

Development of immunoglobulin G subclass antibodies to ovalbumin, birch and cat during the first eight years of life in atopic and non-atopic children

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Immune responses to allergens in young children include both Th1- and Th2-like immunity, which may regulate the secretion of immunoglobulin (Ig) G subclass antibodies differently. The time, route and level of exposure to an allergen may be decisive with regard to whether sensitization or tolerance will ensue. To study this, we investigated the development of IgG subclass antibodies to food and inhalant allergens during childhood. The study group comprised a cohort of 96 children participating in a prospective study. IgG subclass antibodies to ovalbumin, Bet v 1 and cat dander were analyzed at birth, 6 and 18 months and 8 years by ELISA. IgG₁ and IgG₃ subclass antibodies to ovalbumin peaked at 18 months and then declined up to 8 years of age, whereas antibodies to the inhalant perennial allergen cat, but not the inhalant seasonal allergen birch, increased with age. Exposure to cat and birch tended to be associated with high antibody levels to those allergens, whereas antibody levels to ovalbumin were not related to exposure to egg. The presence of positive skin prick tests and circulating IgE antibodies correlated with high levels of IgG subclass antibody responses to the allergens. Atopic symptoms were associated with high levels of IgG subclass, particularly IgG₄, antibodies to the allergens. The difference in antibody levels between atopic and non-atopic children was most marked at 6 months for ovalbumin. For the seasonal inhalant allergen birch, the difference was apparent from 18 months, whereas a difference in antibody levels to the perennial inhalant allergen cat was already present at 6 months. In conclusion, IgG subclass antibodies to food allergens peak in early infancy and are then down-regulated, whereas antibodies to the inhalant perennial allergen cat, but not the inhalant seasonal allergen birch, increase with age. Atopy is associated with high levels of IgG subclass, particularly IgG₄, antibodies to allergens, supporting a deviation of the immune system towards Th2-like responses in atopic children.

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Cross-sectional (1,2) and prospective (3) studies strongly indicate that exposure to allergens early in life may have an impact on the incidence of allergy many years later. In experimental animal models, the primary immune responses to aller-

gens delivered to the respiratory mucosa are characterized by a cross-regulation between competing T-helper 1 (Th1) cell populations secreting interferon- γ (IFN- γ) and Th2 cell populations producing interleukin-4 (IL-4), the

latter thereby stimulating immunoglobulin (Ig) E production (4). In adults, the allergen-responder phenotype is determined by a balance between Th1 and Th2 memory cells (5). It has been suggested that this balance is set during early life by a process of allergen-driven Th1/Th2 selection (6). After initial inhalant allergen encounters, biphasic (sometimes see-sawing) serum IgE responses are seen during early childhood (7), supporting this hypothesis. In non-atopic children, the IgE responses are eventually down-regulated, whereas they reach higher levels and persist in atopic children (7). IgE antibody responsiveness to food allergens, however, may be down-regulated efficiently in both atopic and non-atopic children, after initially higher levels in the former (7). The doses of food allergens may be sufficient for induction of anergy (8).

In mice, in which the Th1/Th2 concept was originally described (reviewed in (9)), Th1-like immunity is associated with antibody production of the complement- and opsonization-activating IgG subclasses IgG_{2a} and IgG_{2b} aiding in phagocyte-dependent defense, while Th2 responses are associated with immunity to extracellular parasites and IgE and IgG₁ subclass responses. In humans, IgG₄ probably corresponds to murine IgG₁ and is Th2-dependent, while the IgG₁ and IgG₃ subclasses are complement-activating and IgG₂ antibodies are mostly directed to carbohydrate antigens (10). *In vitro* studies on mitogen-stimulated human lymphocytes have shown that IL-4 stimulates and IFN- γ inhibits IgE and IgG₄ synthesis (11,12), whereas IgG₂ may be Th1-dependent (13–15). Studies on immune responses to parasites in man also support the theory that Th2-like immunity is associated with IgG₄ antibody production, whereas patients producing antigen-specific IFN- γ responses show IgG₁, IgG₂ and IgG₃ antibodies to the helminths (16,17). We recently observed that birch-induced IL-4 mRNA expression was associated with IgG₄ antibody production to that allergen, while IFN- γ responses correlated to IgG₁ antibody levels (Jenmalm MC et al., *Pediatr Allergy Immunol*, in press). IgG antibody responses to allergens in adults are largely restricted to IgG₁ and IgG₄ isotypes (10), high-level IgG₂ and IgG₃ responses perhaps requiring potent adjuvant stimulation. In support of the association of Th2-like immunity to allergens in allergic adults, atopy is often most clearly associated with high levels of IgG₄ antibodies (18,19), and the presence of IgG₁ antibodies in non-atopic individuals may illustrate immune recognition in the absence of Th2

responsiveness. However, IgG₁ antibody levels are also often enhanced in atopic, as compared to non-atopic individuals, and this could be caused by elevated immunoglobulin synthesis inducing efficiency of human Th2, as compared to Th1 cells, which are limited by their cytolytic activity (20).

