

Serum ovalbumin-specific immunoglobulin G responses during pregnancy reflect maternal intake of dietary egg and relate to the development of allergy in early infancy

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Summary

Background The value of allergen elimination diets during pregnancy for primary prevention of infant allergy has been questioned. However, dietary compliance may influence effectiveness.

Objectives To monitor egg intake during a randomized controlled trial of egg avoidance throughout pregnancy and lactation by serial measurements of serum ovalbumin (OVA) IgG concentration in conjunction with dietary diary record and also, to analyse specific IgG concentrations at birth in relation to infant allergic outcome.

Methods Pregnant women, with personal or partner atopy, were randomized to complete dietary egg exclusion or an unmodified healthy diet before 20 weeks gestation. The infants were evaluated for atopy at 6 months of age. Serum food-specific IgG concentrations were determined by ELISA in maternal samples collected at study recruitment and during labour, and in infant samples at birth (umbilical cord).

Results Serum-specific IgG to OVA, but not the unrelated allergen, cow's milk β -lactoglobulin, decreased over pregnancy in egg-avoiding women only ($P < 0.001$). Cord OVA IgG concentration correlated with maternal IgG at delivery ($r = 0.944$; $P < 0.001$), and for infants born to atopic women, cord concentration was higher than that of their mother's ($P < 0.001$). Infants with the lowest and highest cord IgG concentrations were the least likely, and those with mid-range concentrations were the most likely, to be atopic by 6 months of age ($P = 0.008$).

Conclusion Serum OVA IgG concentration reflects egg consumption, thereby indicating dietary allergen doses to which the developing immune system might be exposed. Trans-placental maternal IgG must be considered among early life factors that regulate infant atopic programming.

Keywords atopy, children, elimination diet, hen's egg ovalbumin, IgG antibodies

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Introduction

The considerable increase in the prevalence of atopic diseases in recent decades, particularly in westernized countries, continues to drive a search for successful allergy prevention/therapeutic strategies [1]. In particular, the established, causal links between allergen exposure and sensitization, and between sensitization and disease expression among those with a genetic predisposition to atopy have encouraged trials of allergen exclusion measures for primary allergy prevention [2–4].

A successful allergen avoidance programme must not only be clinically effective but must also be safe and economical. However, the interpretation of a programme's effectiveness is crucially dependent on subject compliance, which in turn relates to the acceptability and manageability of the inter-

vention. In Southampton, UK, a prospective, randomized controlled trial of egg avoidance from the second trimester of pregnancy throughout lactation as a primary allergy prevention strategy has been ongoing. A number of studies have identified that egg sensitization or allergy in infancy places the child at particular risk of developing later, and possibly lifelong, inhalant sensitivity and asthma [5, 6]. Thus, it was hypothesized that this single, manageable intervention might reduce the incidence of infant sensitization, without compromising mother or baby nutritional welfare.

The first aim of the current work was to monitor subject compliance with the egg exclusion diet. Because self-reporting is prone to intentional, or non-intentional, error and compliance is judged best by an objective method, we utilized measurement of serum-specific IgG concentration, as a reflection of dietary intake, in conjunction with a diary record to assess adherence to the intervention.

The relationship between infant IgG levels at birth and subsequent atopic disease remains to be clarified. Some authors have reported high maternal and cord IgG to food

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and inhalant allergens to be associated with less infant atopy [7, 8], while others have reported either no association [9], or even an increased risk of allergy [10]. Thus, in this cohort, who were expected to have wide-ranging quantities of egg intake, and hence, serum egg ovalbumin (OVA) IgG concentration, we also sought to investigate the relationship between levels of cord egg-specific IgG and the expression of later infant atopy in this study group.

