Eur Respir J
1992, 5, 318–322

Effect of hyposensitization upon the immediate and late asthmatic reaction and upon histamine reactivity in patients allergic to house dust mite *(Dermatophagoides pteronyssinus)*

H.P. Van Bever, W.J. Stevens

Effect of hyposensitization upon the immediate and late asthmatic reaction and upon histamine reactivity in patients allergic to house dust mite (Dermatophagoides pteronyssinus). *H.P. Van Bever, W.J. Stevens.*

ABSTRACT: The effect of hyposensitization (HS) upon the allergen evoked immediate asthmatic reaction (IAR), late asthmatic reaction (LAR) and upon bronchial reactivity towards histamine was examined.

Eighteen young asthmatic patients were studied using a double-blind, placebo-controlled design. All were allergic to house dust mite (HDM) and demonstrated an IAR and a LAR after bronchial provocation with HDM. Furthermore, all, except one child, demonstrated bronchial hyperreactivity towards histamine (median provocative dose producing a 20% fall in forced expiratory volume in one second (PD_{20}) histamine = 0.19 $mg \cdot ml^{-1}$, range: 0.02–8 $mg \cdot ml^{-1}$). The subjects were randomly divided into two groups to receive HDM or placebo injections during one year.

After the one year period, the IAR was less severe in the subjects who received HDM injections (p=0.02), while this reduction was not observed in the subjects who received placebo injections. Also, in the HDM group a small but significant increase of the median PD_{20} HDM was observed (p=0.04). Furthermore, the LAR was less severe in the subjects who received HDM injections (p=0.02), while the subjects who received placebo injections showed the same severity of LAR after one year (p=0.44). Histamine reactivity did not change after HDM injections or after placebo injections.

Thus, HS reduces the severity of the IAR and LAR without any change in histamine reactivity.

Eur Respir J., 1992, 5, 318–322.

Depts of Paediatrics and Immunology
University of Antwerp, Belgium.

Correspondence: H.P. Van Bever
Dept of Immunology-Rheumatology
Universiteitsplein 1
2610 Antwerp
Belgium.

Keywords:
Histamine reactivity
house dust mite
hyposensitization
immediate asthmatic reaction (IAR)
late asthmatic reaction (LAR).

Received: May 15, 1990
Accepted after revision October 10, 1991.

In allergic asthmatics, it has been demonstrated that hyposensitization (HS) with the allergen can reduce the frequency and the severity of the immediate asthmatic reaction (IAR) and the late asthmatic reaction (LAR) evoked by this allergen [1–4]. The mechanisms by which HS reduces the IAR and the LAR are still unknown. Since the appearance of the LAR partly depends upon the degree of nonspecific bronchial responsiveness, it can be speculated that this reduction of the LAR by HS, could be the result of an induced diminished degree of nonspecific bronchial responsiveness [5]. However, the results of studies looking at the effect of HS on nonspecific responsiveness are contradictory. In one study, using carbachol provocations, a reduction of nonspecific responsiveness was observed after HS [6]. In another study, no influence upon nonspecific responsiveness could be detected [7], while MURRAY *et al.* [8] showed a worsening of histamine reactivity in a group of children who were hyposensitized with house dust mite.

At the moment, no reports are available in which the effect of HS upon nonspecific and specific (allergic) bronchial reactivity have been studied simultaneously. The aim of the present study was to look at the effect of HS upon the allergen evoked IAR and LAR and upon histamine reactivity in young asthmatic patients, using a double-blind, placebo-controlled, design.

Subjects and methods

Subject selection

Eighteen young asthmatic patients, mostly children, were selected for this study. All suffered from perennial asthma and all were sensitized to house dust mite (HDM) *(Dermatophagoides pteronyssinus)* as proved by positive prick tests and positive specific immunoglobulin E (IgE) radioallergosorbent test (RAST). To be included in this study all subjects had to have stable asthma, a normal forced expiratory volume in one second (FEV_1) (>70%), and all had to show a LAR after bronchial challenge with HDM. Furthermore, all had to be able to withhold anti-asthmatic drugs before the challenges with histamine

and with HDM. In an attempt to assess the severity of asthma, the subjects 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 with weekly occurring symptoms were classified as "moderate" and those with monthly or less occurring symptoms were classified as "mild".

