

Is the allergen-inhalation challenge predictive of the severity of seasonal asthmatic exacerbations?

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Fifteen asthmatic patients sensitized to *Parietaria* pollen were studied. Before the pollen season they underwent an allergen-inhalation challenge which was preceded and followed by a methacholine-inhalation challenge. Pollen count, symptom score, and drug consumption were monitored daily throughout the study. A severity score was obtained by adding symptom score and drug consumption. Patients underwent a third methacholine challenge during the pollen season, after they had been exposed to a high atmospheric concentration of pollen. The severity score during the first period of the pollen season was significantly correlated with both the early and the late asthmatic responses to the allergen observed before the season ($r^2 = 0.50$; $P < 0.005$). Bronchial sensitivity to methacholine was significantly increased both after allergen challenge and after seasonal exposure, but these increases correlated neither with each other nor with the severity score. We conclude that bronchial responses to experimental exposure to allergens, but not the changes in nonspecific airway responsiveness, can, in part, predict the severity of asthma exacerbation during the pollen season.

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Nonspecific bronchial challenges with histamine or methacholine (MCh) have been widely used to quantify airway hyperresponsiveness and, by inference, the severity of asthmatic disease. Even though an increase of airway responsiveness has been shown to occur after exposure to allergens (3, 5) or after viral infections (10), events that also worsen asthma symptoms, recent studies have shown some discrepancies between the degree of bronchial sensitivity to MCh and the severity of asthmatic exacerbations (11, 14). In allergic asthma, bronchial challenges with allergens may cause a transient early-phase bronchospastic response, often followed by a sustained late-phase response, which is commonly associated with an increase of sensitivity to MCh. This model has been used to study the mechanism of the disease, assuming that the response of the asthmatic airways to the acute experimental exposure to an allergen mimics the response to prolonged natural exposure. If this assumption is valid, the results of allergen-inhalation challenge performed during a period of quiescence of disease should reflect the severity of symptoms.

The present study was undertaken to determine the extent to which the airway response to experi-

mental exposure to allergens and the associated allergen-induced increase of airway responsiveness predict the severity of asthmatic exacerbations that follow natural exposure to allergens. Furthermore, we investigated whether a correlation exists between experimentally and naturally allergen-induced increases of airway responsiveness to MCh.

Material and methods

We studied 15 patients who had suffered from seasonal rhinitis and asthma caused by *Parietaria* pollen for the last 3 years or longer; their characteristics are given in Table 1. To participate in the study, patients had to meet the following criteria: 1) to be in a stable condition and not to have suffered from asthmatic exacerbation and viral infections for the previous 1 month at least; 2) to have positive skin prick test and RAST to *Parietaria*; 3) not to be sensitized to other common allergens (grass, mugwort, birch, olive, house-dust mites, *Alternaria*, and cat and dog dander). Patients were provided with diary cards for recording symptoms and drug consumption. The occurrence of asthmatic symptoms (cough, wheezing, dyspnea) was scored from 1 to 3 on the

Table 1. Patient characteristics

No.	Sex	Age years	IgE PRU	FEV ₁ % of predicted
1.	F	40	> 17.5	102
2.	F	33	13.3	98
3.	F	30	9.4	109
4.	F	30	> 17.5	88
5.	M	42	> 17.5	109
6.	M	28	16.4	108
7.	M	26	17.5	85
8.	F	21	16.2	90
9.	F	39	6.9	87
10.	F	38	11.9	96
11.	M	28	> 17.5	84
12.	M	45	> 17.5	87
13.	F	37	8.7	107
14.	M	23	13.5	94
15.	F	17	10.5	77

PRU: Pharmacia RAST Unit.

basis of their severity and duration, as follows: 1) of mild intensity, i.e. lasting less than 1 h/d and not interfering with daily activity; 2) of moderate intensity, lasting longer than 1 h/d, and not interfering with daily activity; 3) of severe intensity, i.e. interfering with daily activity. Only β_2 -stimulant treatment by aerosol (salbutamol) was allowed to control symptoms during the period of study, and each puff was scored 1. Scores for asthmatic symptoms and drug consumption were added to obtain a severity score. All patients recorded symptoms and drug consumption during the same period of about 2 months, starting after the recovery from allergen challenge and going up to the day of methacholine challenge performed during the pollen season.

