observed in controls followed for a longer period, compared to controls who were only followed for some months.

In 5/15 of the children receiving HS for 1 yr the LAR

**Fig. 1.** FEV₁ (percentage of predicted) during the two bronchial challenges with house dust mite in the 15 subjects, before (●) and after (Δ) 1 yr of hyposensitization. * Indicates significantly different value compared to before HS.

completely resolved and, as a group, we found a less severe LAR after HS. Although the PD₂₀ of the IAR was not changed after 1 yr of HS, the IAR was less severe. As different doses of allergen were used in some children, this is not necessarily a valid comparison. However, in the controls the severity of the IAR was the same during both BPTs (expressed as mean fall in FEV₁), although in some of these children different doses of allergen were also used.

The clinical response to HS was based on the usage of β -agonists, since all other prophylactic therapy was maintained constantly during the trial, keeping the conditions for the BPTs as constant as possible. We found no statistically significant difference between the changes in the usage of β -agonists and loss or persistence of the LAR. This could be due to the small number of subjects, as a tendency to use less β -agonists in subjects who lost their LAR could be observed. On the other hand, if one assumes that the LAR is an expression of bronchial inflammation, it is reasonable to hypothesize that loss of the LAR is not necessarily reflected in the requirement of β -agonists, but might be expected to reduce the usage of anti-inflammatory agents [1].

The results of the present study provide further evidence that the LAR can be resolved by the administration of HS in asthmatic children, sensitized to HDM. Furthermore, this resolution cannot be due to the administration of anti-asthmatic drugs, since our control group, which used similar medication constantly, showed the same reaction pattern in the two BPTs after a mean interval of 6.3 months. Two previous studies have shown a similar tendency. The first study, by Warner *et al.* [1], on 51 asthmatic children receiving HS with HDM, showed that the children who improved clinically had no change in IAR, but the LAR was lost in half of them. In this study a

considerable reduction in the usage of anti-inflammatory agents was observed in the children who lost their LAR. Metzger *et al.* [2], working on 10 asthmatic patients receiving *Alternaria* HS, demonstrated that high doses of HS preferentially reduced the severity of the LAR rather than the IAR.

A previous study, from our group, also showed that the LAR occurred more frequently in a group of children who never received HS, compared to a group of children receiving HS. Furthermore, in this study it was observed that the LAR was less severe in subjects receiving HS [5].

The evaluation of the efficacy of HS in allergic asthma remains difficult, because of the chronicity of the disease and the considerable geographical, climatic, situational and individual variability [6]. On the other hand, BPTs with allergens were found to be highly reproducible in subjects not receiving HS [7,8]. Therefore, by using a BPT in the evaluation of HS, we have an objective and reliable tool to measure the specific sensitivity of a subject to an allergen. Furthermore, it is possible to measure changes in sensitivity and changes of the reaction pattern, before and after HS.

The mechanisms by which HS abrogates the occurrence of the LAR are still unknown. It seems possible that the production of antigen-specific IgG is involved with the reduction of the LAR, although this has not been proved in humans. In the animal model, it has been demonstrated that the presence of IgG can prevent the occurrence of the LAR [9]. However, a relationship between the level of antigen-specific IgG and a clinical benefit of HS has not been documented in humans with asthma or rhinitis [6]. Therefore, it is also possible that other immunological changes induced by HS are responsible for the relief of the LAR [6]. Recently, it has been shown that the seasonal rise of eosinophil chemotactic activity was completely abolished by HS in birch-allergic subjects [10]. This might be one of the phenomena suppressed by HS, thereby suppressing the occurrence of a LAR. The generation of other inflammatory factors could also be suppressed by HS, but this hypothesis needs further study.

It has been suggested that the LAR, rather than the IAR resembles the natural history of bronchial asthma [11]. This is emphasized by the ability of non-bronchodilator or prophylactic drugs (disodium cromoglycate, corticosteroids) to inhibit the occurrence of the LAR, providing these drugs are continued during the BPT procedure [12,13]. This report also suggests that it is possible that the LAR recurs when these drugs are withheld, even for short periods, after having been administered for several months (three control subjects took beclomethasone dipropionate for 9–12 months, which was then stopped 48 hr before the BPT).

In conclusion, we believe that HS should be added to

the list of therapeutic regimens preventing the LAR, and may therefore have a role in the treatment of selected subjects with asthma. However, further studies are needed to determine the exact place of HS in the treatment of asthmatic children.

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