Original Article

Analysis of new respiratory allergies in patients monosensitized to airborne allergens in the area North of Milan

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Summary

Background: A recent study carried out in North of Milan, an area that was recently colonized both by birch and ragweed pollen, showed that subjects developing allergies to these "new" allergens were much older than those becoming allergic to "traditional" airborne allergens, which suggests that predisposition to develop respiratory allergies is probably allergen-specific, persists throughout life, and does not occur until the patient encounters the appropriate allergen for a sufficiently long period.
Objective: The present study aimed to test this hypothesis by following-up a large cohort of patients monosensitized to airborne allergens.
Methods: The prevalence and characteristics of new sensitizations to airborne allergens after >2 years of follow-up were retrospectively assessed in 726 patients monosensitized to grass, pellitory, mite, birch, ragweed, *Alternaria*, mugwort, or plantain living North of Milan.
Results: The overall prevalence of new sensitizations was 35% (256/726), with marked differences between the subgroups ranging from 11% in *Alternaria*-allergic subjects to 46% in grass-allergic subjects. Patients allergic to grass, birch, or pellitory pollen showed a significantly higher propensity to become sensitized to a second airborne allergen than subjects allergic to ragweed, *Alternaria*, and mite. Overall, the "new" allergens (birch and ragweed) caused 228/256 (89%) new sensitizations detected in the whole study group. Ragweed pollen induced 90% (38/42) of new sensitizations in birch pollen allergic patients, and birch pollen induced 80% (8/10) of new sensitizations in ragweed allergic patients. No difference in age at the first visit or in duration of the follow-up between patients developing and not developing new respiratory allergies was observed.
Conclusion: This study shows that: 1) predisposition to develop respiratory allergies is allergen-specific and persists throughout life; 2) proneness to become allergic to certain airborne allergens might be associated with primary sensitization to specific airborne allergens; 3) in monosensitized adults, sensitization to another airborne allergen that has been always present in that particular geographical area is unlikely.

Key Words: Respiratory allergy, Ragweed, Birch, Sensitization, Follow-up, Genetics, Predisposition, Atopy

Introduction

Allergy is the result of the complex interaction between multiple gene products and appropriate environmental triggers [1]. To date, genetics studies have failed to find a single gene responsible for allergic response or disease; instead, many genes seem to contribute to the development of the allergic response, each showing a variable degree of involvement. An association between certain HLA haplotypes and IgE response to specific allergens such as house dust mite, *Parietaria*, and ragweed has been observed [2-6]. Although several aspects of the relationship between allergen exposure and sensitization are still unclear, a close association between both levels [7-9] and duration [10] of exposure and sensitization to a specific allergen seems to exist. In this sense, the exposure of the general population living in a certain geographical area to new ubiquitous airborne allergens might represent an interesting model to get some new valuable information. The area North of Milan was massively colonized by birch pollen during the last 15-20 years (a consequence of the widespread use of this tree to embellish public parks as well as private gardens) and by ragweed pollen during the last 12 years (spread of ragweed started from the surroundings of the International Malpensa Airport, possibly as a consequence of pollen released by the wheels of the planes arriving from the USA) [11]. A recent work found that in this area allergy to these two airborne allergens frequently occurs in older subjects (up to 75 years of age) that had never suffered from allergic diseases before and do not show any detectable risk factor for allergy [11]. These observations strongly suggest that predisposition to develop respiratory allergies is allergen-specific, and persists throughout life, and that no sensitization occurs until the patient is exposed to the "appropriate" allergen for a sufficiently long period. If this were the case, a proportion of patients living in the area North of Milan who are already sensitized to airborne allergens other than birch or ragweed (i.e. the "old" allergens that have always been present) would be expected to develop allergies to the "new" allergens as well, whereas in the same subjects novel sensitizations to "old" allergens should be unlikely. This study analyzed the pattern of new sensitizations in a large cohort of patients with respiratory allergy living in the area North of Milan in order to assess the validity of this hypothesis.

