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Natural exposure to pollen reduces the threshold but does not change the pattern of response to the allergen in allergic subjects

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It is known that exposure to seasonal allergen in sensitized asthmatics increases non-specific bronchial responsiveness, but it is controversial if exposure to seasonal allergen influences the presence and the severity of the late asthmatic response (LAR) to allergen.

Fifteen asthmatic subjects sensitized to grass pollen performed a specific bronchial provocative test (sBPT) with *Phleum pratensis* extract before and during the pollen season. Changes of methacholine were also assessed.

Allergen $PD_{20}FEV_1$ significantly decreased during the pollen season with respect to outside (allergen $PD_{20}FEV_1$, geometric mean: 0·10 *vs.* 0·23 biological units; $P<0·05$), but the pattern of specific airway response did not change. Particularly, a consistent LAR was observed in three subjects outside the pollen season and in two subjects during the pollen season. Seven subjects with isolated early asthmatic response (EAR) outside the season did not show LAR after allergen inhalation during the pollen season. However, four of five subjects with slight LAR outside the pollen season (ΔFEV_1% between 15 and 20%) lost LAR during season. Methacholine sensitivity increased slightly but significantly from outside to during the pollen season. This increase was greater in subjects with LAR outside the pollen season.

The natural exposure to pollen induces an increase in bronchial sensitivity to allergen in sensitized subjects, but it does not induce LAR in subjects without LAR outside the pollen season.

Key words: asthma; grass pollen allergy; bronchial challenge test; bronchial hyperresponsiveness.

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Introduction

The inhalation of allergen in the laboratory can induce a specific airway reaction in sensitized subjects which can result in an early (EAR) and/or a late airway response (LAR) (1). EAR measures the sensitivity of airways to allergen. Conclusive evidence indicates that the EAR is determined by the level of allergic sensitivity and by the level of non-specific airway responsiveness (as determined by airway sensitivity to methacholine or histamine) (2,3). The development of a subsequent LAR is associated with a greater severity of the disease (4). Also, a transient worsening of the severity of asthma, such as that induced from viral infection of the respiratory tract, determines the development of LAR after allergen challenge in subjects with previous isolated EAR (5). Several studies had shown that natural exposure to pollens induces an increase in non-specific bronchial hyperresponsiveness to non-specific stimuli (6,7) and in the level of serum-specific IgE (8,9) in sensitized subjects. It could be expected that the sensitivity of the airways to the inhalation of a specific allergen, expressed as EAR, should be increased after natural exposure to the allergen, but conflicting results have been reported (9–11). To the best of our knowledge, only one paper reported the changes in the degree of the LAR in asthmatic subjects sensitized to the birch pollen at the end of the seasonal exposure to the allergen (9).

The aim of this study was to verify whether the natural exposure to the allergen was able to significantly change the threshold and the pattern of airway response to the inhalation of specific allergen in sensitized subjects. A group of subjects sensitized to grass pollen (*Phleum pratensis*) and with symptoms of asthma and rhinitis during the period of natural seasonal exposure to a such allergen was examined before and during the pollen season using a similar schedule.

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Methods

SUBJECTS

Fifteen subjects [nine male and six female subjects, mean age: 23·7 years (range: 13,36)] with mild asthma during the

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grass pollen season were selected on the basis of a positive skin prick test to Graminaceae in the absence of skin sensitivity to other allergens (namely Dermatophagoides, Alternaria, Parietaria, Olea, and dog and cat danders). Eleven subjects also reported seasonal rhinitis. All subjects were examined in a stable phase of the disease, that is no infections during the previous 4 weeks. Subjects were examined in two different time periods: (i) outside the pollen season (from September to March) in a symptom-free period; (ii) during the grass pollen season (from May to July) when they were symptomatic, mean 7 weeks (range 4–10 weeks) after the beginning of the pollen season. During the season, nine out of 15 subjects used regular therapy: six used anti-histamines; five ketotifen; one cetirizine; one sodium cromoglycate; two low-dose beclomethasone dipropionate. The pollen season was confirmed by measurements of the concentrations of grass pollen in the atmosphere of three main towns in Tuscany during the period of the study and expressed as total pollen counts per m^3 of air in 1 week. Usually, the concentration of grass pollen significantly increased in the last week of April or in the first week of May, and persisted until the second or third week of July. Specific bronchial provocative tests during the pollen season were executed in 2 consecutive years after at least 4 and before 10 weeks from the beginning of season to obtain a long exposition to pollen with small inter-subject differences. The time-courses of pollen seasons are represented in Fig. 1.

