

Increased Bronchial Hypersensitivity after Early and Late Bronchial Reactions Provoked by Allergen Inhalation

L. MACHADO

Department of Pulmonology, Uppsala University, Akademiska Sjukhuset, Uppsala, Sweden

The non-specific bronchial reactivity following bronchial allergen challenge was studied in 40 patients with allergic bronchial asthma, particularly in subjects without definite late reactions 6 h after the provocations (reduction in peak expiratory flow or forced expiratory volume in 1 s of less than 15% of the control value at this time). Among a group of 21 patients submitted to bronchial provocation tests, 13 carried out maximal exercise tests 6 h and 1 week after the allergen challenge. In another group of 19 patients, the bronchial hyperreactivity to methacholine was assessed before and 6 h and 1 week after challenge. Two patients with a dual response (early + late) reacted with bronchial obstruction to the exercise. Exercise tests performed after 1 week did not provoke asthma in any patient. In the methacholine group a marked increase in responsiveness to methacholine 6 h after the provocation was observed in those patients with a dual response who were tested and in those with equivocal late reactions and even in three patients with an isolated immediate reaction. The increased responsiveness was still present in many patients 1 week after challenge. The airway caliber did not influence the degree of responsiveness to methacholine. Nor did the degree of responsiveness have any influence on the patterns of reactions observed after allergen exposure.

It was concluded that in some individuals exposure to the relevant allergen predisposes them to exercise-inducible bronchial obstruction. Further, it was confirmed that non-specific bronchial reactivity can be increased not only in patients with late responses - both definite and equivocal - but also in some patients with immediate reactions alone. It is suggested that only allergens provoking late reactions or an increase in the non-specific reactivity should be considered clinically relevant.

Key words: asthma; hyperreactivity; immediate reactions; late reactions.

Accepted for publication 11 June 1985

In individuals with pollen allergy the non-specific bronchial reactivity to histamine is increased during the pollen season, as was first reported by Altounyan (1). It has since been demonstrated that enhanced hyperreactivity can be produced in allergic patients by bronchial challenges in the laboratory (6). This increased reactivity has been observed only after dual bronchial responses to challenge and not in patients with immediate reactions alone. These observations have been questioned, but more recently Boulet et al. (3) have confirmed that seasonal pollen exposure increases the bronchial

responsiveness to methacholine and that subjects with the most pronounced seasonal responsiveness were those who showed dual reactions when challenged with an allergen out of season.

In the present trial the results of bronchial allergen provocations were studied in 40 patients with allergic asthma. The occurrence of increased reactivity to exercise and to methacholine after allergen challenge was investigated, particularly in patients displaying an immediate reaction alone. Factors which could have influenced the results of the tests, such as the output of the nebulizers, the particle size of

the aerosols, and respiratory patterns during the inhalations, were kept constant during the study.

MATERIAL AND METHODS

Patients

Forty patients with bronchial asthma of allergic origin participated in the study. There were 22 men and 18 women, with a mean age of 30 years (range 17–52 years). All of them had positive RAST and/or positive prick tests for the allergen in question, and the majority of them had elevated total IgE levels (mean 550.5, range 16–2,740 kU/l). All patients were free from symptoms on the days of the tests and none of them had had any upper airway infections later than 2 weeks before the study. Treatment with sodium cromoglycate and local steroids was discontinued for 1 week, with xanthine derivatives for 48 h and with beta-2-receptor stimulators for 24 h before the provocations. None of the patients were taking steroids orally and none of them had received immunotherapy in the last 2 years. The patients were asked not to drink tea or coffee or to smoke for 4 h prior to the methacholine tests. Only patients with a forced expiratory volume in 1 s (FEV_1) of at least 70 % of the predicted value on the days of the tests participated in the study. Patients with FEV_1 changes of more than 8 % between the control day and the day of allergen challenge were excluded.