Patients and Methods

Patients

The study was carried out at the Allergy Unit of the Hospital of Bollate (a town 5 km North of Milan). The data from all 726 (M/F 330/426; mean age 28 years, range 4-67 years at the first visit) patients monosensitized to common airborne allergens first visited between January 1st, 1986, and December 31st, 1999, and who spontaneously presented for a control visit after no less than 2 years were reviewed. Monosensitization was defined as a single positive SPT in keeping with a clinical history of rhino-conjunctivitis, with or without asthma. In order to avoid bias caused by overlapping pollen seasons (e.g. grass/pellitory, ragweed/mugwort) and by sensitization to cross-reacting allergens such as profilin [12,13], to common allergens in ragweed and mugwort pollen [14], or in grass and plantain pollen [15], patients showing multiple sensitizations were excluded. Since the aim of the study was to assess the effects of long-term exposure to ubiquitous allergens present in this geographical area, patients sensitized to airborne allergens to wich not all subjects might be exposed (e.g., cat or dog dander) or to pollen grains that are very rare in this area (e.g. cypress, and olive) were excluded as well. During the follow-up visits patients were thoroughly interviewed in order to detect any possible change in clinical symptoms, particularly in seasonality, and underwent a complete set of skin tests (see below). A new sensitization was defined as the occurrence of both a newly positive skin test and clinical symptoms associated with that particular allergen. In patients who underwent more than one control visit, the follow-up duration was calculated as the time between the first and the last visit.

Skin tests

During all visits (i.e. both the first and the follow-up visits) patients underwent skin prick tests (SPT) with a standard panel of commercial extracts (Allergopharma, Reinbeck, Germany) of the main seasonal and perennial allergens present in this geographical area, including grass, pellitory, ragweed, birch, mugwort, and plantain pollen, *Alternaria tenuis*, and house dust mite, SPT were carried out on the volar side of the forearm using disposable 1 mm-tip lancets (Dome Holister/Stier). Readings were taken at 15 min. Reactions were expressed as mean wheal diameter (adding the longest diameter to the orthogonal diameter and dividing by 2). A diameter of 3 mm or more was considered positive [16]. Histamine 10 mg/ml and saline were used as positive and negative controls respectively.

Statistical methods

The mean ages of patients developing or not developing new allergies within each subgroup were compared by the two-tailed Student's test. Proportions were compared by chi-square test. Probability values less than 5% were considered significant.

Results

Clinical features are summarized in Table 1. The mean follow-up time ranged from 5.9 years in the ragweed subgroup to 8.8 years in the grass subgroup. The overall prevalence of new sensitizations detected at the follow-up visits was 35% (256/726), ranging from 11% in subjects allergic to *Alternaria* to 46% in grass-allergic subjects. Overall, two distinct behaviors could be observed in the different subgroups: a) a high propensity to develop new respiratory allergies characterized patients allergic to grass (157/338 [46%]), pellitory (26/75 [35%]), and birch pollen (41/112 [37%]) whereas b) patients allergic to house dust mite (14/94 [15%]), ragweed (10/65 [15%]), *Alternaria* (2/19 [11%]), mugwort (4/18 [22%]), and plantain (1/5 [20%]) showed a much lower propensity to develop new

Table 1. Clinical features of 726 patients monosensitized to different airborne allergens

Allergen	Nº	Sex (M/F)	Mean age at 1st visit (SD)	Mean follow-up in years (SD)	Prevalence of new allergies
Grass	338	159/179	23.6 (10.8)	8.8 (4.5)	157 (46%)
Birch	112	42/70	37.0 (11.0)	7.0 (3.9)	42 (37%)
Pellitory	75	31/44	30.0 (10.0)	8.0 (4.0)	26 (35%)
Mugwort	18	8/10	34.4 (8.5)	6.9 (3.8)	4 (22%)
Plantain	5	2/3	36.0 (9.1)	7.8 (2.7)	1 (20%)
Mite	65	28/37	37.1 (11.7)	5.9 (3.1)	10 (15%)
Alternaria	19	11/8	13.1 (6.8)	7.5 (3.8)	2 (11%)
Total	**726**				**256 (35%)**

allergies. Patients allergic to grass pollen showed a significantly higher propensity to become sensitized to a second airborne allergen than subjects allergic to ragweed ($p<0.001$), Alternaria ($p< 0.005$), and mite ($p<0.001$). Similarly, patients allergic to birch pollen showed a significantly higher propensity to become sensitized to a second airborne allergen than subjects allergic to ragweed ($p<0.005$), *Alternaria* ($p<0.05$), and mite ($p<0.001$), and pellitory-allergic subjects allergic to ragweed ($p<0.025$) and mite ($p<0.005$).