Outside the pollen season, all subjects showed a positive skin prick test to grass pollen (mean wheal diameter, −nagative control, ≥ 5 mm), and a normal baseline FEV_1 (except subject No. 11) (Table 1). Non-specific bronchial hyperresponsiveness to methacholine was present in 11 of 15 subjects, but respiratory symptoms were absent outside the pollen season.

In the two evaluations (outside and during the season) the subjects performed, on different days and with informed consent, non-specific bronchial challenge test with methacholine and specific bronchial provocative test (sBPT) with allergen. Before each challenge test the pharmacological treatment was withdrawn following the usual recommendations (12).

SPECIFIC BRONCHIAL PROVOCATIVE TEST WITH ALLERGEN

The sBPT was performed with allergens standardized in biological units (BU). Allergen extract solution was delivered by a DeVilbiss 646 jet nebulizer (DeVilbiss Health Care, Somerset, PA, U.S.A.) using a procedure previously described (13). Lyophilized allergen extract (NeoAbellò, Milano, Italy) was dissolved in saline in order to obtain two working solutions with different concentrations (1 and 10 BU ml^{-1}). The nebulizer was filled with 3 ml of diluent (phenol 0·4% in saline) or allergen solution; the nebulizer was connected to a dosimeter (Passerini, Pontedera, Italy) driven by compressed air and activated by the beginning of inhalation. With the nebulizer vent closed, and a 20 psi inlet pressure and 1 sec long nebulization, the output was $10 \pm 1\mu l$, measured by weighing the nebulizer before and after one discharge. The aerodynamic mass median diameter of the aerosol generated was $1{\cdot}2\,\mu m$ (geometric standard deviation, 2·9), measured with a cascade impactor. The output of the nebulizer was regularly checked every 3 months, and any change in the output was modified to obtain the same output of the solution. Each subject wore a nose clip and was instructed to breath via a mouthpiece from functional residual capacity. The nebulizer was filled with 3 ml of allergen solution or diluent

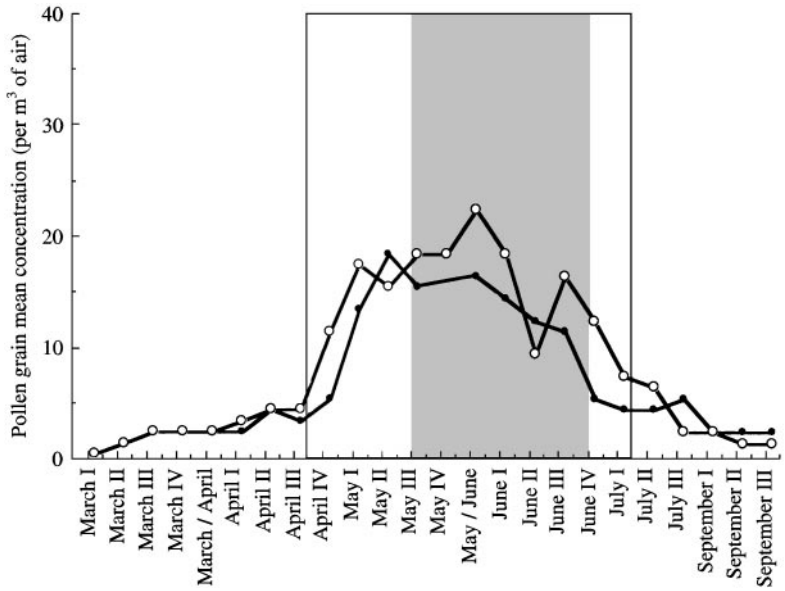

FIG 1. Time courses of pollens during 2 consecutive years in which the study was performed. The white square represents the duration of pollen season and the grey area represents time in which specific bronchial provocative tests were performed.

