Molecular spreading and predictive value of preclinical IgE response to *Phleum pratense* in children with hay fever

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Background: IgE sensitization against grass pollen is a cause of seasonal allergic rhinitis.
Objective: We sought to investigate the evolution at the molecular level and the preclinical predictive value of IgE responses against grass pollen.
Methods: The German Multicentre Allergy Study examined a birth cohort born in 1990. A questionnaire was administered yearly, and blood samples were collected at 1, 2, 3, 5, 6, 7, 10, and 13 years of age. Grass pollen–related seasonal allergic rhinitis (SARg) was diagnosed according to nasal symptoms in June/July. Serum IgE antibodies to *Phleum pratense* extract and 8 *P pratense* molecules were tested with immune-enzymatic singleplex and multiplex assays, respectively.
Results: One hundred seventy-seven of the 820 examined children had SARg. A weak monomolecular/oligomolecular IgE response to *P pratense* was observed very frequently before SARg onset. These initial IgE responses increased in concentration and molecular complexity during the preclinical and clinical process. A typical progression of IgE sensitization was observed: Phl p 1 (initiator in >75% of cases); then Phl p 4 and Phl p 5; then Phl p 2, Phl p 6, and Phl p 11; and then Phl p 12 and Phl p 7. At age 3 years, IgE sensitization predicted SARg by age 12 years (positive predictive value, 68% [95% CI, 50% to 82%]; negative predictive value, 84% [95% CI, 80% to 87%]). At this preclinical prediction time, the number of recognized molecules and the serum levels of IgE to *P pratense* were significantly lower than at 3 or more years after SARg onset.
Conclusions: The IgE response against grass pollen molecules can start years before disease onset as a weak monosensitization or oligosensitization phenomenon. It can increase in serum concentration and complexity through a "molecular spreading" process during preclinical and early clinical disease stages. Testing IgE sensitization at a preclinical stage facilitates prediction of seasonal allergic rhinitis at its molecular monosensitization or oligosensitization stage. (J Allergy Clin Immunol 2012;130:894-901.)

Key words: *Allergenic molecules, allergic rhinitis, children, component-resolved diagnosis, component-resolved therapy, grass pollen, hay fever, immunoglobulin E,* Phleum pratense*, Phl p 1, prediction, timothy grass*

Allergic sensitization to grass pollen and hay fever are highly frequent among adults[1,2] and children[3] in developed countries, and their burden is enormous.[4] Avoidance of grass pollen allergens is difficult, drug treatment is only partially effective, and no cure is available.[5] Allergen-specific immunotherapy (SIT) is effective in the short-term[6,7] and long-term,[8] but its efficacy is partial[9] and debated.[10]

The diagnostic and therapeutic approach to grass pollen allergy is mainly based on grass pollen extract preparations. However, extracts produced from different companies are heterogeneous in their molecular composition.[11] On the other hand, the IgE sensitization profiles of patients with established clinically relevant allergic sensitization to grass pollen are also very heterogeneous.[12] Mismatch in the molecular sensitization profile of an individual patient and the molecular profile of the allergenic preparation might explain a reduced diagnostic and therapeutic performance.[12]

Our understanding of IgE-mediated allergies has greatly improved since the advent of "molecular allergology."[13] Under this approach, molecules, instead of extracts, are used for both the diagnosis (component-resolved diagnosis) and therapy (component-resolved therapy) of allergic diseases.[14] This concept

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Abbreviations used
ISAC: Immuno Solid-phase Allergen Chip
ISU: ISAC Standard Unit
MAS: Multicentre Allergy Study
SARg: Grass pollen–related seasonal allergic rhinitis
SIT: Allergen-specific immunotherapy

foresees characterizing the patient's sensitization profile at the molecular level and, with this information, tailoring the composition of his or her individualized SIT.[14]

At least 13 allergenic molecules of *Phleum pratense* (timothy grass), a representative species of the Poaceae family, have been identified and sequenced,[15] and the prevalence of an IgE response to 8 of them has been repeatedly examined in children and adults.[16] IgE sensitization molecular profiles of children with established disease are complex and heterogeneous,[12] so that tailoring component-resolved therapy at advanced disease stages might be difficult.[14] However, the origins and molecular evolution of IgE responses against grass pollen have never been investigated. Therefore whether IgE sensitization is less complex and heterogeneous at earlier stages of the disease process is presently unknown.

To answer this question, we have taken advantage of the Multicentre Allergy Study (MAS), a birth cohort study starting in 1990. The MAS has scheduled yearly evaluation of upper airway symptoms and repeated peripheral blood drawing at 8 follow-up points during the first 13 years of life.[17] We could therefore retest the sera of participants with a molecular approach and could match this molecular analysis with the clinical history of seasonal allergic rhinitis.