Allergen provocation tests

All provocations were started in the morning, between 8 and 9 a.m. The patients inhaled the allergen extract from a De Vilbiss-40 nebulizer (particle size 0.5 to 5.5 μ m, output 0.175 ± 0.03 ml/min, mean $\pm$ SD). The aerosols were produced by passing oxygen through at a flow of 5 l/min, delivered into a mouthpiece and inhaled by tidal breathing. The patient's nose was clipped during the inhalations. The test started with an inhalation of 1 ml of physiological saline, followed by 1 ml of the extract chosen for inhalation, in concentrations increas-

ing 10-fold at each step, at 15-min intervals, until there was a decrease in the peak expiratory flow rate (PEF) of 20 % or more of the lowest post-saline value, or until the maximum concentration of the extracts was administered. PEF measurements were performed before, immediately after, and 15 min after each inhalation, and then hourly until the evening. FEV_1 was also measured before, immediately after, and 15 min and 6 h after the allergen challenge. The test was considered positive if PEF or FEV_1 was decreased by 15 % or more of the lowest post-saline value.

Exercise tests

The exercise tests were carried out on an ergometer bicycle, at room temperature (18–20°C), starting with a workload of 30 watt for women and 50 watt for men. The resistance was increased by 30 and 50 watt, respectively, at 3-min intervals until symptomatic limitation occurred. The PEF, heart beats, and breathing rates were measured before the test, at 3-min intervals during the test, and 2, 5, 10, 20 and 30 min after the exercise. The test was considered positive if PEF decreased 15 % or more of the pre-exercise value within 30 min after the end of the bicycling exercise.

Methacholine test

The non-specific bronchial reactivity was assessed by a methacholine test, according to the method of Cockcroft and collaborators (5) with some modifications. FEV_1 measurements were performed first until a correct technique was achieved. The test started with an inhalation of 0.5 ml of physiological saline delivered from a De Vilbiss-40 nebulizer, followed by inhalations of 0.5 ml of methacholine solution in doubling concentrations at 3-min intervals, from 0.0625 (or 0.125) to 20 mg/ml, until FEV_1 had decreased to below 80 % of the lowest post-saline value, or until the highest concentration of methacholine was given. FEV_1 measurements were repeated 30 and 90 s following each inhalation. The inhalation procedures and the characteristics of the nebulizers were the same

as those in the allergen provocation tests. The cumulative dose of methacholine necessary to produce a decrease in FEV_1 of 20 % of the lowest post-saline value (PD_{20}) was obtained from the log-dose response curve by linear interpolation of the last two points.

Allergen extracts

Extracts of pollens, animal and house dust mites were purchased from Pharmacia AB, Uppsala, Sweden, and extracts of house dust and moulds (*Cladosporium herbarum*) from ALK, Allergological Laboratory, Copenhagen, Denmark. The dried extracts were kept frozen and were diluted at different concentrations in physiological saline immediately before the provocations.

Study design

The patients were divided into two groups. A group of 21 patients were submitted to a total of 29 bronchial provocation tests with one or more allergens. In this group, 13 patients in whom the provocations resulted in an early bronchial reaction without a definite late response 6 h after the challenge, were submitted to a maximal exercise test at that time. The exercise test was repeated 1 week later at the same time of day, without previous allergen challenge.

In another group of 19 patients, who underwent a total of 20 allergen provocations, the non-specific bronchial reactivity was assessed by the methacholine test 1-3 days before the provocations. On the day of allergen challenge, patients with an immediate reaction, but with PEF or FEV_1 changes of less than 15 % 6 h after the challenge, were retested with methacholine at this time.

The methacholine sensitivity was also measured in the morning 1 week after the bronchial challenge, both in patients with an isolated early reaction and in those with a dual (early + late) reaction. An increase in sensitivity to methacholine was defined as a decrease in PD_{20} after the allergen challenge of one-third or less.