The *Parietaria* bronchial challenge was performed in February, when patients were completely asymptomatic and no *Parietaria* pollen was present in the air; MCh challenge was performed 24 h before and 24 h after the *Parietaria* challenge and repeated during the pollen season, i.e. May. The study was approved by the internal ethics committee, and all patients gave informed consent.

Bronchial challenges

Both MCh and *Parietaria* aerosols were delivered by an ampul-dosimeter device (MEFAR, Brescia, Italy) (7), the same ampul being used for each patient. Forced expiratory volume in 1 s (FEV₁) was measured after each inhalation, and the best of three measurements was taken to construct dose-response curves. The provocative dose causing a 15% fall of FEV₁ (Pd₁₅) from control was calculated by interpolation of the dose-response curves.

MCh challenge was started from a dose of 20 μ g and continued, if necessary, with twofold increments

up to a maximum dose of 5000 μ g. Doses were obtained with three different MCh solutions (1, 10, and 50 mg/ml), and changing the number of breaths. FEV₁ measurements were taken 1 min after each inhalation. When patients did not respond even at the maximum dose of 5000 μ g, this was considered to be their Pd₁₅. Preseason and during-season MCh challenges were performed by the same steps and procedures.

For allergen bronchial challenge, *Parietaria* was predosed in arbitrary units (AU) by means of RAST inhibition technique against an in-house (Bracco, Milan, Italy) sera pool containing a high titer of specific IgE. Double-decreasing solutions from 1000 AU/ml to 8 AU/ml were extemporaneously prepared by adding redistilled water to dried *Parietaria* allergen. After control inhalation of saline, the allergen bronchial challenge was started from the concentration of 8 AU/ml and continued with doubling increases of the allergen concentration until FEV₁, measured 15 min after inhalations, fell below 85% of control. Each concentration was inhaled with 70 quiet breaths corresponding to an effective inhaled volume of 0.5 ml. The maximum concentration at which challenge was stopped if the patient did not respond was 1000 AU/ml, corresponding to an effectively inhaled dose of 500 AU. After the end of inhalation, FEV₁ was monitored hourly for 8 h to detect the occurrence of late-asthmatic response (LAR). The early asthmatic response was expressed as Pd₁₅, and the LAR as the maximum fall of FEV₁ 3–8 h after the end of challenge.

IgE antibodies determination

We measured specific IgE antibodies by the immunoenzymatic technique (Pharmacia, Uppsala, Sweden), using the same batch of allergen for all patients. Results are reported in Pharmacia RAST Units (PRU).

Pollen count

Parietaria pollen counts per m³ air were taken continuously with a volumetric pollen trap (Burkard Manufacturing, Rickmansworth, UK) placed on the roof of the hospital building at a height of approximately 20 m. Microscopic analysis was performed at a magnification of $\times 400$. Results are expressed as total counts per week.

Statistical analysis

Pd₁₅ values were transformed into logarithmic form and are expressed as the geometric mean. For statistical analysis, a cumulative severity score for each patient was obtained by summing all daily scores

Table 2. Results of bronchial challenges and severity score

No.	Allergen Pd ₁₅ , AU	Max FEV ₁ fall, %		MCh Pd ₁₅ , µg			Severity score
		Early	Late	Pre-all.	Post-all.	In season	
1.	18	27	50	80	48	87	105
2.	>500	2	10	5000	335	561	22
3.	36	5	41	145	46	80	70
4.	195	20	22	5000	937	472	24
5.	308	26	31	5000	857	328	33
6.	27	21	16	1172	282	39	65
7.	221	18	7	5000	5000	5000	17
8.	67	30	36	66	19	48	96
9.	221	20	17	1167	5000	510	75
10.	52	23	13	988	303	73	36
11.	128	26	22	5000	521	127	40
12.	152	28	24	221	94	10	10
13.	>500	11	3	5000	5000	5000	26
14.	363	19	7	1400	223	2509	17
15.	85	20	21	1665	278	578	38

MCh Pd₁₅ (geometric mean) was 1140 µg pre-allergen, 372 µg post-allergen, and 262 µg in season ($P < 0.005$, as compared with pre-allergen MCh Pd₁₅ for both).

and drug consumption. Student's paired t-test, Pearson's correlation coefficient and stepwise multiple regression analysis were used when appropriate. P values < 0.05 were considered to be statistically significant.