TABLE 1. Main clinical features of the examined subjects at the first evaluation outside the grass pollen season

No.	Age (years)	Sex	Duration of disease (years)	FEV_1 (% predicted)	SPT (mm)	Diagnosis
1	32	Female	30	111	8	Asthma
2	25	Male	4	117	11	Rhinitis and asthma
3	20	Male	18	97	10	Rhinitis and asthma
4	27	Male	27	107	13	Rhinitis and asthma
5	23	Female	17	122	10	Rhinitis and asthma
6	17	Male	10	110	9	Rhinitis and asthma
7	16	Male	5	111	5	Asthma
8	26	Male	17	108	10	Rhinitis and asthma
9	24	Female	2	94	8	Rhinitis and asthma
10	36	Female	20	88	6	Rhinitis and asthma
11	31	Female	6	70	7	Rhinitis and Asthma
12	13	Male	1	121	12	Rhinitis and Asthma
13	17	Male	3	110	8	Rhinitis and Asthma
14	26	Male	21	97	6	Asthma
15	22	Male	10	132	11	Asthma

SPT = skin prick test with mix Graminaceae (mean wheal diameter).

control filtered through a 0·2 μm millipore filter. After baseline spirometry, the patient inhaled three puffs of diluent, followed at 10 min intervals by incremental doses of allergen to obtain a series of cumulative doses: 0·025, 0·05, 0·1, 0·2, 0·4, 0·8, 1·6, and 3·2 BU. FEV_1 was measured 10 min after the end of each series of allergen inhalations by means of a Biomedin water sealed spirometer connected to an Olivetti microcomputer. The inhalations were continued until FEV_1 fell more than 20% below the post-diluent value or until the top concentration had been administered, and the total dose (TD) of allergen delivered into the airways was calculated. FEV_1 was then measured at 20, 30 and 60 min, and then hourly for 7 h. A fall in FEV_1 greater than 20% between 10 and 60 min was considered to be an EAR, and a fall of FEV_1 greater than 15% between the third and the seventh hour after the challenge was considered to be a LAR, respectively. The dose of allergen causing a 20% fall in FEV_1 from post-diluent value ($PD_{20}FEV_1$ allergen) was derived from a log dose–response curve and was used to evaluate the degree of EAR; the maximal percent FEV_1 fall between the third and the seventh hour was used to express the degree of LAR.

The short-term and long-term reproducibility of EAR and LAR had been previously assessed (14).

METHACHOLINE CHALLENGE TEST

Methacholine was delivered by a DeVilbiss 646 jet nebulizer using the previously described procedure (13). Phosphate buffered saline was inhaled first, followed every 2 min by methacholine inhalation of cumulative doses of 0·04–3·2 mg FEV_1 was measured until it fell more than 20% below the post-diluent value and $PD_{20}FEV_1$ (the cumulative dose producing a 20% fall of FEV_1) was computed. In a previous study, the reproducibility of methacholine $PD_{20}FEV_1$ was assessed: methacholine $PD_{20}FEV_1$ varied $\leq 50\%$ in nine of 12 subjects (variability, 27%).