Study design

Before entering the trial, all patients were challenged with histamine and with HDM. Histamine challenges were always performed the day before the allergen challenge. The patients were then divided randomly into two groups of nine patients to receive double-blind HS with HDM or placebo injections during one year. After one year, the challenges with histamine and with HDM were repeated. During the trial, therapy with disodium cromoglycate and inhaled steroids was kept constant. Based on symptom score, inhaled beta-agonists were used as needed. All patients were seen at the out-patient clinic at two monthly intervals during the study period, the administration of the HS was started (semi-rush) at the out-patient clinic and it was continued by their paediatrician or general physician.

Provocation tests

A standardized histamine challenge and HDM challenge was performed, according to a protocol described previously [9]. Before the provocation tests, all children had stopped inhaled corticosteroids or disodium cromoglycate 48 h and inhaled beta-agonists 24 h. In between histamine and HDM challenge only inhaled beta-agonists were allowed. The last dose of beta-agonists was inhaled at least 12 h before the HDM challenge. None of the patients used systemic corticosteroids, theophylline or antihistamines. Challenges were started if the subject's resting FEV_1 and vital capacity (VC) exceeded 70% of predicted normal and if the subject was free of respiratory infection for at least 3 weeks. After the histamine challenge, performed between 8.30 and 9.30 a.m., lung function was recorded hourly by peak flow measurement until 6 p.m. in order to detect any spontaneous decrease (= control day for the LAR).

Histamine provocation test

After establishing baseline values for VC and FEV_1, the subjects inhaled through a mouthpiece and with the nose occluded, solutions delivered by a Bird Mark 7 respirator with nebulizer (particle size 0.5–5 μm according to the manufacturer) at an approximate mean flow rate of 0.9 $l \cdot s^{-1}$. Before each challenge saline was inhaled during 1 min. To evaluate nonspecific hyperresponsiveness progressive twofold increases in concentration of histamine hydrochloride in saline were inhaled during 1 min, starting at a dilution of 0.06 $mg \cdot ml^{-1}$. VC and FEV_1 were measured immediately and then 10 min after inhalation. Provocation was stopped when FEV_1 decreased to at least 20% from the initial value or from the best value recorded during the provocation procedure. In order to compare degrees of hyperresponsiveness, provocative dose 20 (PD_{20}) histamine was determined. This was based on the first decrease of FEV_1 of >20%; the concentration of histamine causing a 20% decrease of FEV_1 was calculated. According to this challenge procedure, bronchial hyperresponsiveness was defined as a PD_{20} histamine <5.88 $mg \cdot ml^{-1}$.

Bronchial provocation test with house dust mite

For HDM provocation progressive increases in concentration (10, 100, 500 and 1,000 $BU \cdot ml^{-1}$) (BU = biological unit) (21 kU Der P1 = 1,000 BU) of a commercial allergen extract of HDM (*Dermatophagoides pteronyssinus*, Haarlem Allergen Laboratories, HAL, Haarlem, The Netherlands) were inhaled during 1 min, starting at a dilution of 10 $BU \cdot ml^{-1}$. Bronchial responses were measured by serial monitoring of FEV_1 and VC by a dry spirometer, immediately and 10 min after each inhalation procedure. If the decrease of FEV_1 was <20% (compared with the post-buffer control) after inhalation of 1,000 $BU \cdot ml^{-1}$, no further challenge was given and the subject was considered as having no immediate asthmatic reaction (IAR). In order to compare degrees of specific hyperreactivity to HDM between the bronchial challenges, PD_{20} HDM was determined according to the same method used for the histamine reactivity. After finishing the procedure for measuring the IAR, the FEV_1 was recorded at 1, 4, 6 and 8 h, or until a significant LAR occurred. A LAR was defined as a decrease of FEV_1 of at least 20% of the post-buffer value in the observation period starting from 4 h after the beginning of the allergen challenge. A symptomatic LAR was reversed by nebulized fenoterol. In a previous report, we found an acceptable reproducibility of the LAR, using this provocation technique [4].

