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Ovalbumin-specific immunoglobulin G and subclass responses through the first 5 years of life in relation to duration of egg sensitization and the development of asthma

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Summary

Background Egg sensitization, particularly persistent sensitization, is a risk factor for later asthma. However, little is known about accompanying IgG and subclass responses and how they might relate to asthmatic outcome.

Objective To characterize hen's egg ovalbumin (OVA) IgG and subclass responses through the first 5 years of life in relation to duration of egg sensitization and later asthma.

Subjects and methods The subjects ($n = 46$) formed part of a larger cohort, born to atopic parents, who had been evaluated prospectively for the development of asthma. Egg sensitization was classified as transient (positive egg skin prick test at 1 year only) or persistent (positive skin test for at least 2 years). Plasma OVA IgG, IgG1 and IgG4 concentrations at birth (cord), 6 months, 1 and 5 years of age were measured by ELISA.

Results The kinetics of OVA IgG and IgG1 responses, but not IgG4, differed between egg sensitized and non-egg sensitized (NES) children. Only persistently sensitized children had a rise in OVA IgG1 concentration through the first year of life, and at 1 year of age they had significantly higher OVA IgG and IgG1 than either transiently sensitized or NES children. High OVA IgG1 was associated with later asthma: at 1 year of age, OVA IgG1 greater than 14 500 U predicted asthma with a sensitivity 64% and specificity 74%.

Conclusion OVA IgG and subclass responses relate to the duration of egg sensitization. Measurement of OVA IgG1 concentration in infancy might offer a useful adjunct to identify those at an increased risk of asthma.

Keywords asthma, child, IgE, IgG, IgG subclass, Ovalbumin.

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Introduction

There has been a considerable increase in the prevalence of atopic disease in recent decades, particularly in westernized countries [1, 2]. Epidemiological data have suggested that asthma severity may correlate with disease duration [3]. Therefore, since asthma in older children and adults is commonly atopic in nature and has been preceded by other allergic manifestations in infancy ('Atopic March') [4–6], the early identification of those with an increased asthma risk would offer considerable benefits for individual quality of life and national health resources by enabling timely introduction of appropriate intervention strategies to prevent the onset of disease at an early stage in its development.

IgE sensitization to hen's egg has been recognized as a risk factor for the development of later inhalant sensitization and asthma [7, 8]. However, evidence also implicates egg-specific IgG in the allergic process: for example, high levels of egg-specific IgG, IgG1 and IgG4 have been associated with both allergic sensitization and disease [9, 10]. Furthermore, a dichotomous pattern of egg-specific IgG and all subclasses has been observed between subsequent atopic and non-atopic children by 6 months of age [11], implying that differences in specific IgG responses might predate IgE-mediated disease. The relationship between egg sensitization and later respiratory allergic disease has been qualified further by data from a large cohort study reporting that food sensitization persisting for more than 1 year was associated with a fivefold greater risk of later asthma compared with transient sensitization [12]. However, to date, nothing is known about the specific IgG responses that accompany this relationship. Therefore, the aim of this current work was to investigate retrospectively the development of specific IgG, IgG1 and IgG4 responses to hen's egg ovalbumin (OVA) in relation to duration of egg sensitization and the development of asthma in a group of children at genetic risk of atopic disease who had been followed up from birth to 5 years of age.

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Subjects and methods

Overview

Between January 1991 and February 1994, 158 women attending antenatal clinics at the Princess Anne Hospital, Southampton, UK, were enrolled in a prospective study of the development of allergy in infants with a genetic risk of atopy. At least one parent was allergic and asthmatic, as demonstrated by clinical history, histamine bronchial challenge and allergen skin prick tests (SPTs).

The women were recruited to the study with informed consent. The project was approved by Southampton and South-West Hampshire Local Research Ethics Committee.

Infant follow-up

The babies were clinically evaluated for allergic manifestations at 6 months of age and annually from 1 year until 5 years of age by a respiratory paediatrician (J. O. W.). At each review, from 1 year of age, the infants were also skin prick tested to a panel of common inhalant and dietary allergens – grass pollen, tree pollen, cat dander, dog dander, house dust mite (*Dermatophagoides pteronyssinus*), cow's milk, whole hen's egg and wheat. A weal size $\geqslant$2 mm, in the presence of appropriate responses to negative (saline) and positive (histamine) controls, was regarded as positive. Eczema was diagnosed if there was a history/signs of erythematous, papulo-vesicular, pruritic skin eruptions occurring in an age-dependent typical body distribution [13]. At 5 years of age, the children were classified as asthmatic if they had episodic ($\geqslant$3 episodes) [14], or chronic, wheeze. Non-asthmatic children were those with only cough, transient wheeze or who had no respiratory symptoms.