STATISTICAL ANALYSIS

Arithmetic mean (M) and standard deviation (SD) were used to evaluate ΔFEV_1% fall during EAR and LAR. Geometric mean (GM) expressed mean values of methacholine and allergen $PD_{20}FEV_1$ and TD of delivered allergen during specific bronchial challenges. Area under the curve (AUC) of sBPT (between the second and the seventh hour after allergen inhalation) was computed by the method of trapezoidal integration. Statistical analysis was performed using ANOVA and paired *t*-test; a level of probability lower than 5% was considered significant (15). To use statistical parametric methods, logarithmic transformation of methacholine and allergen $PD_{20}FEV_1$ and TD of delivered allergen was performed (16).

Results

Individual and mean values of the response to non-specific and specific bronchial provocative test performed outside and during grass pollen season are reported in Table 2.

Bronchial hyperreactivity to methacholine slightly, but significantly, increased during the grass pollen season [methacholine $PD_{20}FEV_1$, GM $\pm$ geometric SD: from 0·88 (0·11) to 0·50 (0·12), $P = 0{\cdot}01$]. Methacholine $PD_{20}FEV_1$ < 1 mg was observed in nine of 15 subjects outside the pollen season and in 11 of 15 subjects during the pollen season. Six of 15 subjects showed a reduction of one doubling dose of methacholine at least during pollen

TABLE 2. Bronchial responsiveness to methacholine ($PD_{20}FEV_1$) and to allergen (PD_{20} FEV_1) in the evaluations performed outside and during the pollen season

	Methacholine $PD_{20}FEV_1$ (mg)			Allergen $PD_{20}FEV_1$ (BU)			EAR (ΔFEV^1% fall)			LAR (ΔFEV^1% fall)			AUC		
#	Out		In	Out		In	Out		In	Out		In	Out		In
1	0·36		0·23	0·16		0·20	−22		−27	−15		−12	280		288
2	0·66		0·12	0·18		0·01	−27		−31	−17		−1	175		202
3	0·18		0·06	0·20		0·21	−26		−26	−17		+2	179		210
4	>3·2		0·43	0·07		0·07	−30		−37	−5		0	195		201
5	1·53		0·47	0·12		0·08	−39		−28	+12		−6	186		190
6	0·95		0·60	3·21		1·47	−24		−26	−3		−5	197		193
7	0·76		0·46	0·24		0·04	−52		−29	−22		+3	160		207
8	>3·2		>3·2	0·23		0·07	−22		−38	−5		−18	193		179
9	0·56		0·47	0·70		0·06	−45		−32	−21		−20	174		165
10	0·11		0·12	0·02		0·02	−23		−23	−2		−6	203		190
11	0·56		0·25	0·17		0·05	−28		−50	−28		−48	150		134
12	0·20		0·12	0·15		0·04	−47		−26	−18		−8	173		187
13	>3·2		>3·2	0·64		0·13	−23		−19	−10		−16	184		185
14	1·63		2·93	0·09		0·30	−30		−30	−4		−6	194		188
15	>3·2		>3·2	1·84		0·92	−26		−22	−19		−17	166		172
Mean	0·88†	*	0·05	0·23†	*	0·10†	−31	*NS*	−30	−13	*NS*	−11	187	*NS*	193
SD							10		8	8		13	30		32

EAR: early asthmatic response; LAR: laate asthmatic response; AUC: area under curve.
(*$P<0·05$ between outside and during the pollen season †Geometric mean).

season in comparison with the methacholine challenge performed outside the pollen season. It is of interest that FEV_1 values during the season did not change significantly with respect to outside the season [FEV_1% of predicted, from 105·3 (15) outside to 102·1 (17) during the pollen season].

Changes in methacholine responsiveness was related to the pattern of airway response to allergen outside the pollen season: in the seven subjects with dual asthmatic response (DAR) the responsiveness to methacholine significantly increased from outside to during the pollen season [methacholine $PD_{20}FEV_1$: from 0·58 (0·28) to 0·29 (0·40) mg respectively; $P = 0·021$], while in the eight subjects with EAR the responsiveness to methacholine did not significantly increase from outside to during the pollen season [methacholine $PD_{20}FEV_1$: from 1·27 (0·36) outside to 0·79 (0·39) mg respectively; $P = 0·18$].