Until recently, only IgG₁ antibodies to the inhalant allergen house dust mite were demonstrable in young children (21,22). We have developed sensitive methodology enabling the measurement of all the IgG subclass antibodies to rBet v 1 in early infancy (23). The aim of this study was to further investigate the proposed Th1/Th2 cell selection process to food and inhalant allergens in humans. Thus, the development of the typical Th2-induced IgG₄ subclass, as well as the differentially regulated IgG₁, IgG₂ and IgG₃ subclass, antibodies to the food allergen ovalbumin, the seasonal inhalant allergen birch and the perennial allergen cat was investigated in relation to the development of atopic disease, sensitization and allergen exposure in a cohort of 96 children who were prospectively followed from birth to 8 years of age.

Materials and methods

Study group

From November 1987 to January 1989, pregnant women and their families were asked by the midwives of the maternity clinics in the Linköping area, County of Östergötland, Sweden, to participate in the study. Participation was accepted by 96 families, 38 with an atopic mother, 22 with an atopic father and 36 with no parental atopic history. The atopic status of the parents was established by a typical clinical history, e.g. allergic rhinoconjunctivitis, allergic asthma or flexural itchy dermatitis. Presence of IgE antibodies to inhalant allergens was demonstrated in all but five of the atopic mothers and all but two of the atopic fathers by Phadiatop (Pharmacia, Uppsala, Sweden) or skin prick tests. All but four of the 96 children were born at term. Umbilical cord blood was collected at birth and the sera were frozen and stored at -20°C .

Venous blood samples were drawn and skin prick tests performed to common allergens at 6 (range 5–7) and 18 (range 17–21) months of age and at 8 years (range 7 years and 1 month to 8 years and 6 months). All children attended the six-month examination. Six of the 96 children were not available for the 18-month examination. Seven children, of whom one child was absent also from the 18-month examination, did

not attend at the eight-year examination. For technical reasons, blood samples were not available from all examinations, leaving 78 cord serum samples, 93 samples from the six-month follow-up, 86 samples from the 18-month follow-up and 85 samples from the eight-year follow-up.

The families also completed questionnaires at 3, 6, 9 and 12 months and then yearly up to 8 years, regarding symptoms of allergy, feeding and medication of the child, infections, smoking habits of the family and presence of pets at home. Except for four, five and one missing questionnaire at 1, 7 and 8 years of age, respectively, all the questionnaires were returned. The questionnaires were validated by a clinical examination and a history of allergic manifestations at 6 and 18 months of age and at 8 years. Atopic symptoms were reported in the questionnaires for all children who were judged as atopic at the physical examination. The cumulative incidence was based on the questionnaire responses.

Atopic dermatitis was defined as pruritic, chronic or chronically relapsing non-infectious dermatitis with typical features and distribution, as suggested by Hanifin and Rajka (24). Asthma was defined as three or more episodes of bronchial obstruction, at least once verified by a physician. Allergic rhinoconjunctivitis was defined as rhinitis and conjunctivitis appearing at least twice after exposure to a particular allergen and not related to infection. Urticaria was defined as allergic if it appeared at least twice within one hour after exposure to a particular allergen. Probable atopy was used when some, but not all of the diagnostic features described above were present. Furthermore, the children were also divided into stricter groups, i.e. sensitized symptomatic children (with positive skin prick tests and/or circulating IgE antibodies to allergens at least once) and non-symptomatic children.

Skin prick tests were performed in duplicate on the volar aspects of the forearms with egg white extract (Dome/Hollister-Stier/Bayer, Spokane, Washington State, USA) and fresh skimmed cow's milk (lipid concentration 0.5%) at 6 and 18 months. At 8 years, the children were tested with standardized birch, timothy and cat extracts (Soluprick[®], ALK, Hørsholm, Denmark). Histamine hydrochloride (10 mg/ml) was used as a positive control and albumin diluent was included as a negative control. The test was regarded as positive if the mean diameter was ≥ 3 mm.