Blood was collected into heparin at birth (cord), 6 months, 1 and 5 years of age and the plasma prepared and frozen at $-80\,^{\circ}C$.

Study group

From this birth cohort, a subgroup ($n = 46$) were selected for the current analysis. These were children identified as being egg sensitized (ES) (egg SPT positive) at any time point ($n = 21$), who were further categorized into those with transient egg sensitivity (positive egg SPT at 1 year of age only) ($n = 9$) and those with persistent egg sensitivity (positive egg SPT for at least 2 years during 5-year follow-up) ($n = 12$). Control children were those without egg sensitization (non-egg sensitized, NES) and who had blood samples available at the four time points ($n = 25$). They comprised children who had at least one positive SPT to any allergen other than egg at any time point ($n = 14$) (atopic, NES) and a group who never had a positive SPT ($n = 11$) (non atopic, NES) (Fig. 1).

Specific immunoglobulin G and subclass measurement

Plasma ovalbumin IgG (OVA G), IgG1 (OVA G1) and IgG4 (OVA G4) concentrations were measured by ELISA. Briefly, microtitre plates (Nunc MaxiSorp, Roskilde, Denmark) were coated with 100 µg/mL OVA (Grade VII, Sigma-Aldrich, Poole, UK) and stored overnight at 4 °C. Bovine serum albumin (BSA) (Sigma-Aldrich)-coated plates (100 µg/mL) were run in parallel as a control for non-specific binding. After blocking with 3% BSA, a human IgG reference serum (Binding Site, Birmingham, UK), plasma samples (diluted 1 : 50–1 : 100) and buffer without added plasma (blanks) were added to duplicate wells and incubated for 1 h. Bound IgG was detected by horseradish peroxidase (HRP)-conjugated rabbit anti-human IgG (diluted 1 : 6000) (Dako, Ely, Cambridgeshire, UK) and IgG subclasses by HRP-conjugated mouse monoclonal antibodies to IgG1 (diluted 1 : 500) (Clone JDC-1: BD Pharmingen, Cowley, Oxfordshire, UK) and IgG4 (diluted 1 : 1600) (Clone HP6023: Binding Site). Tetramethylbenzidine (BD Pharmingen) was used as the substrate and the absorbance read at 450 nm after stopping the reaction with 1 M sulphuric acid. The absorbances on the BSA-coated plate were subtracted from those on the allergen-coated plate and the value expressed as arbitrary units (AU) by comparison with the standard curve of the reference serum. All samples from an equal number of ES and NES children were run on the same plate, on the same day. Where possible, all measurements for an individual subject were carried out at the same analysis.

Statistics

The data were analysed using non-parametric tests since they were not normally distributed even after log transformation. Measurements that fell below the limit of assay detection

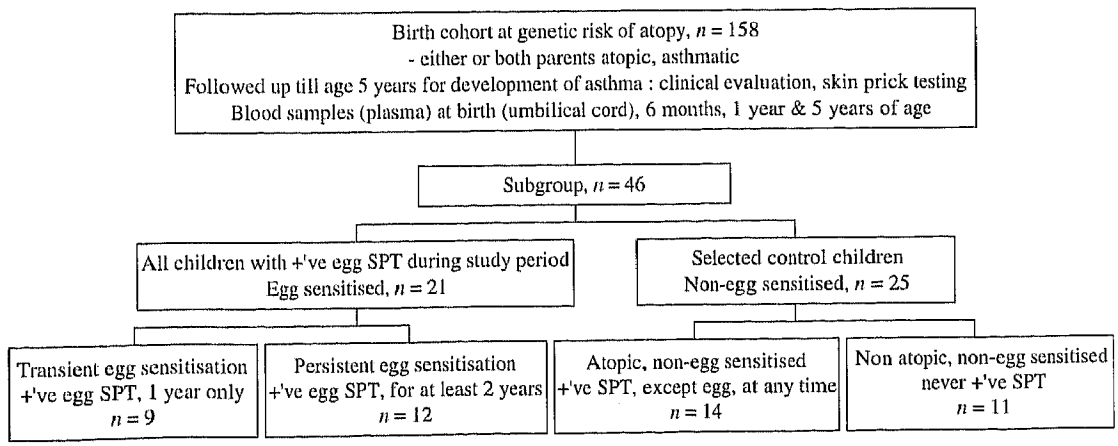


Fig. 1. Consort diagram illustrating subject selection for current analysis.