Methods

Study overview

In a prospective study, pregnant women attending antenatal booking clinics in Southampton and surrounding areas, who had a personal or partner history of allergy, were invited to participate in a randomized controlled study of egg avoidance during pregnancy and breastfeeding as a primary allergy prevention measure. The women ($n = 229$) were randomized to either complete dietary egg exclusion or an unmodified healthy diet from 17 to 20 weeks gestation until the end of lactation. The infants were evaluated at 3, 6, 12 and 18 months of age for the development of allergy by an investigator blinded to the randomization category (Fig. 1).

Subjects

During the initial recruitment visit in the Southampton Child Health Department, parental allergy was established by interview questionnaire [11] and positive skin prick testing to one or more of a panel of common dietary and inhalant allergens (house-dust mite (HDM), cat, dog, timothy grass pollen, birch pollen, horse, alternaria, egg: ALK, Abelló; Denmark). A weal size ≥ 2 mm, in the presence of appropriate positive (histamine) and negative (saline) controls, was considered positive. Women were excluded from the study

if the pregnancy was complicated, if they had birds in the home, were egg allergic themselves or had ongoing dietary restrictions.

The women were recruited to the study with informed consent. The project was approved by Southampton & South West Hampshire (LREC number 52/97) and North & Mid Hampshire (LREC number 021/A) Local Research Ethics Committees.

Dietary management

At recruitment, a research dietitian (K. E. C. G.), dedicated to this project, gave all the women healthy eating advice and guided the intervention group with regard to following an egg-free diet (both overt and hidden forms of egg). A food diary kept during the week prior to recruitment was analysed for nutritional adequacy in general, and for eggs, and egg products, in particular using the 'CompEat 5' dietary analysis programme. A food frequency questionnaire detailing egg intake over the month prior to recruitment was also completed. All subjects were evaluated again at 24 and 32 weeks gestation to ensure satisfactory nutritional intake by interview and analysis of a 7-day food diary completed during the preceding week. Known episodes of accidental egg ingestion by egg-avoiding women were recorded prospectively and the data were collected at these visits. All women were advised to avoid egg in weaning foods until their infant(s) had been assessed at 6 months of age.

Infant follow-up

The infants were evaluated for the development of allergy at 3, 6, 12 and 18 months of age by a research nurse (R. B.) or clinician (G. H. S. V.). Respiratory symptoms and skin rashes were recorded prospectively by the parents and the diary discussed at each review. The infants were examined carefully for signs of allergic disease and at 6, 12 and 18 months of age they were skin prick tested to a panel of common dietary and inhalant allergens (raw cow milk, whole egg, egg white, egg yolk, peanut, HDM, cat, timothy grass pollen, birch pollen: ALK). A weal size ≥ 2 mm, in the presence of appropriate positive (histamine) and negative (saline) controls, was considered positive.

Atopic dermatitis was defined as an erythematous-papulovesicular chronic skin disease with dry skin, itching and typical distribution (cheeks, abdomen, extensor surface of limbs) [12], and in this analysis, an infant atopic phenotype was regarded as a diagnosis of atopic dermatitis and/or at least one positive skin prick test (SPT).

Blood samples

Sera were prepared from maternal blood collected at study recruitment (17–20 weeks gestation) and delivery (term), and infant blood at birth (umbilical cord). Samples were stored at -80°C until use (Fig. 1).

Specific immunoglobulin G measurement

Concentrations of serum IgG-specific for egg OVA and cow's milk β -lactoglobulin (BLG) were measured by ELISA. Briefly, microtitre plates (NUNC, Maxisorp, Sweden) were