Nine patients in the first group were tested with two different allergens. In these patients

the interval between the provocations was longer than 1 week. In the second group, one patient was tested with two different allergens, with an interval of 1 month between these tests.

The study was approved by the Ethics Committee of the Medical Faculty of Uppsala University, and informed consent was obtained from all patients before the tests.

RESULTS

Three patterns of reactions to the allergen provocations were observed: a) isolated early reactions, b) early reactions followed by an equivocal late one (decreases in PEF and/or FEV_1 of between 10 and 15 % during the late phase of the bronchial response) and c) dual reactions (decreases in PEF and/or FEV_1 > 15 % between 3 and 24 h after the provocation).

The results of the allergen provocations in the two groups of patients are presented in Table 1. Fifty per cent of the patients who had an early response also showed a late one, occurring from 3 to 8 h after the provocations. All classes of allergens used for testing were able to give late reactions. In three patients the immediate reactions were followed by an equivocal late response.

Thus, exercise tests were performed after 10 provocations resulting in an isolated immediate reaction and after three provocations followed by a manifest dual response. Exercise led to bronchial obstruction within 15 min of the test in only two patients, both among those with a manifest dual reaction. At the exercise tests repeated 1 week later at the same time of the

Table 1
Results of the bronchial allergen challenges in all patients (49 provocations)

	Pollens	House dust	Mites	Animals	Mould
Negative	0	1	2	2	0
a) Only early reactions	10	5	0	4	0
b) Early + equivocal late	0	1	0	1	1
c) Early + late reactions	8	3	4	6	1

day, no significant changes in PEF or FEV₁ were noted in any subject.

The non-specific bronchial reactivity to methacholine before the challenge was markedly increased in 11 patients, with PD₂₀ below 2 mg. Moderate hyperreactivity, with PD₂₀ between 2 and 8 mg, was observed in three patients. PD₂₀ was > 8 mg in five patients (in 3 cases > 19 mg). The reactivity to methacholine was not dependent on the pre-test airway caliber. FEV₁ (in per cent of the predicted value) was > 90 % in six patients, between 80 and 90 % in three and < 80 % in three patients with PD₂₀ < 2 mg. Among those patients with a PD₂₀ of above 8 mg were two with FEV₁ between 80 and 90 % and three with FEV₁ > 90 % of the predicted value. Among the patients with moderate hyperreactivity before the allergen challenge, FEV₁ was between 80 and 90 % in one patient and > 90 % in two patients.

Only seven of the patients in the methacholine group were using bronchodilators regularly at the time of the study, and in all of them PD₂₀ for methacholine was < 2 mg.

The degree of bronchial reactivity before challenge had no impact on the patterns of reactions to the provocations. Thus, of 11 patients with PD₂₀ < 2 mg, seven reacted to the challenge with a dual bronchial response, one had an immediate reaction followed by an equivocal late one, and three had an isolated early reaction. Of those patients with moderate hyperreactivity, one had only an immediate reaction to the provocations with two different allergens, one reacted with an early followed by an equivocal late response and one had a dual response. Two patients with PD₂₀ > 8 mg had a dual response after challenge, two had an isolated early reaction and one had no reaction at all.

Among the 13 patients in whom PD₂₀ was assessed 6 h after the provocations, three of seven patients with an isolated early response, both of two with an early reaction followed by an equivocal late one, and all four with a dual response, displayed markedly increased bronchial sensitivity, PD₂₀ being at least three times lower than before the challenges (Table 2).

After 1 week, increased responsiveness to

methacholine was still manifest in three of five patients with an isolated early reaction, one of two with an equivocal late one and three of nine patients with a dual response.