(Absorbance™ 0.1) were assigned a value of zero and included in the analyses. The Kruskal–Wallis *H*-test was performed before comparisons between groups were made. In order to compare data from different groups, the Mann–Whitney *U*-test was applied and for data relating to the same subjects the Wilcoxon signed-rank test was performed. For the analysis of IgG subclass concentration with regard to asthma outcome, a receiver–operator characteristic (ROC) curve was constructed. This plot of sensitivity (true positive) against one specificity (false positive) enabled selection of a cut-off value of subclass concentration for the diagnosis of asthma. Differences in the number of subjects falling into one or more categories was measured by χ^2; Fisher's exact test was used when more than 20% of the categories had an expected count of less than five.

These analyses demanded multiple comparisons of the study group. However, such measurements had been planned *a priori*. While the non-parametric methods do not offer adjustments for multiple testing, we considered statistical significance when $P \leqslant 0.01$, although we have included *P*-values where significance was just missed. Moreover, the limitations of multiple testing have been recognized in the bio-clinical interpretation of the data.

Calculations were carried out using SPSS for Windows software (version 10.1).

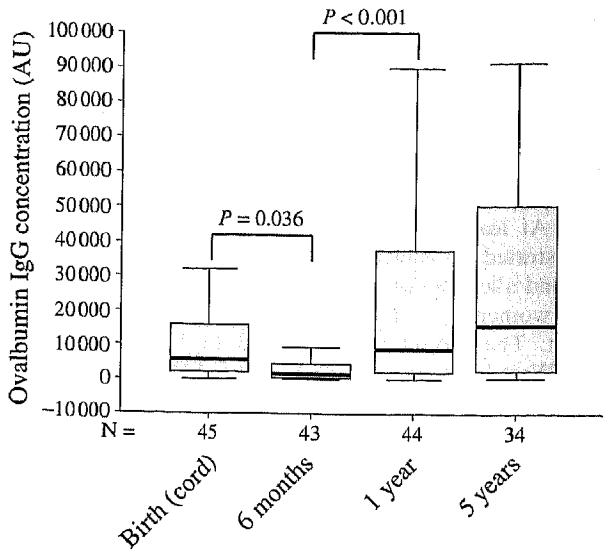


Fig. 2. Concentrations (arbitrary units(AU)) of ovalbumin (OVA)-specific IgG at birth (umbilical cord), 6 months, 1 and 5 years of age within a cohort at genetic risk of atopy. OVA- specific IgG was measured by in-house indirect ELISA. In the plot, the box shows values in the interquartile range between the 25th and 75th percentiles. The central line is the median value and the whiskers extend to the lowest and highest values, excluding outliers. Among the entire group OVA-specific IgG concentration fell between birth and 6 months of age, rising again by 1 year of age.

Results

The respective rates for the detection of OVA IgG and IgG1 were: birth (44/45; 44/45), 6 months (38/43; 41/44) and 1 year (43/44; 42/43). OVA IgG and IgG1 was detected in all samples at 5 years of age. OVA IgG4 was detectable in a smaller proportion of samples, particularly at 6 months and 1 year of age (birth 39/45; 6 months 10/44; 1 year 20/44; 5 years 27/35).

Ovalbumin specific immunoglobulin G and subclass responses in egg sensitized and non-egg sensitized children

Within the study group as a whole, OVA- specific IgG concentration fell slightly between birth and 6 months of age ($P = 0.036$) and rose again by 1 year ($P = < 0.001$), remaining elevated at 5 years of age (Fig. 2). However, analysis of the IgG responses in relation to egg sensitization revealed that this pattern was found predominantly among NES children: within the ES group OVA IgG concentration did not appear to change over the first 6 months of life, rose between 6 months and 1 year ($P = 0.003$) and peaked at 1 year of age. At 6 months and 1 year of age ES children had higher OVA IgG concentration than had NES children, although not reaching statistical significance ($P = 0.028$, 0.038, respectively) (Fig. 3a).