Results

The results of bronchial challenges with MCh and *Parietaria* are shown in Table 2. The mean value of

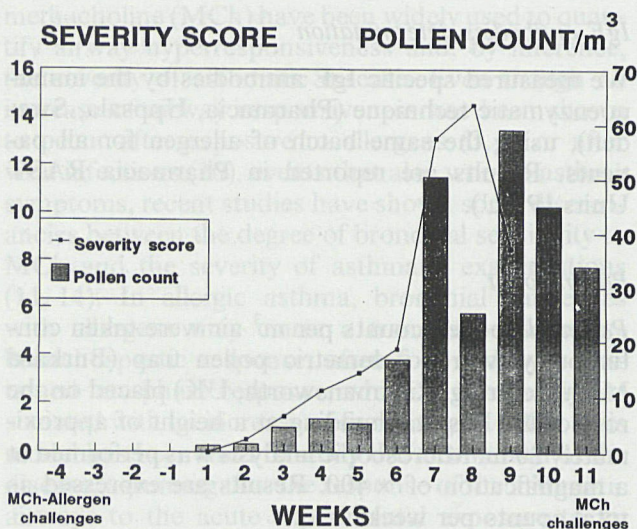


Fig. 1. Weekly average severity score and pollen count. Methacholine and allergen challenges were performed before the pollen season when patients were asymptomatic and no pollen was present in the air. Methacholine challenge was also performed in the 10–11th weeks of the symptom-recording period, when patients had recovered from asthmatic exacerbation. For each patient, the severity score was computed up to the day of methacholine challenge performed during the season.

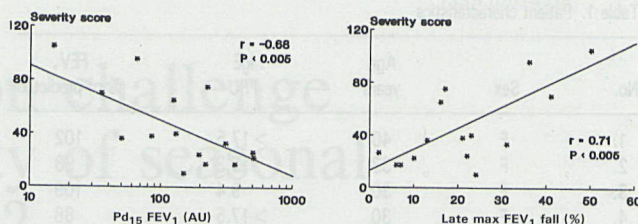


Fig. 2. Relationships between cumulative severity score results of pre-season allergen-inhalation challenge. See text for abbreviations. Note that in both cases $r^2 \sim 0.50$, indicating that half the variance of severity score was explained by regressions.

MCh Pd₁₅ observed after *Parietaria* challenge was significantly lower ($P < 0.005$) than that observed before *Parietaria* challenge. The level of specific IgE antibodies in serum was not correlated with the MCh Pd₁₅ observed before *Parietaria* challenge; therefore, these two were considered to be independent variables. Multiple regression analysis showed that MCh Pd₁₅ at base line was correlated with both allergen Pd₁₅ ($r = 0.68$) and maximum late fall of FEV₁ ($r = -0.71$). Specific IgE antibodies correlated neither with the allergen Pd₁₅ nor with the maximum late fall of FEV₁; this was apparently due to the fact that the IgE serum level was restricted to a narrow range (6.9–17.5 PRU).

At least 3 weeks separated *Parietaria* bronchial challenge from the beginning of natural symptoms. Then the severity score increased progressively with the increase in pollen count during the season (Fig. 1).

The cumulative severity score recorded during the season was significantly correlated with both the *Parietaria* Pd₁₅ ($r = -0.68$; $P < 0.005$) and the severity of the LAR determined before season ($r = 0.71$; $P < 0.005$) (Fig. 2).

