IgG and IgG_4 to 91 allergenic molecules in early childhood by route of exposure and current and future IgE sensitization: Results from the Multicentre Allergy Study birth cohort

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Background: Studies of a limited number of allergens suggested that nonsensitized children produce IgG responses mainly to foodborne allergens, whereas IgE-sensitized children also produce strong IgG responses to the respective airborne molecules.
Objective: We sought to systematically test the hypothesis that both the route of exposure and IgE sensitization affect IgG responses to a broad array of allergenic molecules in early childhood.
Methods: We examined sera of 148 children participating in the Multicentre Allergy Study, a birth cohort born in 1990. IgG to 91 molecules of 42 sources were tested with the ImmunoCAP Solid-Phase Allergen Chip (ISAC; TFS, Uppsala, Sweden). IgE sensitization at age 2 and 7 years was defined by IgE levels of 0.35 kU_A/L or greater to 1 or more of 8 or 9 extracts from common allergenic sources, respectively.
Results: The prevalence and geometric mean levels of IgG to allergenic molecules in nonsensitized children were lower at age 2 years than in IgE-sensitized children, and they were extremely heterogeneous: highest for animal food (87% ± 13%; 61 ISAC Standardized Units [ISU], [95% CI, 52.5-71.5 ISU]), intermediate for vegetable food (48% ± 27%; 13 ISU [95% CI, 11.2-16.1 ISU]), and lowest for airborne allergens (24% ± 20%; 3 ISU [95% CI, 2.4-3.4 ISU]; *P* for trend < .001 [for percentages], *P* for trend < .001 [for levels]). IgG_4 antibodies were infrequent (<5%) and contributed poorly (<3%) to overall IgG antibody levels. IgG responses at age 2 years were slightly more frequent and stronger among children with than in those without IgE sensitization at age 7 years.
Conclusion: The children's repertoire of IgG antibodies at 2 years of age to a broad array of animal foodborne, vegetable foodborne, and airborne allergenic molecules is profoundly dependent on the route of allergen exposure and the child's IgE sensitization status and only marginally involves the IgG_4 isotype. (J Allergy Clin Immunol 2016;138:1426-33.)

Key words: *Allergenic molecules, IgE sensitization, birth cohort, childhood, IgE, IgG, IgG_4, microarray, prediction*

The role played by IgG antibodies in allergic reactions, unlike that of IgE,[1,2] is still debated. IgG antibodies are considered to be nonpathogenic[3] or even protective,[4] and because they occur within the course of and after immunotherapy,[5] they are labeled as blocking antibodies.[6] However, allergen-specific IgG might also play a role in anaphylactic events[7] and could develop in parallel with or even preceding IgE antibodies.[8] IgG has been seen generally as a marker of natural exposure.[9] The IgG response against allergens starts in general as an IgG_1-dominated response and, on continued exposure, can gradually shift toward an IgG_4-dominated response,[10] a phenomenon also characterizing allergen-specific immunotherapy.[11]

The IgG responses to animal foods are frequent in childhood,[12] whereas little epidemiologic information is available about IgG responses to inhalant allergens in early childhood. The effect of allergic predisposition on allergen-specific IgG responses has been investigated only in individual studies, each focusing on 1 or a limited variety of allergenic sources, such as mites,[13] pollen,[14] pets,[15] and a few foods.[16-18] Overall, these studies suggested that allergen-specific IgG responses are stronger or more frequent in IgE-sensitized than nonsensitized subjects.[13-19] Also, it has been suggested that IgG responses in early childhood

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Abbreviations used
ISAC: ImmunoCAP Solid-Phase Allergen Chip
ISU: ISAC Standardized Units
MAS: Multicentre Allergy Study
PR-10: Pathogenesis-related protein 10

to certain allergenic molecules might even predict IgE sensitization later in life.[20,21] Altogether, information on the effect of the route of exposure and allergic predisposition on allergen-specific IgG responses is sparse, and no study in early childhood has simultaneously investigated the IgG repertoire to a wide range of molecules.

Before 2000, most studies of allergen-specific IgG responses were performed with assays based on allergenic extracts. These tests were hampered by a high nonspecific background signal caused by extract contaminants recognized by the normal human IgG repertoire. The availability of purified native or recombinant allergenic molecules printed on microarrays has recently allowed systematic study of the serum antibody repertoire with a few microliters of serum only.[8,15] Using this microarray approach, we have recently compared IgG and IgE responses to a panel of 7 foodborne and 3 airborne pathogenesis-related protein 10 (PR-10) molecules in birch-sensitized and nonsensitized children of the Multicentre Allergy Study (MAS) cohort.[8] This study allowed discrimination of a default weak IgG response driven by foodborne PR-10 molecules and detectable in both healthy and birch-sensitized children from a (pre-)atopic strong IgG response driven by airborne PR-10 molecules and detectable in birch-sensitized children only.[8]

Therefore the present study aimed to test the working hypothesis that IgG responses to environmental noninfectious antigens in infancy are influenced by 2 major determinants: the route of exposure in early childhood to that antigen and the individual predisposition of IgE sensitization. To this end, we examined the IgG responses at 2 years of age against 91 allergenic molecules in a subset of 148 children of the MAS cohort, and we related these responses to the presence of IgE sensitization at 2 and 7 years of age.