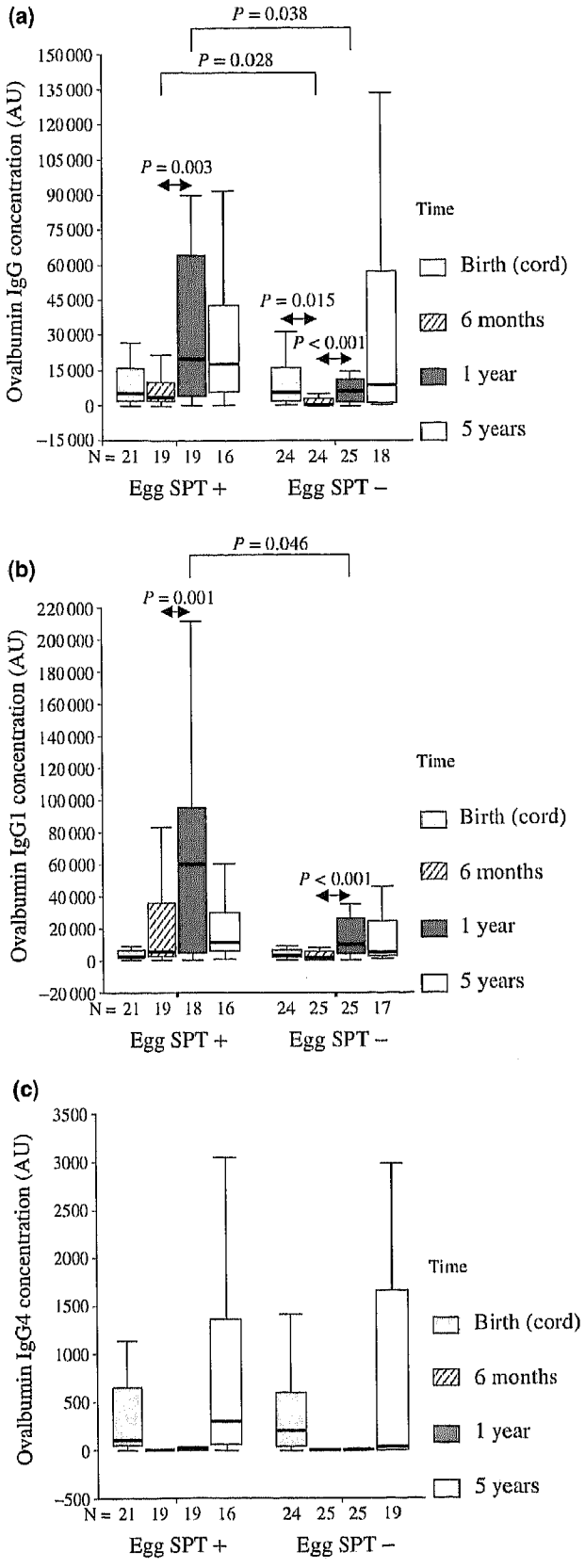
Similar differences between ES and NES children were identified for OVA IgG1. Among ES children OVA IgG1 concentration did not change between birth and 6 months of age, rose between 6 months and 1 year ($P = 0.001$), and peaked at 1 year of age (Fig. 3b). In contrast, there was no difference in the pattern of OVA IgG4 responses between the groups: in both groups OVA IgG4 concentration fell from birth to 6 months (ES and NES: $P = < 0.001$) and rose by 1 year (ES: $P = 0.005$, NES: $P = 0.05$), reaching a maximum at 5 years of age (ES and NES: $P = 0.001$) (Fig. 3c). At all time points OVA IgG4 concentration was substantially lower than OVA IgG1 in both ES and NES children (ES and NES: $P < 0.001$) and did not differ between the two groups (not graphically represented).

Ovalbumin specific immunoglobulin G and subclass responses according to duration of egg sensitization and atopic predisposition

IgG responses were further evaluated according to the four atopic sub-categories, namely, transiently egg sensitized (transient ES), persistently egg sensitized (persistent ES), atopic but non-egg sensitized (atopic, NES) and non-atopic and non-egg sensitized (non-atopic, NES).

Ovalbumin immunoglobulin G and immunoglobulin G1 responses (Figs 4a and b)

In this analysis it was apparent that only non-atopic, NES children demonstrated a fall in specific IgG and IgG1 over the first 6 months of life (OVA IgG, $P = 0.005$; OVA IgG1, $P = 0.091$). In the other three categories there was no change in OVA IgG and IgG1 concentrations between birth and 6 months of age, except for persistently ES children in whom OVA IgG1 rose ($P = 0.028$). At 6 months of age ES children had higher OVA IgG and IgG1 concentrations than the non-atopic NES children, but this was most evident for the persistent ES category (transient ES: OVA IgG, $P = 0.023$, persistent ES: OVA IgG1, $P = 0.001$; OVA G1, $P = 0.005$). In addition, atopic children, even though NES had higher OVA IgG and IgG1 concentrations than their non-atopic counterparts (OVA IgG, $P = 0.001$; OVA IgG1, $P = 0.014$).



In all four categories OVA IgG and IgG1 rose by 1 year of age. However, this was most marked in persistently ES children, who had higher concentrations at 1 year than each of the other categories, although clear statistically significant differences were only observed between persistent ES and non-atopic, NES children (transient ES: OVA IgG, $P = 0.021$; OVA IgG1, $P = 0.051$. atopic NES: OVA IgG, $P = 0.043$; OVA IgG1, $P = 0.026$; non-atopic NES: OVA IgG, $P = 0.001$; OVA IgG1, $P = <0.001$). OVA IgG and IgG1 concentrations of the persistent ES group peaked at 1 year of age. There were no differences in OVA IgG or IgG1 concentrations between transiently ES children and atopic, NES children at any time point. Again, at 1 year of age atopic NES children had higher OVA IgG and IgG1 concentrations than had non-atopic NES children (OVA IgG, $P = 0.014$; OVA IgG1, $P = 0.021$).