Hyposensitization protocol

HS was performed according to a standardized protocol [10]. The children were randomly divided into two groups to receive double-blind HDM injections or placebo injections (identical vials). As placebo we used histamine 0.003 $mg \cdot ml^{-1}$ in buffer solution. This concentration was chosen because it induces a local reaction comparable to that seen after injection of HDM-extract. The determination of this amount was studied in healthy volunteers (unpublished results).

In all children HS was started using a semi-rush procedure in which five injections were given

at 30 min intervals with increasing amounts (0.1, 0.3, 0.5, 0.8, 1.0 ml) of the same aqueous extract of *Dermatophagoides pteronyssinus* (10 BU·ml^{-1}) as used for the bronchial challenges and for skin prick testing (or with placebo). One week later the same amounts were injected with a tenfold higher concentration (100 BU·ml^{-1}) or placebo. 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 dosed 1,000 BU·ml^{-1} or placebo. This treatment took 1 month to reach the final concentration of 1.0 ml of 1,000 BU·ml^{-1}. Therapy was continued by injecting this dose, once after one week, then once after 2 weeks, then once after 3 weeks and finally every 4 weeks. During the trial, the HDM group received a cumulative dose of 16,497 BU.

Statistical analysis

The results were analysed using non-parametric tests (Mann-Whitney rank sum test, Wilcoxon signed rank test). A $p<0.05$ was considered statistically significant.

Results

The groups were comparable according to age, IgE mediated sensitivity towards HDM and severity of asthma (table 1). The usage of anti-asthmatic drugs was also the same in both groups (table 2). All subjects took inhaled beta-agonists regularly, according to the appearance of asthmatic symptoms. In the HDM group, four subjects used disodium cromoglycate, four used inhaled corticosteroids and one child took neither.

Table 1. – Data of the patients at the beginning of the trial, mean and range in parenthesis

	HDM group n=9	Placebo group n=9
Age yrs	9 (7–11.2)	12 (7.9–22)
Total IgE kU·l^{-1}	354 (55–1092)	387 (61–1470)
Specific IgE HDM PRU	34 (4–66)	29 (2–64)
Skin prick test mm	7.25 (3–12)	7.25 (6–9)
Severity of asthma		
Severe	5	6
Moderate	2	1
Mild	2	2

HDM: house dust mite; IgE: immunoglobulin E; PRU: Phadebas radioallergosorbent test (RAST) unit.

In the placebo group, three subjects were on disodium cromoglycate, four on inhaled corticosteroids and two on neither. Before the trial was started, all subjects showed a biphasic asthmatic reaction (IAR + LAR) after inhalation of HDM and no subject showed a spontaneous decrease of the lung function on the control day. Furthermore, all but one subject (patient 5 from the HDM group) showed marked nonspecific hyperresponsiveness towards histamine (median PD_{20} histamine = 0.19 mg·ml^{-1}, range: 0.02–8 mg·ml^{-1}). Between the two groups there was no difference for PD_{20} histamine (p=0.17) (table 2) and for PD_{20} HDM (p=0.8), severity of IAR (p=0.14) and severity of LAR (p=0.56) (tables 3 and 4).

Table 2. – Evolution of the PD_{20} histamine (mg·ml^{-1})

HDM group				Placebo group			
Subject no.	*	Before trial	After trial	Subject no.	*	Before trial	After trial
1	SC	0.02	0.07	1	SC	0.09	0.13
2	SC	0.17	0.12	2	-	0.05	0.80
3	BD	0.25	0.80	3	BD	1.10	0.28
4	-	0.37	0.25	4	SC	0.21	0.02
5	BD	8.00	6.40	5	BD	0.10	0.40
6	SC	1.00	1.74	6	BD	0.05	0.08
7	BD	0.06	0.11	7	-	0.13	0.37
8	SC	0.48	0.48	8	BD	0.25	0.25
9	BD	0.90	0.40	9	SC	0.17	0.10
Median		0.37	0.40	Median		0.13	0.25

*: maintenance treatment, disodium cromoglycate (SC) or inhaled beclomethasone dipropionate (BD). Comparison before and after the trial: HDM group p=0.89; placebo group p=0.67. Comparison between the two groups: before the trial p=0.17; after the trial p=0.25. HDM: house dust mite; PD_{20}: provocative dose producing a 20% fall in forced expiratory volume in one second.