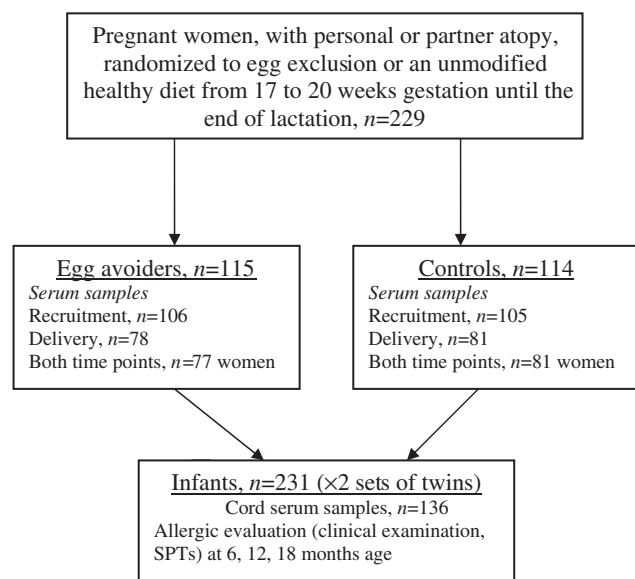


Fig. 1. Consort diagram illustrating study structure, participant numbers and corresponding serum samples.

coated with 100 µg/mL OVA, BLG or bovine serum albumin (BSA) (as a control for non-specific binding) (all Sigma Aldrich, Poole, UK) and stored overnight at 4 °C. After blocking with 3% BSA, a reference serum pooled from non-food allergic, healthy women (diluted 1:50–1:6400), test serum samples (diluted 1:100) and buffer without added serum (blanks) were added to duplicate wells. Bound IgG was detected by HRP-conjugated rabbit anti-human IgG (Dako, Ely, UK). *O*-phenylenediamine hydrochloride (OPD) (Sigma Aldrich) substrate was added and the absorbance was read at 492 nm after stopping the reaction. The absorbances on the BSA-coated plate were subtracted from those on the allergen-coated plates and the values expressed as arbitrary units (AU) by comparison with the reference serum standard curve.

All samples from an individual subject were run on the same plate, on the same day. Samples from an equal number of egg-avoiding and control women were run on the same plate.

Statistical analyses

The OVA IgG data were normally distributed following log transformation, and thus, parametric statistical analyses were applied to this data set. The mean change in OVA IgG within study groups was analysed using the paired *t*-test, while the within-group changes were compared between study categories using the two-sample *t*-test. Pearson's *r* correlation coefficient was used to evaluate the relationship between maternal and infant OVA IgG titres at delivery.

The data for cow's milk BLG IgG, egg intake during pregnancy and number of dietary accidents were not normally distributed, even after log transformation. Thus, non-parametric statistical analyses were applied to these variables; specifically, the Mann–Whitney *U*-test was used for between-group analyses and Spearman's rank *r_s* correlation coefficient to evaluate the relationships between change in dietary egg intake or accidents during pregnancy and the change in maternal OVA IgG titre.

Differences in the number of subjects falling into one or more categories were measured by the χ^2 test. Two-sided significance of $P < 0.05$ was used for all the tests performed. Calculations were carried out using SPSS software (Version 11.5 for Windows).

Results

OVA-specific IgG concentration decreased between study recruitment (17–20 weeks gestation) and delivery in egg-avoiding women ($n = 77$) (mean change, -0.77 ; 95% CI -0.90 to -0.64 ; $P < 0.001$), but not in control women ($n = 81$) (mean change, -0.004 ; -0.17 to 0.16 ; $P = 0.963$). The decrease among egg-avoiding women was significantly greater than in the control women (mean difference, -0.77 ; -0.97 to -0.55 ; $P < 0.001$) (Fig. 2).

OVA IgG titres fell during pregnancy in 73 of 77 (95%) egg-avoiding women, despite 84% of the participants in this category reporting known accidental egg exposures during the study period. However, the fall in IgG titre concurred with dietary diary data, indicating a significant reduction in overall egg intake in the intervention group only.