Conventional ELISA for the detection of IgG, IgG₁ and IgG₄ antibodies to ovalbumin, rBet v 1 and cat dander

The determination of IgG subclass antibodies to ovalbumin, rBet v 1 and cat dander was performed as described in detail elsewhere (23). Briefly, Costar 3590 microtitre plates (Life Technologies AB, Täby, Sweden) were coated with 25 µg/ml ovalbumin (grade VI, Sigma-Aldrich, Stockholm, Sweden), 1 µg/ml rBet v 1 (kindly provided by Professor D. Kraft, Institute of General and Experimental Pathology, AKH, University of Vienna, Austria) or 10 µg/ml cat dander extract (purified as previously described (25) and a kind gift from Dr C. Schou, ALK, Hørsholm, Denmark) in phosphate-buffered saline (PBS). Free binding sites were blocked with human serum albumin (HSA) (Chemicon, Stockholm, Sweden). Human serum samples or buffer without added serum (blanks) were added to duplicate wells, and then monoclonal antibodies to the human IgG subclasses (IgG (clone HP6064), IgG₁ (clone HP6069) and IgG₄ (clone HP6011), Bio Zac AB, Järfälla, Sweden) were added. Alkaline phosphatase-conjugated rabbit anti-mouse IgG (Sigma-Aldrich, Stockholm, Sweden) was applied, and p-nitrophenyl phosphate (pNPP) (Sigma-Aldrich, Stockholm, Sweden) used as substrate. The optical density (OD) was read at 405 nm. Values were expressed as arbitrary units (AU) deduced from the ODs of the reference serum curve, after subtracting the blanks. The reference sera, which were calibrated according to a semi-quantitative method as described previously (23), were from an individual with known high antibody titres to ovalbumin and from patients undergoing birch and cat immunotherapy, respectively. The titres in the reference sera were, for antibodies to ovalbumin: IgG₁ 2789 AU, IgG₂ 296 AU, IgG₃ 56 AU and IgG₄ 4884 AU; for antibodies to rBet v 1: IgG₁ 413 AU, IgG₂ 115 AU, IgG₃ 1.0 AU and IgG₄ 1845 AU; for antibodies to cat dander extract: IgG₁ 366 AU, IgG₂ 139 AU, IgG₃ 6.6 AU and IgG₄ 868 AU.

Enzyme-amplified ELISA for the detection of IgG₂, IgG₃ and IgG₄ antibodies to ovalbumin, rBet v 1 and cat dander

A more sensitive enzyme-amplified ELISA was employed for the measurement of IgG₂ and IgG₃ antibodies and for samples where IgG₄ antibodies were not measurable with the conventional ELISA. Coating, blocking and addition of human serum samples, monoclonal antibodies to IgG₂ (clone HP6014), IgG₃ (clone HP6050) and IgG₄, and alkaline phosphatase-conjugated rabbit anti-mouse IgG were as described above. For the substrate reaction, AMPAK kits

Table 1. Number of children with positive skin prick tests to egg (at 6 and 18 months), cat and birch (at 8 years) in relation to the presence of circulating IgE antibodies to the allergens.

Allergen	Age	Circulating IgE detected	Skin prick test	
			Positive	Negative
Egg	6/18 months	Yes	7	2
		No	5	78
Cat	18 months	Yes	5	5
		No	1	65
Cat	8 years	Yes	7	2
		No	0	76
Birch	18 months	Yes	1	6
		No	0	69
Birch	8 years	Yes	7	1
		No	2	75

(Dakopatts AB, Älvsjö, Sweden) were used. NADPH in diethanolamine buffer was allowed to react with the phosphatase for 20 min followed by 10 min incubation with amplifier solution. OD was measured at 492 nm. Values were expressed as arbitrary units (AU) deduced from the ODs of the reference serum curve, after subtracting the blanks. Generally, intra-assay coefficients of variation were < 5% and inter-assay coefficients of variation were < 15% in the conventional and enzyme-amplified ELISAs.

Determination of IgE antibodies to birch, cat and egg white

Levels of IgE antibodies to birch, cat and egg white were analyzed with the high-sensitivity protocol (incubation of serum samples overnight) of Magic LiteTM, a commercial chemoluminometric method, according to the recommendations of the manufacturer (ALK, Hørsholm, Denmark). At 6 months, the sera were tested for the presence of IgE antibodies to ovalbumin only, at 18 months to all allergens and at 8 years to the inhalant allergens only.

Statistics

As the levels of IgG subclass antibodies to the allergens were not normally distributed even after logarithmic transformation, non-parametric tests, corrected for ties, were used. Comparisons between unpaired groups were analyzed with the Mann-Whitney *U* test, comparisons between paired groups with the Wilcoxon signed rank test and correlations with Spearman's rank order correlation coefficient test. A probability level of < 5% was considered to be statistically significant. Calculations were performed with a statistical package StatView 4.5 for Macintosh (Abacus Concepts Inc, Berkeley, California, USA).

Ethics

The study was approved by the Regional Ethics Committee for Human Research at the University Hospital of Linköping.