Ovalbumin immunoglobulin G4 responses (Fig. 4c)

The change in OVA IgG4 concentration across the 5-year period did not relate to atopic category: in every group there was a drop from birth to 6 months (significance level ranging between $P = 0.002$ and 0.018) and rise between 1 and 5 years of age (significance ranging between $P = 0.012$ and 0.043). In addition, OVA IgG4 concentration of persistent ES subjects rose between 6 months and 1 year of age ($P = 0.015$). No significant difference in OVA IgG4 concentration was observed between the atopic categories, although at 1 year of age persistent ES subjects had higher OVA IgG4 concentration than had non-atopic NES subjects ($P = 0.022$) (Fig. 4c).

Ovalbumin specific immunoglobulin G and subclass responses in relation to clinical outcome

Among this study group, 23 children (23/44, 52%; 95% confidence interval (CI), 37.9–66.2%) developed asthma and 24 children (24/46, 52%; 95% CI, 38–66%) developed eczema that persisted during the 5-year follow-up (skin disease on at least three visits). The development of asthma was associated with chronic eczema ($P = 0.016$, χ^2). The respective numbers of children with eczema or asthma who were atopic (positive SPT to egg or other allergen) were: 21/24; 88% and 18/23, 78%. However, only persistent egg sensitization was associated with chronic eczema ($P = 0.002$, Fisher's exact test).

Children with chronic eczema had higher OVA IgG at 6 months, 1 year and 5 years of age ($P = 0.058$; 0.019; 0.021, respectively) and higher OVA IgG4 at birth, 6 months and 5 years of age ($P = 0.02$; 0.035; 0.023, respectively). Significantly higher levels of OVA IgG1 were measured at 1 year of

Fig. 3. Concentrations (arbitrary units (AU)) of ovalbumin (OVA)-specific IgG (a), IgG1 (b) and IgG4 (c) at birth (umbilical cord), 6 months, 1 and 5 years of age. The data are grouped according to the presence or absence of egg skin prick test (SPT) sensitivity. OVA-specific IgG and subclasses were measured by in-house indirect ELISAs. The box–plot configuration is as described for Fig. 2. Only non-egg sensitized (NES) children displayed a fall in egg OVA-specific IgG and IgG1 concentrations over the first 6 months of life. The profile of OVA IgG4 responses over the first 5 years of life was similar in egg sensitized (ES) and NES children. In both groups levels fell between birth and 6 months of age ($P < 0.001$), rose by 1 year (ES: $P = 0.005$; NES: $P = 0.05$), and again by 5 years of age ($P = 0.001$).

age only ($P = 0.006$) (Fig. 5a). While no associations between OVA IgG or OVA IgG4 concentrations and asthma were observed, asthmatic children had higher concentrations of plasma OVA IgG1 at birth ($P = 0.049$), 1 year ($P = 0.026$) and 5 years of age ($P = 0.036$) compared with healthy children (Fig. 5b).

For the analysis of OVA IgG subclasses and asthma, an ROC curve was constructed for OVA IgG1 concentrations at 1 year of age (area under curve 0.703 [95% CI, 0.54–0.87]) (Fig. 6). Measurements at this age were selected since at this time point inter-group differences were observed most commonly. The plot showed that if an OVA IgG1 concentration of 14 500 AU was selected, plasma concentrations above this cut-off value were able to predict asthma ($P = 0.017$) with a sensitivity of 64% (95% CI, 43–80%) and specificity of 74% (95% CI, 51–88%).