The airway response to allergen showed significant changes between outside and during the pollen season. Allergen TD [0.33 (0·35) outside and 0.14 (0·33) during the pollen season, $P = 0·02$] and allergen $PD_{20}FEV_1$ significantly decreased during the pollen season [0·23 (0·35) outside and 0.10 (0·38) during the pollen season, $P = 0·04$] (Fig. 2). The individual analysis showed that nine out of 15 subjects had a decrease ≥ 50% of the allergen $PD_{20}FEV_1$ from outside to during the pollen season. This statistical difference was due to the seven patients with LAR outside the pollen season [$PD_{20}FEV_1$, from 0·25 outside to 0·08 during the pollen season, $P = < 0·05$). No change was evident in the remaining eight patients with only EAR ($PD_{20}FEV_1$, from 0·22 outside to 0·11 during the pollen season).

On the contrary, LAR did not show significant change either as maximum percentage fall of FEV_1 (-13 ± 8% outside and -10 ± 3% during the pollen season, P = NS), and as AUC (187 ± 30 outside and 193 ± 32 during pollen season, P = NS).

On the basis of the pattern of response to sBPT outside the pollen season, the subjects can be separated into three groups: (i) seven subjects with isolated EAR; (ii) five patients with a slight LAR (fall of FEV_1 between -15% and -20%); (iii) three subjects with a consistent LAR (fall of $FEV_1 > 20$%).

From outside to during the pollen season the first group showed a similar pattern of airway response to sBPT: five out of seven subjects maintained a EAR, and two showed a slight LAR during season. In the third group of patients two out of three maintained the LAR, and one changed to EAR during season. However, the second group of subjects showed a shift to an isolated EAR in four out of five subjects and just one subject maintained the same pattern during the pollen season.

A significant relationship was found between the changes of methacholine hyperresponsiveness and the changes in the pattern of response to sBPT from outside to during pollen season ($P = 0·01$).

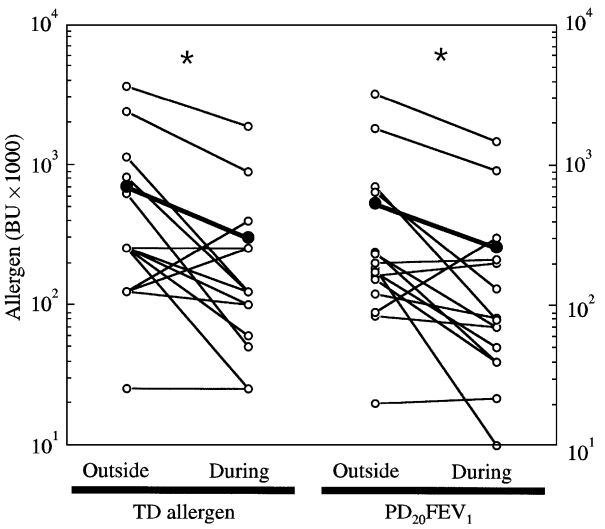

FIG 2. Comparison of total dose (TD) and $PD_{20}FEV_1$ of allergen inhaled during sBPT performed outside and during pollen season in 15 asthmatic subjects (open circles). Closed circles represent the geometric means (* $P<0{\cdot}05$).

Discussion

Our study shows that airway sensitivity to inhaled allergen increases during pollen season without changes in the pattern of response.

A significant increase of non-specific bronchial responsiveness to methacholine during the pollen season occurred, similarly to in other studies (6,7,17). Only 40% of the examined subjects had a significant increase in bronchial responsiveness to methacholine from outside to during the grass pollen season. On the other hand, the increase in methacholine responsiveness was greater in subjects who showed DAR than isolated EAR outside the grass pollen season. These results confirm a previous observation (6) and are suggestive of a positive relationship between the presence of LAR and a higher bronchial sensitivity to non-specific stimuli after natural exposure to the allergen.