Results

Clinical diagnosis and allergic sensitization

The cumulative incidence of atopic disease during the eight-year follow-up period was 38% (37 of 96). Fifty children (52%) did not develop any allergic manifestations and nine (9%) were classified as probably atopic. The corresponding rates for the various clinical features of atopic disease were 26% atopic dermatitis, 15% asthma, 3% allergic rhino-conjunctivitis and 2% urticaria. Five children had both atopic dermatitis and asthma, one both allergic rhino-conjunctivitis and urticaria and one both eczema and allergic rhino-conjunctivitis. In all, 24 children, of whom 15 had atopic symptoms, seven had no symptoms and two had probable symptoms, were sensitized, i.e. had detectable IgE antibodies and/or positive skin prick tests to allergens on at least one occasion. Ten of the 25 children with atopic dermatitis, eight of the 14 asthmatic children, all the children with allergic rhinoconjunctivitis and one of the children with urticaria were sensitized. The number of children with positive skin prick tests to egg, cat and birch at the different ages in relation to the presence of circulating IgE antibodies to the allergens are shown in Table 1.

Kinetics of IgG subclass antibodies

IgG antibodies to the allergens were mostly of the IgG₁ subclass (Fig. 1a–c), except for ovalbumin at 8 years, at which time the IgG₄ component was larger.

The levels of the IgG subclass antibodies to ovalbumin, cat and rBet v 1 generally decreased from birth to 6 months and then increased from 6 to 18 months (Fig. 1). From 18 months to 8 years, the levels of all IgG subclass antibodies to cat, and IgG₂ and IgG₄ antibody levels to ovalbumin continued to increase, while the levels of the major IgG subclass antibody, i.e. IgG₁, to ovalbumin and rBet v 1 decreased, as well as IgG₄ antibody levels to rBet v 1. The levels of the IgG subclass antibodies to ovalbumin and cat correlated at different ages (Table 2). This was not the case for antibodies to rBet v 1, except for IgG₄ at 18 months and 8 years ($r=0.30$, $p < 0.01$).

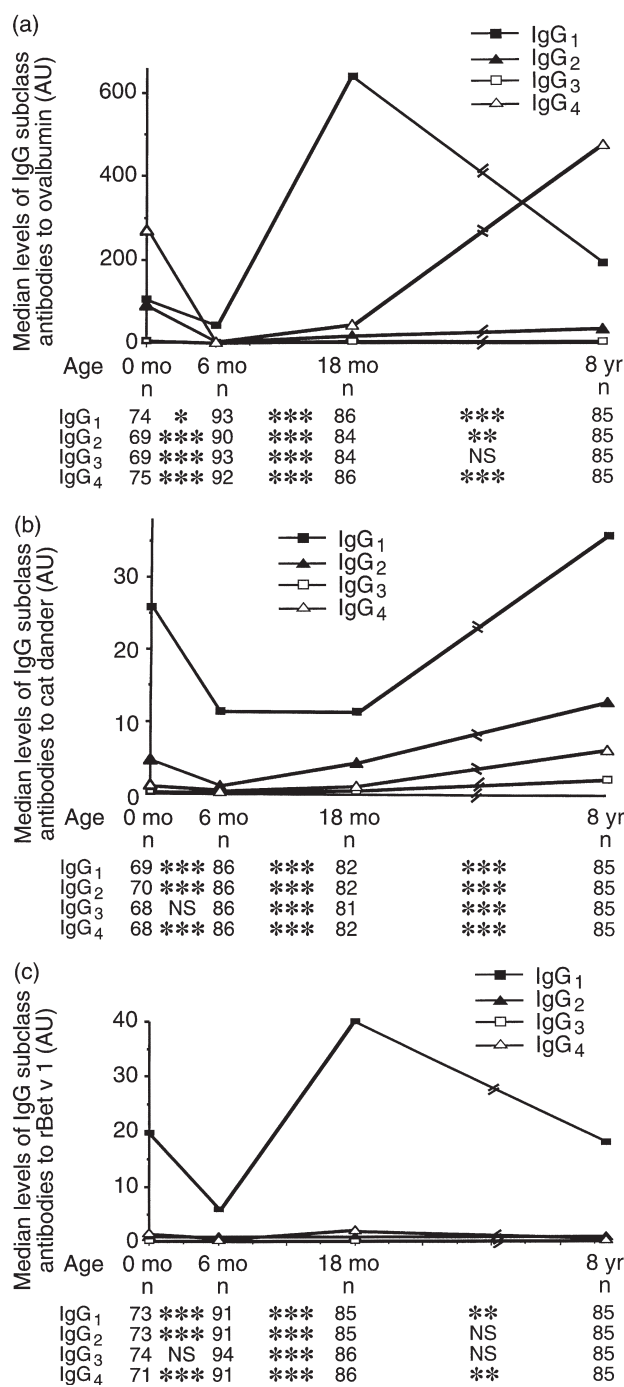


Fig. 1. Median levels of IgG subclass antibodies to ovalbumin (a), cat (b) and rBet v 1 (c) from birth to 8 years of age. The p values for changes with age are indicated for each subclass. n.s., not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Differences in the levels at 0 and 6 months were analyzed with the Wilcoxon signed rank test, whereas changes from 6 to 18 months and 18 months to 8 years were analyzed with the Mann-Whitney U test.