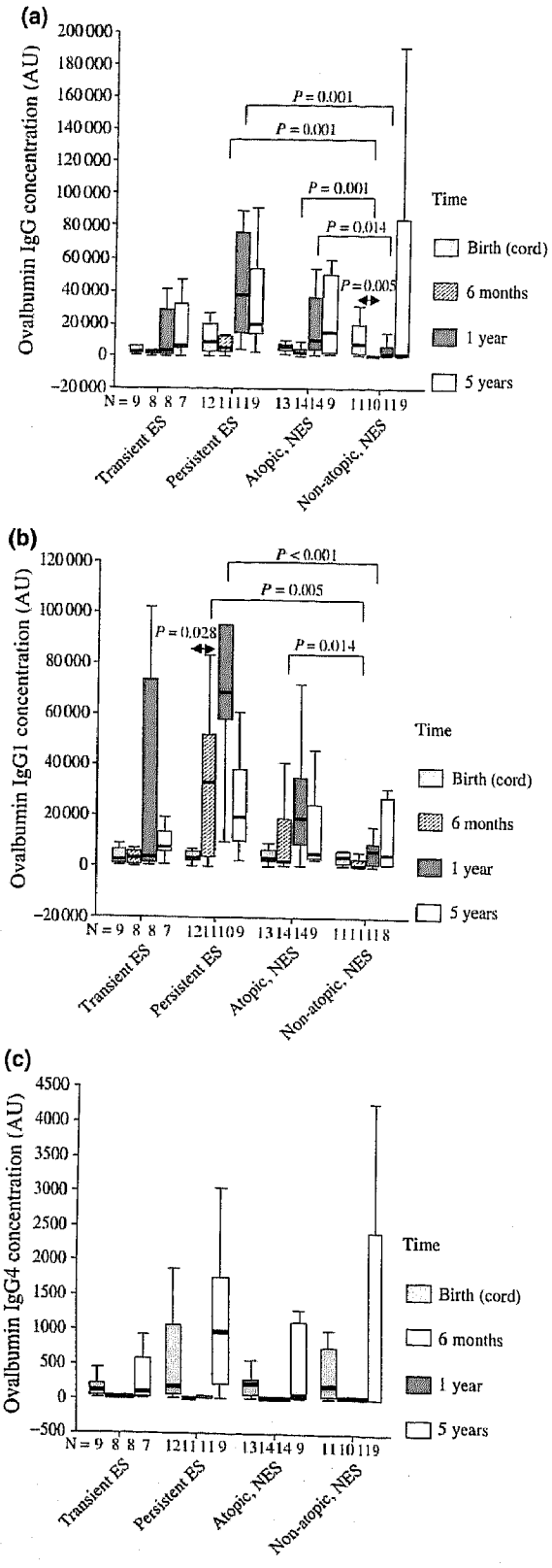


Fig. 4. Concentrations (arbitrary units (AU)) of ovalbumin (OVA)-specific IgG (a), IgG1 (b) and IgG4 (c) at birth (umbilical cord), 6 months, 1 and 5 years of age. The data are grouped for children with transient egg skin prick test (SPT) sensitization (transient egg sensitized (ES)), persistent egg sensitization (persistent ES), SPT sensitivity to an allergen other than egg (atopic, non-egg sensitized (NES)) and always SPT negative (non-atopic, NES). The box-plot configuration is as described for Fig. 2. Among the control NES groups, only the non-atopic category had a significant fall in specific IgG between birth and 6 months of age. Similarly, only persistent ES children had a rise in OVA IgG and IgG1 over the first year of life, resulting in significantly higher levels at 6 months and 1 year age compared with non-atopic, NES children. OVA IgG4 responses were unrelated to the presence or duration of egg sensitization. Levels fell after birth (transient ES, $P = 0.018$; persistent ES, $P = 0.008$; atopic, NES, $P = 0.002$; non-atopic, NES, $P = 0.005$) and rose between 1 and 5 years of age (transient ES, $P = 0.043$; persistent ES, $P = 0.012$; atopic, NES, $P = 0.012$; non-atopic, NES, $P = 0.043$).

Discussion

This study has evaluated retrospectively IgG and IgG subclass responses to the common dietary allergen, hen's egg OVA, over the first 5 years of life in a group of ES and NES children who had been followed up to monitor the development of asthma. Among this birth cohort who all had a genetic risk of asthma (at least one atopic, asthmatic parent), differences in the humoral profile were identified that related to the duration of egg sensitization (transient vs. persistent) and also to the development of eczema and asthma. Our data relied on positive SPT responses for the definition of egg sensitization, rather than measurement of specific IgE concentration. However, this may be justified in view of the correlations between positive skin testing to purified, well characterized allergens (as were used in these subjects) and specific IgE concentration, as well as clinical reactivity [15, 16]. Moreover, data from this birth cohort have shown already associations between positive egg SPT, the presence and severity of allergic skin disease and characteristics of allergen-specific T cell responses [17, 18].