Compared with values at base line, that is, before allergen and season, MCh Pd₁₅ was significantly decreased during the season ($P < 0.005$, Fig. 3). This

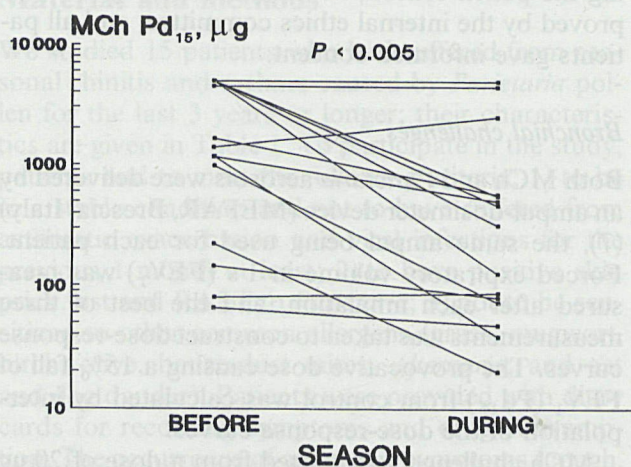


Fig. 3. Sensitivity to MCh observed before and during season.

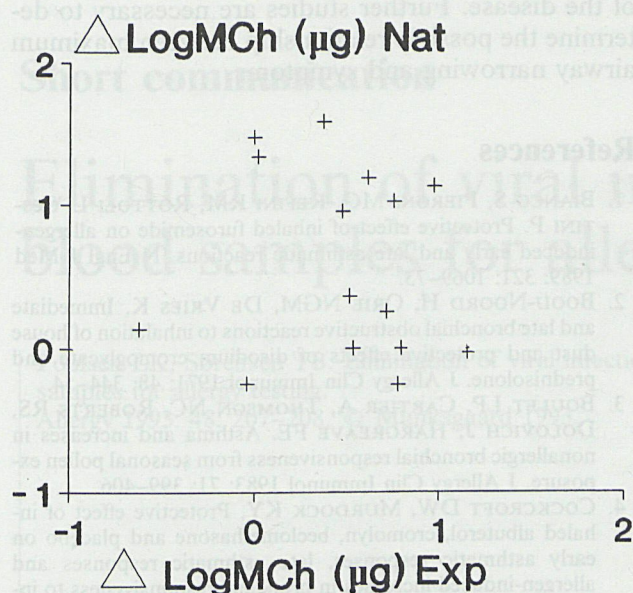


Fig. 4. Relationship between experimentally (Exp) and naturally (Nat) allergen-induced increase of bronchial hyperresponsiveness to methacholine. Differences between pre- and post-allergen-challenge methacholine Pd_{15} were plotted against differences between pre- and during-season methacholine Pd_{15} , but no significant correlation was observed.

decrease was not correlated with the decrease of MCh Pd_{15} induced by the allergen challenge performed before the season ($r = -0.11$; $P > 0.7$) (Fig. 4). Furthermore, neither experimentally nor naturally induced changes in MCh Pd_{15} were significantly correlated with the cumulative severity score ($r = -0.35$; $P > 0.1$ and $r = -0.38$; $P > 0.1$, respectively). Base-line FEV_1 was also decreased during season from 3.31 ± 0.66 (SD) to 3.16 ± 0.54 l ($P < 0.01$), but this difference did not correlate with the cumulative severity score ($r = -0.13$; $P > 0.6$).

Discussion

In this study we have shown that in allergic asthma both the early-phase and the late-phase responses to allergen-inhalation challenge performed before the pollen season can partially predict the severity of seasonal exacerbations.

The bronchial provocation challenge with the sensitizing allergen has been used for the diagnosis of allergic asthma and for assessing the efficacy of antiasthmatic drugs or immunotherapy (1, 2, 13). The use of allergen-inhalation challenges for these purposes is justified by clinical and pathologic similarities between the late-phase reaction and the naturally occurring asthma attack. The clinical similarities are the sustained time course and the relative insensitivity to bronchodilators (4); the main pathologic similarity is the inflammatory reaction in the periphery of the lung (8, 9). However, whether the results

of allergen bronchial challenge also reflect the severity of symptoms associated with natural asthma has not been established.