After a time period of natural exposure to a seasonal allergen, hypersensitivity to the inhalation of the allergen can occur, requiring less inhaled antigen to produce a positive response during the challenge test. This 'priming effect' of allergen in seasonal asthma was studied by several investigators, who obtained conflicting results. One study showed no effect on bronchial sensitivity to the allergen in nine subjects with ragweed-induced asthma, by measuring the EAR to the allergen outside and during the ragweed pollen season (10). Kelly *et al.* (11) and Crimi *et al.* (9) showed an increase of bronchial sensitivity to the allergen after the pollen season with respect to sBPT performed before the pollen season. Our results show that an increase of bronchial sensitivity to the allergen, expressed as a decreased allergen $PD_{20}FEV_1$, occurs during natural exposure to the pollen with respect to sBPT performed outside the grass pollen season. This increased sensitivity to the allergen may be due to the increase in airway inflammation induced by natural exposure to the allergen and consequently an increased allergic sensitivity of the airways. In fact, markers of activation of airway inflammatory cells increase in the serum (18,19) and in BAL fluid (20) of asthmatic subjects during natural exposure to the allergen. It is of interest that the differences in EAR outside and during the pollen season are due to patients with LAR outside the season and not to patients without LAR.

In our study, the pattern of specific airway response did not significantly change from outside to during pollen season, in contrast to the results obtained by Crimi *et al.* (9), who showed an increase in severity of LAR in sBPT performed after the birch pollen season. A possible difference is that in our study patients performed evaluations during the pollen season, while in the study by Crimi patients could be studied at the end of the season, after a longer exposure time. It is problematic that drugs that some patients used during the pollen season could influence bronchial reactivity. In fact, inhaled low-dose beclomethasone and cromons need many weeks and continuous treatment to change bronchial reactivity (21). Furthermore, anti-histamines as cetirizine have no effect on EAR and LAR (22).

The clinical significance of the different pattern of response to sBPT in the laboratory is not known. Because LAR is associated with an increase in non-specific bronchial hyperresponsiveness (17) and with recruitment of inflammatory cells in the airway (23), it is considered to be a better reminiscent of spontaneous asthma than EAR, and it is believed to be suggestive of a greater severity of asthma. However, the relationship between LAR induced in the laboratory and the outcome of spontaneous asthma has not been proven yet, and no relevant difference could be observed between subjects with isolated EAR and subjects with DAR regarding the severity of symptoms, bronchial responsiveness and response to the treatment (24,25). If airway inflammation increases during natural exposure to the allergen, LAR severity could increase during the pollen season in allergic asthmatics. Our data did not support this hypothesis, because LAR occurrence and severity (as expressed by the maximal percentage fall in FEV_1 between the third and the seventh hour and by AUC) did not change significantly during the pollen season, and if the subjects were exposed for a consistent period time to allergen, 7 weeks after the beginning of pollen season (range 4–10 weeks). Moreover, if the five subjects with slight LAR (ΔFEV_1% from 15 to 20%) were considered, we found that LAR was not present during the grass pollen season. In these subjects the reduction in the LAR severity could be ascribed to the lower TD of allergen delivered in the airway in sBPT during season. In the same subjects the occurrence and severity of LAR is dose-dependent, as proven by some authors who were able to induce an LAR in subjects with isolated EAR by an increase in the TD of allergen after pretreatment with short-acting β_2-agonists (26). It is of interest that in our study patients with LAR outside the pollen season have a significant decrease in $PD_{20}FEV_1$ during the pollen season. This could influence the development of subsequent LAR.

In conclusion, we have shown that in sensitized subjects natural exposure to allergen induces a reduction in the threshold of response to the inhaled allergen during sBPT, probably due to increased non-specific bronchial hyperresponsiveness and airway inflammation during the season. The absence of an increase in LAR severity during the season does not support the hypothesis that LAR is a marker of a greater airway reaction to the inhaled allergen.

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