The distribution of new sensitizations by airborne allergen, and the relative importance of the "new" allergens (ragweed and birch) in causing new sensitizations in the different subgroups are shown in Table 2. Overall, the "new" allergens caused 228/256 (89%) new sensitizations detected in the whole study group. If subgroups were examined separately, de-novo sensitization to "old" allergens was observed only in mite allergic subjects (notably, a subgroup that, as a whole, showed scarce predisposition to develop new allergies). Ragweed pollen induced 90% (38/42) of new sensitizations in birch pollen allergic patients, and birch pollen induced 80% (8/10) of new sensitizations in ragweed allergic patients.

No difference in age at the first visit or in duration of the follow-up was observed in the different subgroups between patients developing and not developing new respiratory allergies (Table 3).

Moreover, since control visits were carried out on patient demand the influence of time on the results was further assessed by plotting the cumulative new sensitizations against time from first visit to follow-up. No difference in the prevalence of new sensitizations was observed between patients showing a different follow-up duration (Figure 1).

Table 2. Distribution of 256 new respiratory allergies diagnosed at the follow-up visit

Allergen	No	Grass	Pellitory	Mite	Birch	Ragweed	Alternaria	%
Grass	157		7	5	69	76	0	92%
Pellitory	26	4		0	11	11	0	85%
Mite	14	3	3		4	4	0	57%
Birch	42	1 (+1)	2 (+1)	0		38	0	90%
Ragweed	10	1 (+1)	0	0	7 (+1)		1	80%
Alternaria	2	0	0	0	0	2		100%
Mugwort	4	0	0	0	3	1	0	100%
Plantain	1	0	0	0	0	1	0	100%
Total	**256**							**89%**

(+1): One patient developed 2 new allergies.
%: Relative importance of birch or ragweed in inducing new sensitizations

Table 3. Comparison between patients not developing and developing new respiratory allergies

	Age at 1st visit (SD)			Follow-up duration in yrs (SD)		
Primary Allergen	**No new allergy**	**New allergy**	**p**	**No new allergy**	**New allergy**	**p**
Grass	22.8 (11.3)	24.4 (10.1)	NS	8.7 (4.8)	8.8 (4.2)	NS
Pellitory	30.4 (11.1)	29.7 (8.2)	NS	8.1 (4.4)	8.3 (3.3)	NS
Mite	18.4 (10.9)	17.6 (6.8)	NS	8.1 (4.1)	8.6 (4.5)	NS
Birch	38.5 (10.7)	34.6 (9.7)	NS	7.4 (4.1)	6.5 (3.8)	NS
Ragweed	37.2 (10.0)	36.6 (12.1)	NS	6.0 (3.1)	5.2 (3.1)	NS
Mugwort	33.8 (7.7)	36.5 (12.1)	NS	6.9 (3.9)	7.2 (3.5)	NS
Alternaria	12.8 (7.2)	15.0 (2.8)	NS	7.0 (3.7)	11.5 (2.1)	NS

Discussion

A recent study carried out in North of Milan, an area that was colonized during the last two decades both by birch and ragweed [17-19], found that patients becoming allergic to these "new" airborne allergens were in average much older than those developing allergies to "old" allergens such as grass, pellitory, mite or Alternaria [11]. These observations suggested that in each individual patient predisposition to respiratory allergies is both allergen-specific and life-long lasting; in other words, subjects prone to become allergic to a certain allergen will not develop any allergy until the "appropriate" allergen is encountered. In order to test this hypothesis, the present study evaluated retrospectively the pattern of new sensitizations in a large cohort of patients diagnosed as being allergic to a single airborne allergen. The study was not designed to have a strict epidemiological value as it was performed on a selected population that did not include patients sensitized to animal dander, olive pollen, cypress pollen, and, more importantly, the large proportion of allergic subjects showing multiple SPT reactivity to airborne allergens. In particular, the latter might represent a subset showing a higher propensity to develop new respiratory allergies than monosensitized patients. Nonetheless, the exclusion of