Consistent with previous studies [19, 20] OVA IgG concentration of the study group fell slightly from birth to 6 months of age, reflecting the natural attrition of transplacentally acquired maternal antibody. Concentrations rose again by 1 year, most likely because of the introduction of egg into the infants' diet. However, on analysing the data in relation to egg sensitization status, this anticipated pattern was only observed for NES subjects, and in particular, non-atopic, NES children. For the remaining categories OVA IgG concentration did not change over the first 6 months of life,

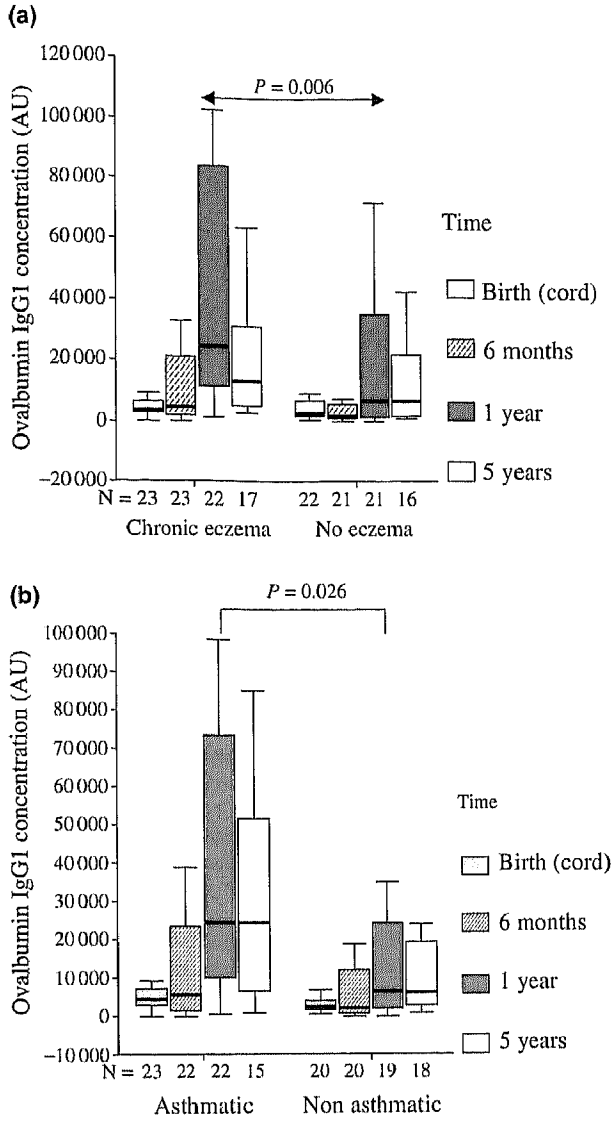


Fig. 5. Concentrations (arbitrary units (AU)) of ovalbumin (OVA)-specific IgG1 at birth (umbilical cord), 6 months, 1 and 5 years of age in relation to the development of eczema (a) and asthma (b). The box-plot configuration is as described for Fig. 2. The diagram shows that children who developed eczema and asthma had higher OVA IgG1, especially at 1 year of age.

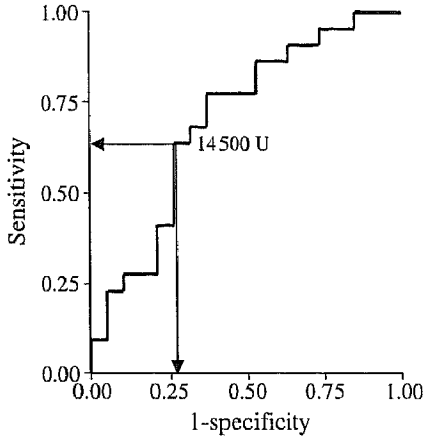


Fig. 6. Receiver–operator characteristic curve for ovalbumin (OVA) IgG1 measurements at 1 year of age and the prediction of asthma outcome. A cut-off of 14500U gave a sensitivity of 64% (95% confidence interval (CI), 43–80%) and specificity of 74% (95% CI, 51–88%) for the prediction of asthma at 1 year of age.

an observation that can only be accounted for by the endogenous production of OVA IgG. The infant weaning practices of this cohort were not monitored, but as a group at high risk of allergy, delayed introduction of egg into the infants' diet was advised. In the absence of ingested egg, exposure to OVA could have occurred through maternal sources, either antenatally or via breast milk [21, 22], or by adventitious contact with the antigen, for example, during food preparation [23].

OVA IgG1 and IgG4 subclass concentrations were also measured. Evidence suggests that IgG1 is T helper (TH)1-, and IgG4 TH2 -dependent: *in vitro* studies on mitogen-stimulated human lymphocytes have shown that IL-4 stimulated, and IFN-γ inhibited, IgE and IgG4 synthesis [24, 25], while birch allergen-induced expression of IL-4 correlated with serum specific IgG4, and IFN-γ secretion with IgG1 responses [26]. Therefore, measurement of these two subclasses facilitated assessment of IgG responses in terms of TH1/TH2 deviation.