Antibody levels in relation to allergen exposure

There was no correlation between age of exposure to egg and antibody levels to ovalbumin. Thus, egg had not yet been introduced into the diet of

Table 2. Correlation coefficients between the levels of IgG subclass antibodies to ovalbumin and cat at 6 and 18 months and at 8 years.

	IgG	IgG ₁	IgG ₂	IgG ₃	IgG ₄
Ovalbumin					
6–18 months	0.27*	0.23*	0.31*	–	0.32*
6 months – 8 years	0.27*	–	0.37**	–	0.47***
18 months – 8 years	0.71***	0.60***	0.58***	0.63***	0.62***
Cat					
6–18 months	0.44**	0.50**	–	–	–
6 months – 8 years	0.31**	0.31**	–	–	–
18 months – 8 years	0.52***	0.44***	0.44***	0.36***	0.45***

Only statistically significant relationships are shown (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

several of the egg-sensitized children with high IgG and IgE antibody levels at 6 months.

The 25 children who had been exposed to a cat at home had higher levels of IgG and IgG₁ antibodies to cat at 6 ($p < 0.001$) and 18 months ($p < 0.01$), with similar trends for IgG₂ ($p = 0.09$ at both ages), but not IgG₃ and IgG₄ antibodies. At 8 years, there were similar non-significant trends for IgG ($p = 0.13$) and IgG₁ ($p = 0.12$) antibodies.

Children with both atopic disease and detectable IgE antibodies and/or positive skin prick tests to allergens on at least one occasion had higher IgG antibody levels to rBet v 1 ($p < 0.05$) at 6 months, with a similar trend at 8 years, if the blood sample was drawn during the spring or summer, but not later. This was not the case in non-atopic children.

Antibody levels in relation to sensitization

Children with positive skin prick tests to egg had higher levels of IgG and all IgG subclass antibodies to ovalbumin at 6 months ($p < 0.001$ to $p < 0.01$), and of IgG, IgG₁ and IgG₃ at 18 months and 8 years ($p < 0.01$ to $p < 0.05$), as compared to those with negative tests. The results were largely similar when comparing children with and without circulating IgE antibodies to egg, except for more pronounced differences for IgG₄. The IgE and IgG subclass antibody levels to ovalbumin correlated ($\rho = 0.25$ – 0.43 , $p < 0.001$ to $p < 0.05$).

Skin sensitivity to cat was associated with high levels of IgG₁, IgG₃ and IgG₄ antibodies to cat at 6 months ($p < 0.05$ to $p < 0.01$), of IgG and IgG₁ at 18 months ($p < 0.05$) and of IgG, IgG₁ and IgG₄ at 8 years of age ($p < 0.05$). The results were largely similar when comparing children with and without detectable IgE antibodies to cat. There were modest correlations between IgE antibody levels to cat at 18 months and IgG and

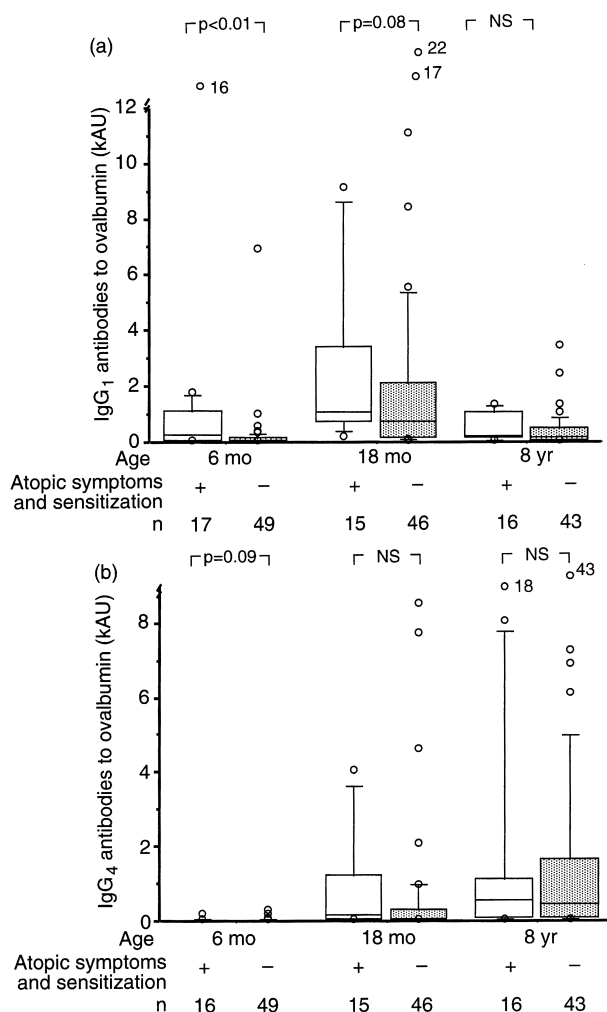


Fig. 2. Levels of IgG₁ (a) and IgG₄ (b) antibodies to ovalbumin at 6 and 18 months and 8 years of age, in children with and without atopic symptoms and sensitization. The 10th, 25th, 50th, 75th and 90th percentiles are indicated, as well as outliers. At 6 months of age, 13 of 16 atopic children had detectable IgG₄ antibodies, compared to 21 of 49 non-atopic children.