The change in OVA IgG concentration correlated with the overall change in egg intake ($r_s = 0.286$, $P = 0.024$), but not to

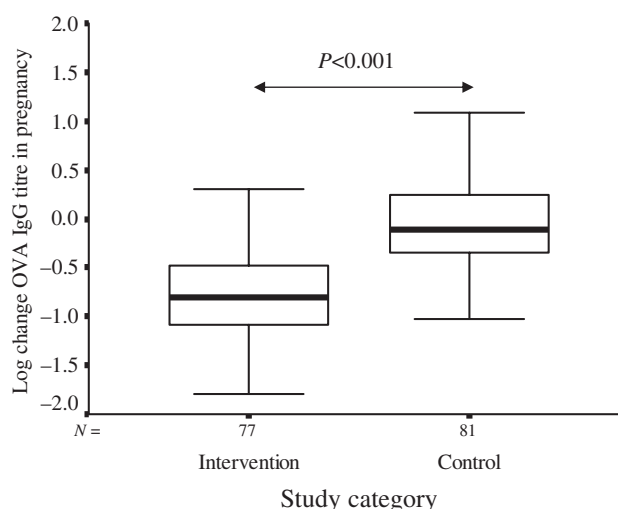


Fig. 2. Change in serum ovalbumin (OVA) IgG concentration over pregnancy compared in women randomized to egg avoidance (intervention) or a normal (control) diet from 17–20 weeks gestation. Women avoiding dietary egg, but not control women, had a decrease in serum OVA-specific IgG concentration between study recruitment (17–20 weeks gestation) and delivery of the infant ($P < 0.001$ vs. $P = 968$). This change in titre was significantly greater ($P < 0.001$) than that measured among control women. OVA-specific IgG was measured by ELISA and the box-plot shows log values of IgG titre change 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.

the number of reported accidental exposures ($r_s = 0.088$, $P = 0.506$). Among egg-avoiding subjects with a rise in OVA-specific IgG titre ($n = 3$), dietary diary records indicated that in all three cases, the women had abandoned the elimination diet. Thus, in only one of 77 women (1.3%) (no significant change in IgG titre) did IgG measurements during pregnancy fail to correspond with dietary assessments.

Intervention women had higher OVA IgG at recruitment than had control women ($P < 0.001$) and consequently there was no inter-group difference at delivery ($P = 0.415$). By comparison, the change in cow's milk BLG IgG concentration over pregnancy, measured in matched serum pairs from a randomly selected subgroup of the cohort ($n = 32$), showed no significant difference between egg-avoiding and control categories ($P = 0.758$) (not graphically represented). Neither absolute OVA-specific IgG concentrations at study recruitment and delivery nor the change during pregnancy were related to maternal atopic status.

Cord OVA IgG concentration directly correlated with maternal serum OVA IgG concentration at delivery ($r = 0.954$; $P < 0.001$). There was no difference in cord OVA IgG concentration between infants born to egg avoiding, as compared with control, mothers ($P = 0.478$), which could be accounted for by the disproportionately high recruitment levels of intervention women. Similarly, there were no differences in cord measurements between infants born to atopic, as compared with non-atopic, mothers ($P = 0.691$). Infants born to atopic women had higher cord OVA IgG concentration than their mothers had at delivery ($P < 0.001$). However, while the maternal/matched cord IgG disparity was greater in atopic mother–baby pairs than in non-atopic pairs, this comparison did not reach statistical significance ($P = 0.574$) (Fig. 3).