The kinetics of IgG1, the predominant IgG subclass, followed IgG closely, with concentrations reaching a maximum by 1 year of age. OVA IgG4, present in significantly smaller quantities than specific IgG1, fell to a nadir at 6 months and rose to a maximum concentration much later, at 5 years of age, a pattern also observed by previous authors [11], and which may be explained by delayed maturation of IgG4 production [27].

Differences in specific IgG responses between ES and NES children have been reported in earlier studies [11, 20]. However, none of these have distinguished between transient and persistent egg sensitization. In this study, differences in both the pattern, and quantity, of IgG and subclass responses were observed between these two categories. A rise in OVA IgG1 concentration from birth to 6 months of age was unique to the persistently ES group. Also, at 6 months of age, these subjects had higher OVA IgG and IgG1 concentrations than non-atopic, NES children, and at 1 year of age had higher levels than all the other categories. In contrast, children with transient egg sensitization displayed little, or no, difference in specific IgG and subclass responses compared with the NES control categories.

Somewhat surprisingly, since IgG4 synthesis relates to allergy-promoting TH2 cytokine (IL-4, IL-13) pathways, the OVA IgG4 profiles showed little difference between the atopic categories. Only persistently ES children had higher levels compared with non-atopic, NES children at 1 year of age. However, other authors have also found food-specific IgG1 subclass antibodies to discriminate better than IgG4 antibodies between IgE-sensitized and non-sensitized children [28].

Thus, taken together, these data show that persistently ES children have a distinctive pattern of OVA IgG responses, with enhanced production of both IgG1 and IgG4 subclasses, particularly at 1 year of age. This enhanced humoural responsiveness could be caused by a general increased promotion of Ig synthesis by TH2 cells [29], which characterize the atopic phenotype. Alternatively, it is possible that the persistent ES

group might have experienced differences in the dose or pattern of maternally derived OVA exposure, either through pregnancy or via breast milk, since the women were not advised on any dietary manipulation. However, to date, there is no literature to support an impact of early life dietary allergen exposure on the duration of sensitization. Moreover, a relationship of atopy *per se* with antibody production is also suggested by our observation that atopic children, even though NES, also had higher OVA IgG and IgG1 concentrations than non-atopic children at both 6 months and 1 year of age.

The clinical relevance of this finding is that egg skin sensitization might be able to be classified as transient or persistent, with major implications for stratification of risk of allergic disease, by concurrent consideration of IgG humoural responses [12]. The numbers in this study were limited and therefore clinical extrapolation must be guarded. However, the potential benefit for asthma prevention through early intervention implied by these observations demands further work with increased subject numbers.

A principle aim of this work was to investigate whether differences in OVA-specific IgG/subclass profiles accompany the development of asthma. In this group of subjects, the children who developed asthma had higher levels of OVA IgG1, but not IgG or IgG4, than non-asthmatics at birth, 1 and 5 years of age. Since childhood asthma is frequently atopic in nature [30, 31], and these data have shown atopy (even in the absence of egg sensitization) to be associated with a raised OVA IgG1 concentration in the first year of life, the current observation is not unexpected. This profile compares with that of eczematous children who demonstrated higher IgG and IgG4 that healthy controls over the first 5 years of life, but higher OVA IgG1 at 1 year only. These observations suggest that alterations of OVA IgG/subclass responses might predate the expression of allergic disease, and concur with a Swedish study that reported atopic symptoms in the first 8 years of life to be preceded by raised OVA IgG1 (also IgG and IgG3) concentration at 6 months of age [11].

Since raised cord blood IgE is not a sensitive predictor of later atopy [32, 33], finding a more reliable marker of atopy would have considerable benefit for implementation of secondary allergy prevention strategies. Therefore, the association of high OVA IgG1 concentration and asthma is of particular interest as a potential early marker for the later onset of respiratory allergic disease among infants with a parental history of atopy. Indeed, the ROC curve showed that OVA IgG1 measurement at 1 year of age (in excess of 14 500 U) could predict the subsequent development of asthma with a sensitivity of 64%, a predictive performance that outranks that of (total) IgE measurement, either at birth or in infancy [34, 35]. However, since OVA IgG1 was also increased at 1 year of age in children with atopic eczema, and since atopic eczema is itself a risk factor for later asthma [36, 37], any future use of such analyses to independently assess asthma risk will require further elaboration of these findings by prospective evaluation of a high atopy-risk birth cohort and comparison of the IgG/subclass profiles among eczematous children with and without asthma.

In summary, these data raise the possibility that measurement of egg OVA- specific IgG and subclasses in infancy might offer a useful serological adjunct to clinical evaluation and skin prick testing for the early identification of those at a particular risk of developing later allergic sequelae. Together they could facilitate timely implementation of measures that might aid in halting the Allergic March.

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