IgG subclass antibody levels to cat at all ages ($\rho=0.27-0.44$, $p < 0.001$ to $p < 0.05$). The IgE antibody levels at 8 years correlated with IgG, IgG₁ and IgG₄ antibody levels at all ages ($\rho=0.23-0.31$, $p < 0.01$ to $p < 0.05$).

Children with positive skin prick tests to birch had higher levels of IgG and all IgG subclass antibodies to rBet v 1 at 8 years ($p < 0.01$ to $p < 0.001$), but not at 6 and 18 months, compared to those with negative tests. Similar results were obtained comparing children with and without circulating IgE antibodies to birch. The IgE antibody levels to birch at 8 years correlated with IgG and IgG subclass antibody levels at that age, but not at 6 and 18 months ($\rho=0.22$, $p < 0.05$ for IgG₃; $\rho=0.41-0.55$, $p < 0.001$ for the other IgG subclass antibodies).

IgG subclass antibodies in relation to atopic symptoms

The levels of IgG, IgG₁ and IgG₃ antibodies to ovalbumin were higher at 6 months in children who developed atopic symptoms during the first eight years of life than in the non-atopic children ($p < 0.05$). These differences were no longer apparent at 18 months and 8 years. When children who had both atopic symptoms and were sensitized (positive skin prick tests and/or circulating IgE antibodies to allergens at least once) were compared with non-atopic children, the differences were even greater, including trends for IgG₄ antibodies at 6 months ($p=0.09$) and IgG₁ antibodies at 18 months ($p=0.08$) (Fig. 2).

Similarly the levels of IgG₄ antibodies to cat were higher at 6 months in the children who developed atopic symptoms than in the non-atopic children ($p < 0.01$). The levels were even higher in the sensitized symptomatic children, including higher levels of IgG₄ antibodies at 18 months ($p < 0.05$) (Fig. 3). In order to compensate for possible differences in exposure to cat, the ratios between IgG subclass antibody levels were calculated. Sensitized children with atopic symptoms had a lower IgG₂/IgG₄ antibody ratio at 18 months ($p < 0.01$) and 8 years ($p < 0.05$) than the non-atopic children.

Children who developed atopic symptoms also had higher levels of IgG, IgG₁ and IgG₂ antibodies to rBet v 1 at 18 months ($p < 0.05$) and IgG₄ antibodies at 8 years ($p < 0.05$), as compared to non-atopic children. Again, when sensitized atopic children were compared with the non-atopic children, the differences were more pronounced (Fig. 4). IgG₁ and IgG₄ antibody levels were higher at 18 months ($p < 0.01$) and IgG, IgG₁ and IgG₂ ($p < 0.05$) as well as IgG₄ ($p < 0.001$) were higher at 8 years. Sensitized asthmatic children had higher levels of IgG₄ antibodies than the non-atopic children at 18 months ($p < 0.05$) and 8 years ($p < 0.01$). Interestingly, non-sensitized asthmatic children had higher levels of IgG₂ antibodies to rBet v 1 than the non-atopic children at 18 months ($p < 0.001$). The three children with allergic rhinoconjunctivitis had higher levels of IgG, IgG₁, IgG₂ ($p < 0.05$) and IgG₄ ($p < 0.001$) antibodies to rBet v 1 than the non-atopic children at 8 years.

Discussion

Our findings on the development of IgG subclass antibody responses to ovalbumin and cat during the first years of life largely agree with previous prospective (26) and cross-sectional (27) studies,