Only one study (16) reported a significant association between the frequency of asthma attacks in the previous year and the development of the late-phase response to allergen-inhalation challenge. In that study, the subjects were children with perennial asthma caused by house-dust mite; thus, inhalation challenges were not performed in the absence of natural exposure to the sensitizing allergen. The results of inhalation challenges may be highly sensitive to the variability of natural exposure at the time of studies. In order to minimize this source of variance, we studied patients with seasonal asthma and performed allergen-inhalation challenges when natural exposure was nil. On the other hand, the severity of symptoms may be affected by pharmacologic treatments and differences in individual exposure to allergen. We minimized the variability connected with the pharmacologic treatments by avoiding anti-inflammatory therapy with cromolyn or steroids. For this purpose we limited the period of study to the first weeks of pollen season, before severe attacks occurred and steroid treatment was eventually needed.

We found that the severity of the exacerbation of asthma during the first weeks of the pollen season was similarly correlated with the early-phase and the late-phase responses to the experimental inhalation of the sensitizing allergen performed before the season. This finding suggests that some events occurring during the development of the asthmatic response to experimental inhalation of allergen reflect, over a shorter period of time, those occurring after the longer natural exposure to pollen. However, about one-half of the variance of the severity score was not predicted by the results of allergen challenges. In addition, the increase in airway sensitivity to MCh observed after natural exposure was not correlated with the increase that followed allergen-inhalation challenge. It is conceivable that some inconsistencies between the effect of experimental and natural exposure to allergen result from differences in natural exposure among patients.

In this study we have found that neither the absolute values of sensitivity to MCh determined during the season nor its increase as compared with pre-season values were correlated with the severity of disease during seasonal exacerbation.

This finding agrees with the results of Josephs et al. (11), who failed to show a relationship between sensitivity to MCh and asthmatic symptoms. It has been previously shown (6) that both the early-phase and the late-phase responses to perennial allergen-inhalation challenge can be predicted by taking into account nonspecific airway responsiveness and

serum specific IgE antibodies. However, neither factor taken alone could well predict the responses to experimentally inhaled allergen. Similarly, it is also possible that symptoms during the pollen season reflect the combined effect of changes in the amount of IgE antibodies and changes in nonspecific airway responsiveness. The latter are probably associated with or follow from the development of allergic bronchial flogosis. Therefore, as with the response to experimentally inhaled allergen, in an unselected atopic population neither the degree of MCh sensitivity nor the amount of IgE antibodies, taken alone, can be expected to predict the severity of asthma. Unfortunately, in our *Parietaria*-sensitized patients, high levels of serum specific IgE were present already before the season, and we were unable to detect a further increase during the season. Therefore we cannot exclude the possibility that changes in the level of IgE antibodies might correlate with the severity of asthmatic exacerbations.

Sensitivity to MCh may be of little utility for predicting the severity of asthma also because symptoms may be determined not only by the sensitivity of airway smooth muscle to a given agonist, as reflected by the position of the dose-response curve, but also by the maximum degree of airway narrowing that a patient can achieve (15). The experimental and the natural exposure to allergens may cause the activation of allergic-inflammatory responses that may increase airway responsiveness through different mechanisms. Prejunctional mechanisms, such as recruitment and activation of inflammatory cells, may lead to an increase of sensitivity to MCh, thus shifting to the left the dose-response curve and decreasing the Pd (12). Postjunctional mechanisms, such as increase of smooth muscle contractibility, reduction of elastic load to the airways, swelling of the airway wall, and intraluminal exudate and secretions, may cause an increase of the maximal attainable level of airway narrowing (12).

In conclusion, the results of the present study confirm that allergen-inhalation challenge is a reasonably good experimental model of clinical asthma and, when performed before the pollen season, may predict the severity of asthma exacerbation during the season. By contrast, changes in nonspecific airway responsiveness, as inferred from changes in sensitivity to MCh, do not appear to reflect the severity

of the disease. Further studies are necessary to determine the possible relationship between maximum airway narrowing and symptoms.

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