METHODS

Study cohort

The MAS, a prospective observational birth cohort study, recruited 1314 of 7609 infants born in 1990 on 6 delivery wards in 5 German cities (Berlin, Dusseldorf, Mainz, Freiburg, and Munich). A detailed description of the stratified sampling scheme and study subjects is given elsewhere[17] and in the Methods section in this article's Online Repository at www.jacionline.org. The study was approved by the local ethics committee. Each parent provided written informed consent at the time of enrollment. Blood samples were collected at 1, 2, 3, 5, 6, 7, 10, and 13 years of age.

Definitions

A standardized parental questionnaire/interview was used yearly (including International Study of Allergy and Asthma in Childhood questions) to assess allergic symptoms. We examined symptoms of allergic rhinitis related to grass pollen, as defined by the presence of "reported sneeze attacks or a runny, blocked, or itchy nose in the absence of common cold"[18] in the months of June, July, or both preceding the follow-up visit. A subject was considered to be affected by grass pollen–related seasonal allergic rhinitis (SARg) if symptoms were reported in 3 or more follow-ups between 3 and 13 years of age or at least 2 of the 3 follow-ups between 11 and 13 years of age. The age at onset of SARg was defined as the age at the first follow-up in which symptoms of SARg had been reported.

IgE assays

All the available serum samples were tested for IgE antibodies against the extracts of *P pratense* with the ImmunoCAP Fluorescence Enzyme Immuno-Assay (Thermo Fisher Scientific, Uppsala, Sweden). Results were expressed in kilounits per liter (detection range, 0.35-100 kU_A/L). Sera with a concentration of specific IgE antibodies greater than 80% of the upper detection limit of the assay were diluted 1:5 to obtain a precise determination. A result of 0.35 kU_A/L or greater was considered positive. Sera with positive results to the extract of *P pratense* were tested again, if still available, with a microarray assay (Immuno Solid-phase Allergen Chip [ISAC]; Thermo Fisher Scientific, Vienna, Austria) to characterize the molecules recognized by their IgE antibodies. The details of the methods are reported elsewhere and in the Methods section in this article's Online Repository.[19] In its version containing 103 molecules, each one arrayed in triplicates, the ISAC test includes 8 molecules of *P pratense* (rPhl p 1, rPhl p 2, nPhl p 4, rPhl p 5 b, rPhl p 6, rPhl p 7, rPhl p 11, and rPhl p 12). A result of low, middle, or high positivity was considered positive.

TABLE I. Characteristics of the population

Variable	Study population	Excluded	*P* value*
No. of subjects	820	494	
Male sex (%)	427/820 (52.1)	257/494 (52.0)	.99
Parental history of allergy (%)	443/816 (54.3)	237/487 (48.7)	<.05
German nationality	764/800 (95.5)	435/469 (92.8)	.04
Older siblings	344/820 (42.0)	194/494 (39.7)	.48
Parental education (≥12 y [%])	445/794 (56.0)	218/478 (45.6)	<.001
Breast-feeding (up to 6 mo)	622/813 (76.5)	271/479 (56.6)	<.001
Mother smoking (at child's age of 5 y)	274/820 (33.4)	141/285 (49.5)	<.001
SARg	177/820 (21.6)	NA	
Age at onset (y), median (interquartile range)	7 (5-9)		
Incidence rate (% [95% CI])	2.3 (1.9-2.6)		

NA, Not applicable.
**P* values are for comparison between subjects of the MAS cohort included and excluded from the study (χ^2 test).

Statistics

The data were analyzed by 2 of us (P.M.M. and V.P.). The data from sera obtained after the initiation of grass pollen SIT (see also the Methods section in this article's Online Repository) were excluded from any statistical analysis. The Shapiro-Wilk test was used to evaluate normal distribution of quantitative variables. The average concentration of IgE to *P pratense* extract in positive sera was calculated as the geometric mean value. Comparisons between participants included and excluded from the analysis were examined by using χ^2 tests. Frequencies for SARg and each *P pratense* molecule were calculated. In survival analyses patients were censored if they had not experienced the end point of interest at the end of the follow-up period. Kaplan-Meier estimates of overall survival time were compared by using the log-rank test; 25% SARg-free time was used as one of our ranking systems of *P pratense* molecular order. Two additional ranking system orders were based on average yearly incidence and on the appearance order of each molecule. Because the length of the follow-up period differed among the patients, all analyses taken into account comprised 5 years before and 5 years after the onset at maximum. Multilevel models were used for multiple values of the same patients during the follow-up. Multilevel mixed-effects linear regression (xtmixed command) and multilevel mixed-effects Poisson regression (xtmepoisson command) were used to evaluate the effects of delay from onset corrected for age at onset on logarithmic transformation of IgE to *P pratense* and the number of *P pratense* molecules, respectively. Separately, models for relationships before and after onset were provided. Multilevel mixed-effects logistic regression was used to evaluate the risk of SARg in the next 3 years for IgE-positive subjects compared with IgE-negative subjects corrected by age. Three different models were provided to evaluate other possible confounding or risk factors. For all multilevel analysis, random effects are estimated by using a multiple of identity matrix (identity option).