DISCUSSION

The reactivity of the bronchial smooth muscle is increased in most asthmatic individuals, as can be demonstrated by inhalation of bronchoconstrictive substances, such as histamine or a cholinergic agent. These stimuli trigger asthma chiefly through irritant parasympathetic receptor mechanisms (13). The same mechanism is involved in the bronchial constriction provoked by naturally occurring irritants such as smoke, dust, and cold air. Exercise induces asthma by respiratory heat exchanges causing a decrease in airway temperature (7). Inhalation tests with histamine and methacholine are more sensitive than exercise tests in demonstrating the occurrence of a non-specific bronchial hyperreactivity (8, 11, 12). Anderton et al. (2) found no increase in the bronchial response to exercise when the PC₂₀ for histamine was > 3 mg/ml.

Triggering of the early asthmatic response by allergen inhalation does not only depend on the dose of allergen inhaled and the level of sensitivity to the tested allergen, but also on the degree of non-specific bronchial hyperreactivity (10).

The degree of hyperreactivity of the bronchial tree correlates well with the severity of the asthma and the treatment necessary to control

Table 2

Responsiveness to methacholine after bronchial allergen challenge. Figures in brackets represent the number of patients tested with methacholine

	Negative	Only early	Early + equivocal late	Dual
No. of reactions	1	7	2	10
Increased reactivity after 6 h	0 (1)	3 (7)	2 (2)	4 (4)
Increased reactivity after 1 week	0 (1)	3 (5)	1 (2)	3 (9)

symptoms (9). In the present study, all patients requiring regular treatment showed markedly increased hyperreactivity to methacholine. Subjects with $PD_{20} > 2$ mg required bronchodilators only occasionally, or needed no medication at all.

Other authors found that in bronchial challenges with allergens, the bronchial reactivity is increased 7–8 h after challenge in all patients with a late reaction (4, 6) and in almost all of those with an equivocal late response (6). This increase could be observed up to 7 days following a single allergen inhalation. The duration of the increase in histamine reactivity corresponded to the magnitude of the late response (4).

The results of our study confirm the occurrence of increased non-specific reactivity in patients with equivocal late reactions and in those with a dual response after allergen challenge. In three patients, however, the increased responsiveness to methacholine 6 h after challenge was observed even after an isolated early reaction. The airway caliber had no impact on the reactivity, since the FEV_1 values before the methacholine test control and before the test performed 6 h after allergen challenge were the same in two patients, and only in one patient was FEV_1 4% lower than on the control day.

In some individuals the increased bronchial reactivity was still present 1 week after challenge. Theoretically, this could have been due to ongoing allergen exposure. This was suspected to be the case in one patient with an isolated early reaction. In the other cases, the single allergen challenge was the most probable cause of the observed increase in non-specific bronchial reactivity. Moreover, development of increased sensitivity to methacholine 1 week after challenge was not found in any patient who had not had a significant increase after 6 h.

Exercise tests led to detection of hyperreactivity in two patients with a dual response, but the results suggest that allergen exposure can be a predisposition for exercise-inducible asthma in some individuals, since the same exercise without previous allergen challenge did not produce bronchial obstruction.

It has been known for some time that there is

a correlation between the severity of asthma and the occurrence of a late reaction to bronchial allergen provocation (14). There is a parallelism between the amelioration of symptoms and disappearance of late reactions after hyposensitization treatment (15). Hargreave and co-workers (9) claimed that patients with increased bronchial hyperreactivity had more severe asthma, demanding more intense treatment, and Boulet et al. (3) found that patients with dual reactions after allergen challenge were more prone to developing bronchial hyperreactivity during the pollen season. Our results show not only that patients with evident late reactions and some of those with an equivocal late reaction become hyperreactive, but also that some of those with an isolated early reaction develop or increase their reactivity as a result of experimental allergen exposure. It is thus concluded that allergens provoking a dual response or increasing the non-specific bronchial reactivity during the late phase are those which should be considered relevant to the development of asthma.

ACKNOWLEDGEMENTS

Eva Persson and Barbro Ekblad, Registered Nurses, are gratefully acknowledged for their assistance with provocation tests. This study was supported by grants from the Swedish Medical Research Council, the Swedish National Association against Heart and Chest Diseases and the Bror Hjerpstedt Research Fund of Uppsala University.