After one year of HS there was no change in histamine reactivity in both groups (table 2). On the other hand, changes were observed in the reaction pattern towards HDM in the subjects who received HDM injections, while this could not be noted in the subjects who received placebo injections. The IAR became less severe in the children who received HDM injections but not in the children who received placebo injections (table 3). However, when the severity of the IAR after the trial was compared between the two groups, no difference was found (p=0.2). Furthermore, a small but significant increase of the median PD_{20} HDM was observed in the patients who received HDM injections (p=0.04), while the PD_{20} HDM was not influenced by placebo injections (p=0.11) (table 3). However, these results should be interpreted with caution because statistical comparison could only be made in six subjects who received HDM injections (table 3). The other three developed no IAR after the trial (PD_{20} HDM >1,000), which means that their PD_{20} HDM could not be calculated. When the PD_{20} HDM after the trial was compared between the two groups (HDM injections *versus* placebo injections), no difference was found (p=0.24).

Five of the nine subjects who received HDM injections lost their LAR, while this was observed in only one of the nine patients who received placebo injections (table 4). Furthermore, two of the children of the HDM group became unresponsive towards HDM. The severity of the LAR was significantly reduced in

the HDM group. In the placebo group no change in the mean severity of the LAR was noted (table 4). When the severity of the LAR after the trial was compared between the two groups, it was found that the children who received HDM injections showed a less severe LAR than the children who received placebo injections (p=0.02).

Table 3. – Evolution of the PD_{20} HDM (BU) and severity of the IAR (expressed as maximal fall of % FEV_1 during the IAR)

HDM group Subject no.	PD_{20} before	IAR before	PD_{20} after	IAR after
1	200	-50	200	-45
2	100	-20	454	-22
3	256	-39	740	-27
4	238	-42	500	-20
5	689	-29	>1000	-7
6	5	-44	741	-27
7	8	-24	357	-28
8	417	-24	>1000	-19
9	588	-34	>1000	-19
Median	238	-34	477	-22
Comparison before and after: PD_{20} p=0.04; IAR p=0.02				
Placebo group Subject no.	PD_{20} before	IAR before	PD_{20} after	IAR after
1	71	-28	526	-20
2	8	-25	238	-42
3	360	-21	323	-31
4	357	-28	312	-32
5	345	-29	455	-22
6	476	-21	285	-35
7	111	-20	416	-24
8	303	-33	435	-23
9	285	-35	385	-26
Median	303	-28	385	-26
Comparison before and after: PD_{20} p=0.11; IAR p=0.64				

IAR: immediate asthmatic reaction; FEV_1: forced expiratory volume in one second; BU: biological unit. For further abbreviations see legend to table 2.

Clinical evolution

During the trial, in all subjects the respiratory symptoms were well controlled by anti-asthmatic drugs. Drug treatment was kept constant, except for beta-agonists. Five of the children who received HDM injections were able to decrease their daily usage of beta-agonists, while four needed the same amount of beta-agonists as before the trial was started. No patient receiving HS with HDM showed a worsening of asthmatic symptoms. In three children receiving placebo injections the usage of beta-agonists could be decreased, while five needed the same dosage of beta-agonists. One child receiving placebo injections showed deterioration of his asthmatic disease and needed to increase his inhalations with beta-agonists.