METHODS

Study design and setting

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, Düsseldorf, Mainz, Freiburg, and Munich). A detailed description of the stratified sampling scheme and study subjects is presented elsewhere.[22,23] The study was approved by the local ethics committee. Each parent provided written informed consent at the time of enrollment. For the present study, only children whose serum data on IgE sensitization were available at both 2 and 7 years of age and whose serum was still available at 2 years of age were included.

Definition of IgE sensitization

IgE sensitization at 2 or 7 years of age was defined as the presence of specific IgE antibodies in serum (cutoff, ≥0.35 kU_A/L; ImmunoCAP, Thermo Fisher Scientific [TFS], Uppsala, Sweden) to at least 1 of the common allergenic extracts tested in the MAS birth cohort study at age 2 years (milk, egg, soy, wheat, house dust mite, cat, birch, and grass) or 7 years (milk, egg, soy, wheat, house dust mite, cat, dog, birch, and grass), respectively.[24]

Assessment of IgG and IgG_4 antibodies against allergenic molecules

IgG antibodies to native and recombinant allergenic molecules were tested in sera diluted 1:50 with a multiplex microarray approach (ImmunoCAP Solid-Phase Allergen Chip [ISAC] 103 and 112; TFS), according to the manufacturer's instructions. The 91 molecules that were available and comparable in both multiplex assays were taken into account for the present analysis. Results were expressed in ISAC Standardized Units (ISU), with a cutoff point for positivity of 0.1 ISU.[15] IgG_4 antibodies against the same molecules have been tested with ImmunoCAP ISAC 112 (TFS), and the results have been expressed in ISU (cutoff for positivity, ≥0.1 ISU).[15]

Assessment of IgG antibodies against allergenic sources

Results obtained at the molecular level were also used to analyze outcomes at the source level. Molecules belonging to sources with irrelevant exposure in the first 2 years of life in Germany (eg, Brazil nut, pellitory, and olive pollen) were excluded from analyses at the source level. When 2 highly cross-reacting molecules belonging to the same source were tested (eg, Phl p 5 and Phl p 6), one of them was excluded (eg, Phl p 6). Highly cross-reacting molecules belonging to different categories with different routes of exposure (eg, PR-10 molecules, profilin, and serum albumin) have also been excluded, with the only exception being Bet v 1, which has been used as a marker of sensitization to birch pollen. Overall, analyses at the source level included 35 molecules and 16 sources (see Fig E1 in this article's Online Repository at www.jacionline.org). The presence of serum IgG to a given molecule was defined by a level equal to or greater than 0.1 ISU, as previously described.[8,15] IgG responses to a given allergenic source were defined by the presence of IgG antibodies against at least 1 of its examined species-specific molecules. The IgG concentration to each allergenic source was calculated by summing up the positive levels of IgG antibodies to each of the non–cross-reacting molecules belonging to that allergenic source.

Statistical methods

Comparison of prevalence values. Comparisons of the frequency of general characteristics between participants included and excluded from the present analysis were examined by using χ^2 tests. Comparisons of the general characteristics and prevalence of IgG responses to allergenic molecules and sources between IgE-sensitized and nonsensitized participants at 2 or 7 years of age were examined by using χ^2 test or Fisher exact tests, when required.

Comparison of levels of IgG to allergenic sources. Geometric mean levels of positive IgG responses to allergenic sources were calculated within the subpopulations of IgE-sensitized and nonsensitized subjects and expressed in ISU. A *t* test was used to compare the logarithmic average values of IgG levels to allergenic sources of sensitized versus nonsensitized children at 2 and 7 years of age.

Comparison of prevalence of IgG responses to the 3 allergenic categories. The mean prevalence (and its SD) of IgG responses to the allergenic molecules within the 3 categories of animal foodborne, vegetable foodborne, and airborne allergens were calculated in IgE-sensitized and nonsensitized children at 2 years of age. These mean values were then compared, and a *P* value was estimated by using a mixed logistic model in which subjects accounted for the random effects. A mixed logistic regression was also used to evaluate the trend of IgG prevalence across the 3 categories of exposure. In this last analysis the 3 categories were hierarchically ordered according to the proportion and dose of ingested allergen: animal foodborne (ingested early in infancy and at high frequency and dose), vegetable foodborne (ingested later in infancy or at a lower frequency and dose), and airborne (inhaled at minimal doses).

Comparison of levels of IgG responses to the 3 allergenic categories. The overall concentration of IgG antibodies to animal foodborne, vegetable foodborne, and airborne allergens were calculated in each participant by summing up the positive levels of IgG