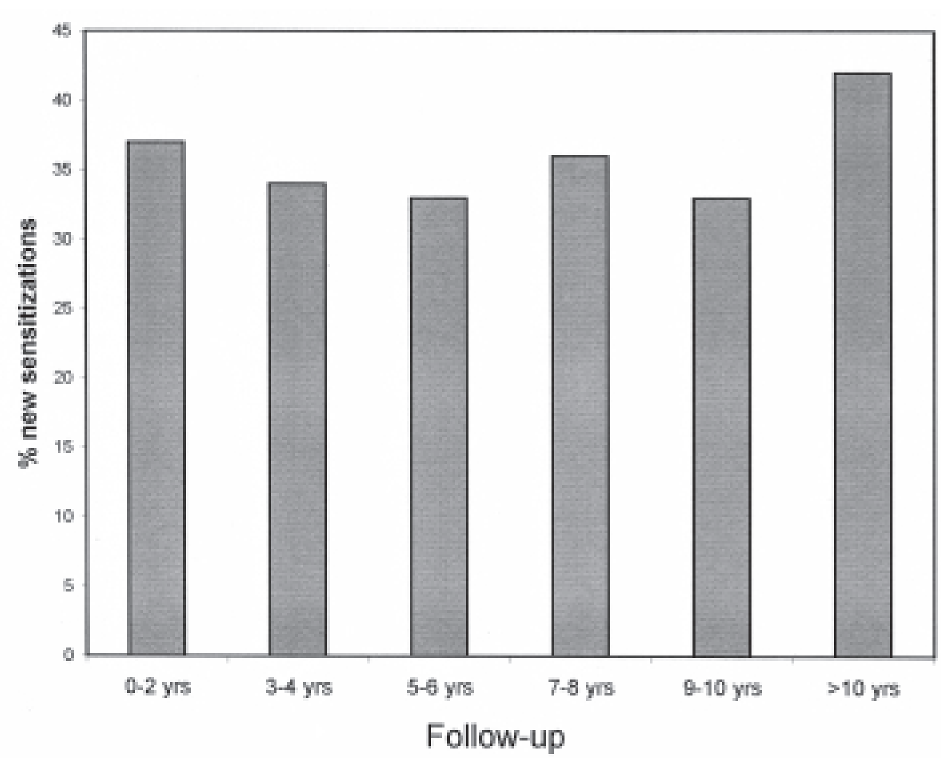

Figure 1. Prevalence of cumulative new sensitizations by follow-up duration.

multisensitized subjects was essential to avoid possible bias and confusion caused by sensitization to cross-reacting allergens present in a number of pollen grains (including ragweed and birch) such as profilin [12,13].

Despite the retrospective design of the study, with control visits carried out on patients' demand, follow-up duration was fairly consistent in all subgroups and long enough to evaluate the effects of long-term exposure to all airborne allergens considered. Not surprisingly, in view of the more recent appearance of ragweed pollen as an allergenic source in this area, ragweed-allergic subjects showed a shorter mean disease duration and, consequently, a slightly shorter mean follow-up duration than patients in all other subgroups; nonetheless, a mean follow-up of 5.9 years in this subgroup should have been long enough to detect further sensitizations to airborne allergens to which patients had been in most cases exposed throughout their whole lives.

The overall prevalence of new sensitizations observed was 35%. Such proportion is not surprising since the study was carried out on atopic subjects and, importantly, follow-up visits were performed on patients' demand. In a population-based study the proportion of subjects developing new sensitizations would probably have been lower; however, an epidemiological survey was out of the scope of the present study. This prevalence is in keeping with the one observed (43.6%) in children monosensitized to airborne allergens after 2-10 years of follow-up [20]. Altogether, the findings of this study fully confirmed the initial hypothesis. Birch and ragweed pollen accounted for 90% of the new sensitizations detected in 256 patients, and if these two allergens were excluded only 22 cases of new sensitizations in 549 allergic patients (4%) would have been recorded. Although it cannot be excluded that some of those who developed birch and ragweed pollen allergy had been exposed to these airborne allergens elsewhere, it seems unlikely that this may explain the findings of this study. Interestingly, the proneness to develop new allergies differed markedly from one subgroup to another. In particular, patients sensitized to some "old" allergens, such as mite and Alternaria, and to a "new" allergen (ragweed) showed a much lower propensity to become sensitized to different allergens than subjects allergic to other "old" allergens (grass, pellitory) or to the other "new" allergen (birch). In a previous study mite-sensitive patients showed a high proneness to become multisensitized [20]; the fact that the large majority of patients included in the present study were adults may explain this difference. The findings of this study suggest that sensitization to different airborne allergens might be associated with a different (specific) genetic background that confers a variable predisposition to develop further allergies. The statistical analysis excluded the possibility that new sensitizations were associated with a younger age at first visit or with a longer follow-up period.

In conclusion, this study shows that: 1) predisposition to develop respiratory allergies is allergen-specific and lasts for life; 2) sensitization to specific airborne allergens might be associated with the proneness to become allergic to certain other airborne allergens; 3) in monosensitized adults, de-novo sensitization to other airborne allergens that has been always present in that particular geographical area is rare.

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