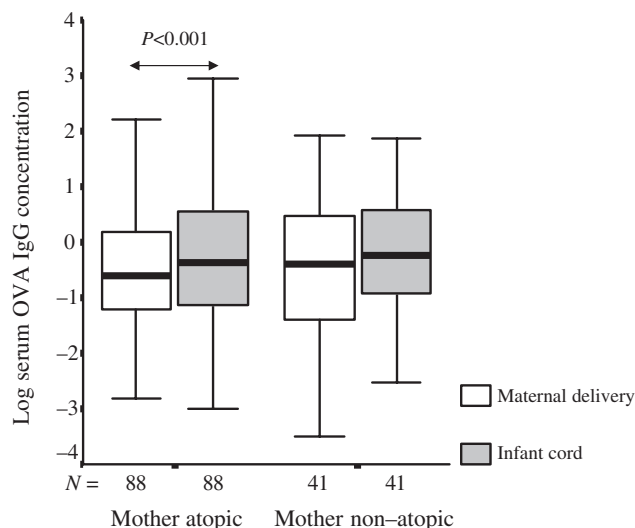


Fig. 3. Ovalbumin (OVA) IgG concentration in cord and matched maternal sera at delivery in relation to maternal atopic predisposition. Infants born to atopic women, but not those born to non-atopic women, had significantly higher OVA-specific IgG concentration in umbilical cord blood (grey boxes) than in matched maternal sample at delivery (empty boxes) ($P < 0.001$). However, this difference was not significantly greater than that observed in non-atopic mother–baby pairs ($P = 0.574$). The box-plot configuration is as described for Fig. 2.

Infant atopic outcome

Clinical data for the infant subgroup considered in this work (those with IgG measurements at birth, $n = 136$) were analysed. A positive egg SPT was noted at 6-, 12- and 18-month follow-up in 16.4%, 15.6% and 10.1% of infants, respectively. At all time-points, a positive egg SPT was significantly associated with a positive SPT to any allergen ($P < 0.001$ at each time-point) and with atopic dermatitis ($P < 0.001$ at 6, 12 months; $P = 0.007$ at 18 months). An atopic phenotype, as defined by atopic dermatitis and/or positive SPT, was identified in 47%, 55% and 42% of these infants at 6, 12 and 18 months of age, respectively.

There was no difference in the number of infants born to egg-avoiding, as compared with control, mothers who developed an allergic phenotype over the first 18 months of life (6 months, $P = 0.794$; 12 months, $P = 0.457$; 18 months, $P = 0.126$). Also, there was no significant difference in OVA-specific IgG levels at birth between those infants with an atopic outcome over the first 18 months of life and those without (6 months, $P = 0.938$; 12 months, $P = 0.582$; 18 months, $P = 0.869$). However, in order to compare the relationship between differing cord blood levels (for example, very low or very high concentrations) and infant allergic outcome, the OVA-specific IgG measurements were ranked by grouping into quartiles, where the first quartile represented the lowest, and the fourth quartile represented the highest, concentrations. This analysis was applied first to the entire subgroup, and revealed no significant relationship between varying specific IgG levels and atopic outcome in infancy (6 months, $P = 0.276$; 12 months, $P = 0.290$; 18 months, $P = 0.076$). However, in order to account for the possibility that the dietary intervention could confound interpretation of the relationship between specific IgG levels at birth and allergic outcome, the analysis was then applied to the infants of the control women only. Among this group with

unmodified environmental dietary allergen exposure, infants with an atopic phenotype at 6 months age were most likely to have middle values of cord OVA IgG, and least likely to have either the lowest or highest concentrations ($P = 0.008$) (Fig. 4a). A similar bell-shaped pattern was noted at 12 months age, although it failed to reach statistical significance ($P = 0.086$) (Fig. 4b), while at 18 months, a non-atopic outcome appeared to be associated only with the highest titres of OVA-specific IgG ($P = 0.043$) (Fig. 4c).

Discussion

Forthcoming data from this intervention trial may offer a definitive analysis of the influence of maternal dietary allergen exclusion from early in gestation on infant atopic outcome. However, knowledge of subject dietary compliance is necessary for appropriate interpretation of study outcome data, and also to gain an appreciation as to whether such an intervention has long-term potential application. In this trial, compliance was promoted by exclusion of a single dietary allergen and through intensive dietetic input. Nevertheless, despite these measures, 84% of the women had a known dietary indiscretion, suggesting that complete dietary exclusion is virtually impossible, even under the most ideal circumstances.