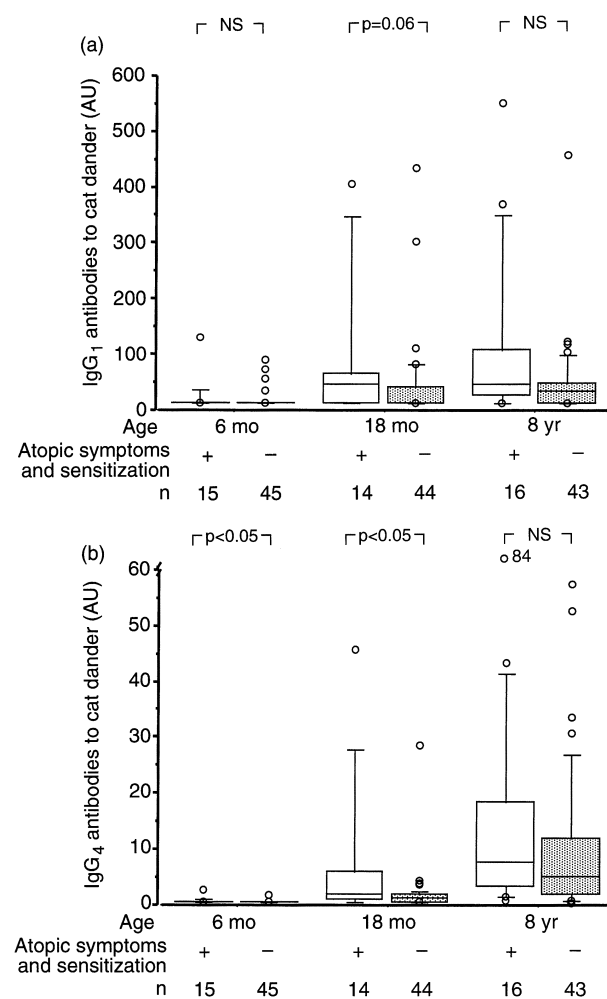


Fig. 3. Levels of IgG₁ (a) and IgG₄ (b) antibodies to cat at 6 and 18 months and 8 years of age, in children with and without atopic symptoms and sensitization. The 10th, 25th, 50th, 75th and 90th percentiles are indicated, as well as outliers. At 6 months of age, three of 15 atopic children had detectable IgG₄ antibodies, compared to one of 45 non-atopic children.

showing that antibody responses to food allergens peak in early childhood, whereas responses to rye grass and mite increase with age. For the inhalant seasonal allergen birch, however, the IgG₁ and IgG₄ antibodies peaked at 18 months. The IgG₁ antibody levels to birch then decreased from 18 months to 8 years in both atopic and non-atopic children, while a down-regulation of IgG₄ antibody levels was seen only in the non-atopic children, similar to what has previously been observed for serum IgE responses during early childhood (7). This may be due to an allergen-driven Th1/Th2 selection (6). In contrast, the levels of IgG₄ antibodies to cat increased up to 8 years of age in both atopic and non-atopic children. This may be due to more chronic exposure to a perennial allergen

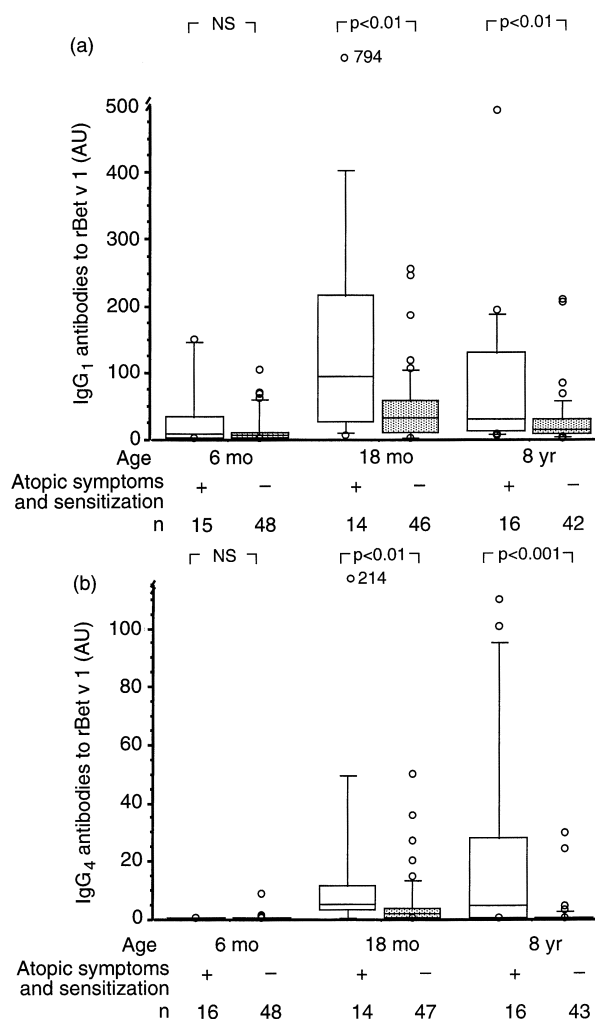


Fig. 4. Levels of IgG₁ (a) and IgG₄ (b) antibodies to rBet v 1 at 6 and 18 months and 8 years of age, in children with and without atopic symptoms and sensitization. The 10th, 25th, 50th, 75th and 90th percentiles are indicated, as well as outliers.

(28). The IgG subclass antibody responses to ovalbumin developed more slowly than the responses to β -lactoglobulin (29), which is in agreement with previous studies (26,30,31), and may reflect the time of their introduction into the diet.