Table 4. – Evolution of the reaction pattern and severity of the LAR (expressed as maximal fall of % FEV_1 during the LAR)

HDM group Subject no.	Reaction pattern before	LAR before	Reaction pattern after	LAR after
1	IAR + LAR	-53	IAR + LAR	-43
2	IAR + LAR	-30	IAR + LAR	-33
3	IAR + LAR	-26	IAR	-8
4	IAR + LAR	-64	IAR + LAR	-21
5	IAR + LAR	-25	Negative	-8
6	IAR + LAR	-36	IAR	-9
7	IAR + LAR	-33	IAR	-6
8	IAR + LAR	-22	Negative	-9
9	IAR + LAR	-42	LAR	-46
Median		-33		-9
Comparison between the LAR before and after: p=0.02				
Placebo group Subject no.	Reaction pattern before	LAR before	Reaction pattern after	LAR after
1	IAR + LAR	-64	IAR + LAR	-36
2	IAR + LAR	-29	IAR + LAR	-53
3	IAR + LAR	-20	IAR + LAR	-30
4	IAR + LAR	-37	IAR + LAR	-47
5	IAR + LAR	-42	IAR + LAR	-39
6	IAR + LAR	-25	IAR + LAR	-38
7	IAR + LAR	-32	IAR + LAR	-55
8	IAR + LAR	-28	IAR	-13
9	IAR + LAR	-25	IAR + LAR	-35
Median		-29		-38
Comparison between the LAR before and after: p=0.44				

LAR: late asthmatic reaction. For further abbreviations see legend to table 3.

Discussion

Previous studies demonstrated a reduction of the frequency and/or the severity of the LAR by HS [1–4]. In the present study, it was additionally shown that the severity of the IAR was reduced by HS, although the results were less obvious because the severity of the IAR after the trial was not different between the HDM group and the placebo group. The PD_{20} HDM also shows a small, but significant, increase in the subjects who received HDM injections, although statistical calculation, using the Wilcoxon signed rank test, could only be performed in six subjects. Therefore, more studies are needed to confirm this observation.

At the moment, it seems logical to assume that only the frequency and severity of the LAR is reduced by HS. This reduction of the LAR is not related to a change in histamine responsiveness, since this remained unchanged after one year of HS. It remains, nevertheless, possible that prolonged administration of HS, during several years, could reduce histamine responsiveness.

The underlying mechanisms of the effect of HS upon allergen reactivity are still unknown. Only a few

studies have been carried out to investigate the effect of HS on nonspecific bronchial responsiveness and the results of these studies are contradictory [6–8]. Recently, RAK *et al.* [11] demonstrated a lesser increase of histamine sensitivity during the birch pollen season in those birch allergic asthmatics who were pretreated with HS, although no statistical difference could be noted when compared to a group of patients who were not treated with HS. These authors concluded that the limited effect of HS on nonspecific bronchial responsiveness could be explained by the intensive birch pollen season and by the fact that only a short course of preseasonal HS was given.

An increase of nonspecific bronchial responsiveness after allergen exposure has been observed in laboratory situations [12] and after natural exposure [13]. The increase of nonspecific bronchial responsiveness could be attributed to the occurrence of the LAR and not to the IAR [12]. Furthermore, in HDM sensitive asthmatics, a decrease in nonspecific responsiveness was seen after prolonged allergen avoidance [14]. COCKCROFT [15] hypothesized that, in perennial asthma, a vicious circle is established when natural exposure to an antigen leads to nonspecific airway hyperresponsiveness. This airway hyperresponsiveness, in turn, causes an increased response to both subsequent exposure to the allergen and to subsequent exposure to non-allergic stimuli, leading to further hyperresponsiveness. In HDM sensitive patients it appears difficult to influence nonspecific bronchial responsiveness since these patients are continuously exposed to HDM in an uncontrolled manner. Furthermore, it can be assumed that the degree of nonspecific reactivity is only partly influenced by allergen exposure and that other triggers (viral infections, air pollution, exercise, passive smoking, fog) are also responsible for the maintenance of a certain degree of nonspecific bronchial hyperresponsiveness. Since HS only influences HDM reactivity, it seems logical to expect only a decreased reactivity towards HDM after HS with HDM extract. The only expected effect of HS on nonspecific bronchial reactivity might be an inhibition of the allergen triggered increase in histamine sensitivity, observed after a LAR [12]. However, this phenomenon was not investigated in the present study but it seems logical to assume that, since the LAR occurs less frequently after HS, the LAR-induced increase in nonspecific bronchial responsiveness also becomes less severe after HS.