Egg exclusion resulted in a significant fall in OVA IgG concentration over gestation. A fall in IgG titre was recorded in all but four (of 77) egg-avoiding women, and in three of these subjects dietary records indicated that the women had abandoned the dietary exclusion. Furthermore, the change in specific IgG concentration over pregnancy correlated with recorded change in egg intake. Accordingly, *in vitro* IgG measurements support dietary records and together they confirm reliably that the participants were making considerable adjustments to their egg consumption. The change was unrelated to the number of reported accidents. However, because the mean cumulative accidental egg protein dose was 3.37 g of egg protein, equivalent to less than 1/2 of one whole egg, this may have been insufficient to have an impact on the declining IgG profile consequent on the substantial reduction in intake overall. Thus, despite inevitable dietary lapses, consistent dietary exclusion measures can still induce a fall in specific IgG levels.

This work has not only reported an objective measure of patient compliance, but unlike other studies [13, 14], has also established that the dietary restriction was limited to egg. Participation in a study of this type runs the risk of subjects, whether intervention or control, avoiding other well-known allergenic foods in an attempt to improve their infant's allergic outcome, which could cause nutritional compromise if undertaken without dietetic supervision. Cow's milk BLG is a common allergen – but cow's milk is also a nutritious food product, particularly during pregnancy and breastfeeding. Reassuringly, measurement of the BLG IgG concentration revealed no difference between egg-avoiding and control groups in the change in BLG titre through pregnancy. Indeed, dietary diary analyses of the whole cohort have shown no difference in cow's milk intake between the two groups. Rather, the diet and serological data together confirm that