The IgG responses to the inhalant allergens were largely restricted to the IgG₁ subclass early in life, confirming previous reports (21,22), with IgG₄ antibody responses detectable with the conventional ELISA only from 18 months. The antibody levels correlated at different ages, suggesting that children with strong antibody responses early in life continue to be high responders. It was not surprising that this was less clearly seen for the seasonal allergen birch than for ovalbumin and cat.

The antibody response to ovalbumin was not influenced by exposure, which is in marked contrast with the development of IgG subclass antibodies to β -lactoglobulin (29). Egg had not yet been introduced into the diet of several of the egg-sensitized children with high IgG and IgE antibody levels at 6 months, as previously observed (7). Ovalbumin appears to be a more potent allergen than β -lactoglobulin, and low amounts present in the breast milk may stimulate antibody synthesis in the baby (32).

Children with a cat at home had higher IgG and IgG₁ antibody levels to cat at 6 and 18 months, whereas only a trend was observed at 8 years. Probably all children are exposed to cat allergen at school age, since high levels are commonly found in public places (33). The IgG₄ antibody levels to cat were not influenced by the presence of a cat at home, but more with atopic symptoms. Atopic children also tended to have increased antibody levels to birch during spring and summer. We have observed a similar trend has for cord blood IgG antibody levels to birch in the children born to atopic, but not to non-atopic, mothers (23).

Positive skin prick tests and circulating IgE antibodies to allergens were associated with high levels of IgG subclass antibodies to the allergens, as previously reported (26). Furthermore, atopic symptoms were associated with high levels of IgG subclass antibodies to ovalbumin at 6 months of age, but not later, supporting efficient down-regulation of immune responses to food allergens in both atopic and non-atopic children later in life (7). In contrast to IgG₁ and IgE antibody responses, IgG₄ were not down-regulated. This could reflect chronic stimulation (28) and a tighter regulation of IgE, as compared to IgG₄, after Th2-induced switching has occurred (34).

Atopic symptoms were also associated with high IgG₄ antibody levels to cat at 6 and 18 months. The IgG₄ antibody levels were particularly high in the sensitized symptomatic children. It was not possible to correct for the presence of a cat at home, since the groups were too small for meaningful statistical comparisons. Therefore, we compared the ratios between IgG subclass antibody levels. Interestingly, the ratio between IgG₂ and IgG₄ antibody levels differed mostly in relation to atopy. The former subclass may be stimulated by IFN- γ (13,15) as well as by an unknown switch factor secreted from Th1-like cells from adults and neonates (14).

For antibodies to birch, the most marked differences between atopic and non-atopic children were seen in the IgG₄ antibody subclass at 8 years of age. Similar to responses to cat, the

antibody levels were particularly high in the sensitized symptomatic children. In contrast, bronchial obstruction occurring during the first eight years of life in the non-sensitized children was associated with high levels of IgG₂ antibodies to birch at 18 months, but not at 8 years. Wheezing in non-atopic children is often associated with viral respiratory infections, and children with such symptoms generally outgrow their symptoms, while wheezing in atopic children often develops into persistent asthma (35). Viral infections may stimulate Th1-like immunity, which enhances IgG₂ antibody production *in vitro* as discussed above. Therefore, the high IgG₂ antibody levels to birch seen in wheezing children early in life may result from the presence of virally induced Th1-like cytokines in the airways.

These findings may reflect qualitatively different immune responses in atopic and non-atopic children, with a bias towards a production of type 2 cytokines in the former (36,37). Enhanced humoral responsiveness in atopic individuals could be caused by increased immunoglobulin synthesis inducing efficiency of human Th2, as compared to Th1 cells, which are limited by their cytolytic activity (20). As IgG₄ switching is selectively stimulated by Th2-type cytokines (11,12,17), higher antibody levels of this subclass in atopic children were expected. We have previously observed this also to be the case for IgG₄ antibodies to β -lactoglobulin (29). This was not clear for ovalbumin, and may partly be caused by the poorly developed IgG₄ antibody response to this allergen at 6 months, when the differences were seen. It is tempting to speculate that during strong immune stimulation, i.e. in the presence of high levels of pet allergen or allergen recognition in the presence of viral infections, non-atopic children respond to allergens with IgG₂ antibody production, possibly enhanced by Th1-type cytokines (13–15).

In conclusion, IgG subclass antibodies to food allergens peaked in infancy, whereas antibodies to the inhalant perennial allergen cat, but not the inhalant seasonal allergen birch, increased with age. Exposure to cat and birch tended to be associated with high antibody levels to those allergens, whereas antibody levels to ovalbumin were not related to exposure to egg. Atopic symptoms and the presence of positive skin prick tests and circulating IgE antibodies to allergens were associated with high levels of IgG subclass antibody responses to allergens. For the inhalant allergens, the difference was most marked for the IgG₄ subclass. Our findings support a deviation

of the immune system towards Th2-like responses in atopic children.

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