In conclusion, the present study shows that HS with HDM reduces the severity of the allergen evoked LAR. There was also a small but significant effect upon the severity of the IAR and the PD_{20} HDM, but no effect upon histamine reactivity. When the two groups were compared after the trial, only the LAR was less severe in the subjects who received HDM injections, while no difference was observed in severity of IAR or in PD_{20} HDM.

References

1. Warner JO, Price JF, Soothill JF, Hey EN. – Controlled trial of hyposensitization to *Dermatophagoides pteronyssinus* in children with asthma. *Lancet,* 1978; ii: 912–915.
2. Metzger WJ, Donnelly A, Richerson HB. – Modification of late asthmatic responses (LAR) during immunotherapy for *Alternaria*-induced asthma. *J Allergy Clin Immunol,* 1983; 71 (Suppl.): 119 (Abstract).
3. Van Bever HP, Bosmans J, De Clerck LS, Stevens WJ. – Modification of the late asthmatic reaction by hyposensitization in asthmatic children allergic to house dust mite (*Dermatophagoides pteronyssinus*) or grass pollen. *Allergy,* 1988; 43: 378–385.
4. Van Bever HP, Stevens WJ. – Suppression of the late asthmatic reaction by hyposensitization in asthmatic children allergic to house dust mite (*Dermatophagoides pteronyssinus*). *Clin Exp Allergy,* 1989; 19: 399–404.
5. Crimi E, Brusasco V, Losurdo E, Crimi P. – Predictive accuracy of late asthmatic reaction to *Dermatophagoides pteronyssinus. J Allergy Clin Immunol,* 1986; 78: 908–913.
6. Bousquet J, Clauzel AM, Hejjaoui A, Dhivert H, Godard P, Chanal I, Michel FB. – Nonspecific bronchial hyperreactivity in asthmatic subjects after immunotherapy with a standardized mite extract. *Am Rev Respir Dis,* 1987; 135 (Suppl.): A315.
7. Taylor WW, Ohman JL, Lowell FC. – Immunotherapy in cat-induced asthma. Double-blind trial with evaluation of bronchial responses to cat allergen and histamine. *J Allergy Clin Immunol,* 1978; 61: 283–287.
8. Murray AB, Ferguson AC, Morrison BJ. – Nonallergic bronchial hyperreactivity in asthmatic children decreases with age and increases with mite immunotherapy. *Ann Allergy,* 1985; 54: 541–544.
9. Stevens WJ, Vermeire PA. – Bronchial responsiveness to histamine and allergen in patients with asthma, rhinitis, cough. *Eur J Respir Dis,* 1980; 61: 203–212.
10. Stevens WJ, Verhelst JA, Van Den Bogaert W, Bridts CH. – Clinical and biological evaluation of semi-rush and ordinary immunotherapy schemes in type I allergic respiratory diseases. *Allergy,* 1985; 40: 447–452.
11. Rak S, Löwhagen O, Venge P. – The effect of immunotherapy on bronchial hyperresponsiveness and eosinophil cationic protein in pollen-allergic patients. *J Allergy Clin Immunol,* 1988; 82: 470–480.
12. Cartier A, Thomson NC, Frith PA, Roberts R, Hargreave FE. – Allergen-induced increase in bronchial responsiveness to histamine: relationship to the late asthmatic response and change in airway caliber. *J Allergy Clin Immunol,* 1982; 70: 170–177.
13. Boulet LP, Cartier A, Thomson NC, Roberts RS, Dolovich J, Hargreave FE. – Asthma and increases in nonallergic bronchial responsiveness from seasonal pollen exposure. *J Allergy Clin Immunol,* 1983; 71: 399–406.
14. Platts-Mills TAE, Tovey ER, Mitchell EB, Moszoro H, Nock P, Wilkins SR. – Reduction of bronchial hyperreactivity during prolonged allergen avoidance. *Lancet,* 1982; ii: 675–678.
15. Cockcroft DW. – Mechanism of perennial allergic asthma. *Lancet,* 1983; i: 253–256.