REFERENCES

1. Altounyan, R. E. C.: Changes in histamine and atropine responsiveness as a guide to diagnosis and evaluation of therapy in obstructive airways disease. In Pepys, J. & Frankland, A. W. (eds.): Disodium cromoglycate in allergic airways disease, pp. 47–53. Butterworths, London, 1970.
2. Anderton, R. C., Cuff, M. T., Frith, P. A., et al.: Bronchial responsiveness to inhaled histamine and exercise. *J. Allergy Clin. Immunol.* 63, 315, 1979.
3. Boulet, L.-P., Cartier, A., Thomson, N. C., Roberts, R. S., Dolovich, J. & Hargreave, F. E.: Asthma and increases in nonallergic bronchial responsiveness from seasonal pollen exposure. *J. Allergy Clin. Immunol.* 71, 399–406, 1983.
4. Cartier, A., Thomson, N. C., Frith, P. A., Roberts, R., Tech, M. & Hargreave, F. E.: Allergen-induced increase in bronchial responsiveness to histamine: rela-

- tionship to the late asthmatic response and change in airway caliber. *J. Allergy Clin. Immunol.* 70, 170-177, 1982.
5. Cockcroft, D. W., Killian, D. N., Mellon, J. J. A. & Hargreave, F. E.: Bronchial reactivity to inhaled histamine: a method and clinical survey. *Clin. Allergy* 7, 235-243, 1977.
 6. Cockcroft, D. W., Ruffin, R. E., Dolovich, J. & Hargreave, F. E.: Allergen-induced increase in non-allergic bronchial reactivity. *Clin. Allergy* 7, 503-513, 1977.
 7. Deal, E. C. Jr., McFadden, E. E. Jr., Ingram, R. H. Jr. & Jaeger, J. J.: Hyperpnea and heat flux; initial reaction sequence in exercise-induced asthma. *J. Appl. Physiol.* 46, 476-483, 1979.
 8. Eggleston, P. A.: A comparison of the asthmatic response to methacholine and exercise. *J. Allergy Clin. Immunol.* 63, 104-110, 1979.
 9. Hargreave, F. E., Ryan, G., Thomson, N. C., et al.: Bronchial responsiveness to histamine or methacholine in asthma: measurement and clinical significance. *J. Allergy Clin. Immunol.* 68, 347-355, 1981.
 10. Killian, D., Cockcroft, D. W., Hargreave, F. E. & Dolovich, J.: Factors in allergen induced asthma: relevance of the intensity of the airways allergic reaction and non-specific bronchial reactivity. *Clin. Allergy* 6, 219-225, 1976.
 11. Kiviloog, J.: Brochial reactivity to exercise and methacholine in bronchial asthma. *Scand. J. Respir. Dis.* 54, 347, 1973.
 12. Mellis, C. M., Kattan, M., Keens, T. G. & Levinson, H.: Comparative study of histamine and exercise challenges in asthmatic children. *Am. Rev. Resp. Dis.* 117, 911-915, 1978.
 13. Simonsson, B. G., Jacobs, F. M. & Nadel, J. A.: Role of autonomic nervous system and the cough reflex in the increased responsiveness of airways in patients with obstructive airway disease. *J. Clin. Invest.* 46, 1812-1818, 1967.
 14. Warner, J. O.: Significance of late reactions after bronchial challenge with house dust mite. *Arch. Dis. Child.* 51, 905-911, 1976.
 15. Warner, J. O., Price, J. F., & Hey, E. N.: Controlled trial of hyposensitization to *Dermatophagoides pteronyssinus* in children with asthma. *Lancet* 28, 912-915, 1978.

Address:

Lavinia Machado
Dept. of Pulmonology
Uppsala University
Akademiska Sjukhuset
Uppsala
Sweden

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.