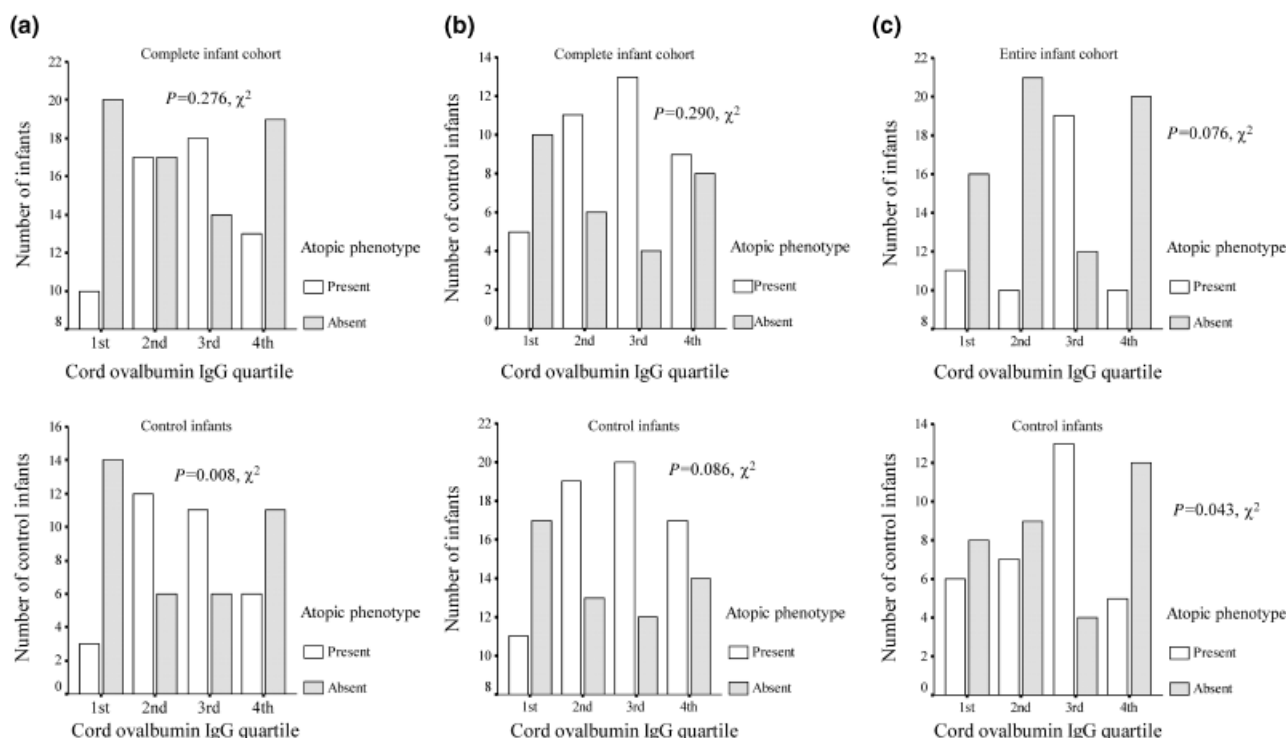


Fig. 4. Infant allergic outcome over first 18 months of life in relation to cord ovalbumin (OVA)-specific IgG concentration. Umbilical cord blood OVA-specific IgG concentrations were ranked by grouping into quartiles. The distribution of IgG quartiles at birth was then compared among atopic (skin prick test positivity and/or atopic dermatitis) (clear bars) and non-atopic (shaded bars) infants at 6, 12 and 18 months of age. The analysis was performed both for the entire infant group (top diagrams) and for the infants of control women only (lower diagrams), as these control infants had not experienced any dietary environmental manipulation. There was no significant relationship between IgG concentration quartile at birth and atopic outcome at each time-point for the complete birth cohort. However, for the control infant group only, at 6 months of age (a) the number of infants expressing an atopic phenotype was least among those with the lowest (first quartile) and highest (fourth quartile) IgG measurements, while infants with mid-range IgG levels (second and third quartiles) more frequently had allergic features (χ^2 ; $P=0.008$). A trend for a similar, bell-shaped pattern was noted at 12 months of age (b) ($P=0.086$), while at 18 months of age (c) only the higher cord levels of OVA IgG appeared to discriminate between an atopic (third quartile) or non-atopic (fourth quartile) outcome.

these study subjects were not making independent dietary adjustments.

At study recruitment, the intervention group women had significantly higher OVA-specific IgG concentration than had the control women. Because specific IgG reflects egg intake, it was proposed that this might be a consequence of inter-group dietary differences. Indeed, batch analysis of the subjects' egg consumption during the month prior to study commencement revealed that the egg-avoiding women had a higher egg intake than their control counterparts recruited during the same time period, and this may have skewed the specific IgG measurements.

Maternal IgG responses in pregnancy were closely related to infant IgG levels at birth, as evidenced by the significant, direct correlation between matched maternal and cord-specific IgG concentrations. Accordingly, humoral changes consequent on maternal dietary manipulation must also be 'experienced' by the fetus/newborn. We observed that infants of atopic women had significantly higher levels of OVA IgG at birth than their mothers had at delivery, although the magnitude of this difference was not significantly greater than that of non-atopic mother–baby pairs. Nevertheless, since the current literature has reported maternal atopy to be associated with higher levels of IgG in cord blood, against both food and inhalant allergens [7, 15], a bio-clinical relevance of this finding must not be discounted. IgG is actively transported across the placenta by a receptor-

mediated mechanism [16]. In the asthmatic lung, the high-affinity receptor for another immunoglobulin (Fc ϵ RI for IgE) is present in increased numbers on dendritic cells [17] and this is thought to be a feature of the microenvironment of the atopic individual [18]. It is conceivable that a similar phenomenon might exist in the pregnancy-associated environment of the atopic mother, whereby enhanced placental IgG receptor expression could result in increased trans-placental IgG transport and elevated cord levels in the infants of atopic mothers. Future studies, with increased subject numbers, planned in our department may clarify the proposed relationship.

A priority of this work was to examine the association between cord-specific IgG levels and infant atopic outcome since, to date, beneficial [7, 8], adverse [10] and no [9] effects of high cord blood allergen-specific IgG concentration on the development of later infant allergic disease all have been reported. The current analysis was confined to the data relating to infants of control mothers in order to avoid potential confounding factors associated with the intervention. For example, the progressive decline in IgG concentration throughout pregnancy, with unknown effects on IgG subclass distribution and range of OVA epitopes recognized, might impinge on the relationship between IgG levels and infant allergic outcome. Also, the change in pattern of antigen exposures consequent on incomplete dietary elimination might have independent influences on infant atopic program-

ming. In animal studies, low-dose and intermittent initial allergen exposures have induced immune priming rather than antigen tolerance [19, 20].

Consistent with earlier studies, there was no significant difference in overall IgG levels at birth between control children developing, and not developing, atopy over the first 18 months of life. However, when cord measurements were ranked by grouping into quartiles, then at 6 months age it was apparent that the infants with the lowest (first quartile) and highest (fourth quartile) concentrations at birth were least likely, and those with mid-range concentrations were most likely, to express an atopic phenotype at this time-point. By 18 months of age, a non-atopic outcome was associated only with the highest cord IgG levels.

Because our data have shown that OVA IgG reflects maternal dietary egg intake, these concentration measurements give a surrogate for overall egg allergen exposure dose in early life – although they offer no indication of the pattern of exposure – and suggest that both low and high exposures may be protective for some children in early infancy. Such a 'bell-shaped' relationship between exposure dose and allergic outcome is reminiscent of recent data regarding the inhalant cat allergen, Fel d 1, which reported the maximum prevalence of cat sensitization to occur with moderate exposure levels [21].

An association between the lowest IgG/exposure levels and an absence of allergy is in keeping with studies reporting a dose–response link between HDM exposure and both sensitization and asthma [2, 3]. Similarly, children with the lowest exposure to cat did not have detectable-specific IgG, or IgE, and were asymptomatic [21]. The most valid interpretation of these findings is that such low-level exposures fail to induce an immune response.

Conversely, an absence of allergy among those with very high occupational exposure to allergen, for example, animal laboratory workers and beekeepers, has been recognized. Moreover, this protective effect has been attributed to IgG, and in particular, IgG4 [22, 23]. Indeed, successful bee-venom and HDM immunotherapy has been linked to the nature of the corresponding IgG response [24–26] and it has been observed that children born to rye grass-sensitized women who have undergone immunotherapy during pregnancy, with accompanying rise in specific IgG, have a reduced risk of becoming rye grass sensitized themselves, compared with children of untreated, sensitized women. Such data imply that IgG might have an active role in tolerance induction rather than simply being a marker of different immune responses. As yet, the mechanism is unknown, but this could include antigen blocking, antigen elimination or down-regulation of allergen-specific T cell and B cell activation via the immunoreceptor tyrosine-based inhibitory motif (ITIM) associated with the low-affinity IgG receptor, FcγRIIb (CD32b), present on these cells [27, 28]. This might explain why an atopy-protective association persists at 18 months of age for the highest cord IgG levels, but not for the lowest levels. It is conceivable that the immuno-modulatory functions of high IgG programme tolerant (T_H -1/ T_H -0) immune responses to later allergen exposure, whereas those infants not exposed to allergen via maternal routes, and who have yet to generate an immune response, remain at risk of developing an atopic (T_H -2 skewed) pattern of responses to initial dietary exposures during later infancy [29].

Two valuable conclusions arise from these data. Firstly, longitudinal measurements of specific IgG demonstrate clearly that OVA IgG concentration is a measure of overall dietary egg consumption. Thus, prospective dietary diary record and serological assays together provide essential confirmation of maternal adherence to an exclusion diet through pregnancy. Moreover, they may give an indication of the levels of dietary allergen to which the developing immune system is exposed. Secondly, the data suggest that trans-placental maternal IgG, particularly in the context of maternal atopic predisposition, might have immuno-modulatory functions. Thus, the interplay between the fall in specific IgG levels induced by the intervention and the change in allergen exposure parameters must be considered in the final analysis.

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