The role of IgG in allergy and tolerance to common food allergens

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Contents Page

1. Executive Summary

Background

The clinical significance of food-specific IgG subclasses in food allergy and tolerance remains unclear. This project investigates the role of IgG in egg and peanut allergy, and in a further group with resolved allergy.

Method

We recruited subjects with peanut (n=59) and/or egg allergy (n=40) from our allergy clinic and clinical database. Subjects had a recent clear history of an acute allergic reaction to either peanut or egg and evidence of egg or peanut specific IgE. Subjects were characterised by clinical interview and blood was taken for RAST IgE assays and ELISA IgG assays. Subjects with egg allergy underwent oral challenge. Assays were developed to measure amounts of serum IgG, G1 and G4 to Ovalbumin (OVA , the major egg allergen) and crude peanut extract.

Results

Egg-specific IgE and Skin Prick Test (SPT) weal diameters were significantly higher in the allergic group compared to the resolved group. OVA and peanut-specific IgG was detected in all allergic and non-allergic subjects alike. OVA IgG levels were higher than crude peanut extract (CPE) IgG in non allergic controls (median μg/ml IgG = 15.9 vs. 2.2, IgG1= 1.3 vs. 0.6, IgG4 = 7.9 vs. 0.7; $p<0.01$). There were no differences in OVA IgG levels between egg-allergic (n=40), egg-resolved (n=22) and control (n=18) subjects. In contrast, peanut-specific IgG were significantly higher in peanut-allergic subjects compared to non-allergic controls and to non-peanut-sensitised but egg-allergic subjects (n=26; median μg/ml IgG = 17.0, IgG1 = 3.3, IgG4 = 5.2). Overall, the range of IgG4 was greater than IgG1, and IgG4 was the dominant subclass in >60% of all subjects.

Discussion

Serum specific IgE and positive specific SPT indicate sensitisation to a specific food allergen. In egg allergy, we have shown the level of specific IgE or size of SPT wheal can distinguish between clinically active allergy and resolution.

We have developed an ELISA system whereby measurements of specific IgG can be made in absolute values rather than arbitrary units; this has the advantage of providing a linear measurement in μg/ml which can be compared between IgG subclasses and for different antigens. Further, as other groups adopt this technique our results may be compared with theirs.

We identified that levels of OVA-specific IgG are similar in egg-allergic, egg-resolved and non-allergic control subjects, suggesting that levels of OVA-specific IgG are a marker of exposure, rather than clinical egg allergy. Higher peanut-specific IgG levels are associated with clinical allergy, compared to non allergics but the range of IgG titres of the allergic and control groups overlap significantly. Hence, these measurements are not of diagnostic value.

OVA IgG is detected in all subjects but levels of OVA specific IgG were 10-fold higher than peanut specific IgG in the same non-atopic controls. This may be explained by the relatively early introduction of higher doses of egg into children's diets compared to peanut. High levels of milk-specific IgG have also been reported in infancy, during the time of highest milk ingestion.

Conclusion

Strong IgG responses to egg may be a normal physiological response to a protein frequently ingested from infancy, whereas upregulated IgG responses in peanut allergy may be indicative of a dysregulated immune response to peanut allergens. Test for specific IgG to egg and peanut are of no diagnostic use.

2. Glossary and definitions

Glossary

OVA Ovalbumin

OM Ovomucoid

CPE Crude peanut extract

TET Tetanus

ELISA Enzyme linked immunosorbent assay

Ig Immunoglobulin

SPT Skin prick test

IL- Interleukin

IFN Interferon

RES Resolved group

CTL Control (non-atopic) group

POS Allergic group

NPAEA Non-peanut allergic, egg allergic (i.e. subjects with egg allergy but not peanut allergy)

NEAPA Non-egg allergic, peanut allergic (i.e. subjects with peanut allergy but not egg allergy)

Definitions

Sensitisation

The presence of specific IgE to an allergen detected in an individual, this can occur with or without clinical allergy

Clinical allergy

The presence of clinical allergic reactions such as itchy skin wheals, swelling or wheeze shortly after contact with a typical allergen in an individual with evidence of specific IgE to that allergen

Immuno Cap or RAST

Generic term for test measuring serum specific IgE

Statistical definitions

Sensitivity=proportion of children with the disease who are also test positive

Specificity=proportion of children without the disease who are test negative

3. Background, aims and objectives

This project addresses the research question “to establish the role of IgG in the development of allergic sensitisation and reactions to foods” set by the FSA in their Research Requirements Document, Issue 8, 2002.

Little attention has been paid to the role of IgG in food allergy. This project focuses on peanut and egg, foods commonly causing allergic disease, and investigates the role of IgG in allergic patients, in sensitised and tolerant subjects, and in patients whose allergy has resolved.

The role of IgG

Studies on the pathogenesis of food allergy have focussed on the role of specific IgE where there is clear evidence for its role in food allergy, but there are few data on the role of specific IgG. For example, the ETAC (Early Treatment of the Atopic Child) study showed that severity of atopic dermatitis related to sensitisation to egg or cow’s milk (1), and in peanut allergy, a size of skin prick test or level of serum specific IgE has been identified above which patients nearly always have clinical allergy (2). In contrast there are few papers on food IgG. There is evidence of intrauterine allergen exposure in that house dust mite allergen (Der p 1) has been detected in amniotic fluid and foetal circulation (3) so it seems likely that food allergens also cross the placenta. IgG against caesin, beta-lactoglobulin (bLG) and alpha-lactalbumin have been demonstrated in sera from newborns, but there were no differences in levels between children with or without allergic disease (4). The presence of food-specific IgG in serum of babies raises the possibility that passively acquired maternal IgG may protect against development of allergy. Studies on children of atopic parents showed the presence of milk and egg specific IgG antibody at 3 months of age, falling by the age of five years (5), but no attempt was made to link this to the development of disease. However, in another study, high levels of IgG anti-IgE were shown to protect children with increased risk of allergy from early development of atopic disease (6).

Specific IgG is produced from infancy in most children regardless of atopic status and development of peak levels depends on timing and quantity of exposure. The

magnitude of such IgG responses is related to atopic status. Specific IgG to the major cow's milk allergen beta-lactoglobulin is the first to appear in infancy, in allergic and non-allergic children (7) OVA-specific IgG falls between birth (maternal levels) and six months but then rises rapidly to peak in infancy, and then gradually falls in later childhood (7,8). High levels of OVA IgG1 predominate in the first 18 months followed by dominance by OVA IgG4 at 8 years, this occurs in populations selected to be at risk of allergy (9) and also unselected populations (9). The peak levels of bLG and OVA specific IgG occur in infancy when milk and egg exposure begin. In accordance with the allergic march, peak levels of IgG to indoor and outdoor allergens (cat, grass and house dust mite), to which children are exposed later in childhood, occur at later ages (10). This suggests that the production of specific IgG is stimulated by allergen exposure and is not limited to those who develop atopy or allergic disease.

However, in many studies high specific IgE in allergic individuals is matched by a high specific IgG (8,9). Further, there is evidence that children who mount a more vigorous specific IgG response to food and aeroallergen in infancy are more likely to develop symptomatic allergic disease later in childhood (8). Interestingly, infants who have a vigorous early life IgG response to food allergens are more likely to develop later sensitisation to unrelated *aero*allergens. In a prospective study of IgG levels in a birth cohort, high levels of OVA specific IgG1 predicted later development of asthma (8). This may represent dysregulation of a vigorous immune response where high IgG production accompanies that of IgE.

There is no information on whether IgG responses are initiated in the gut mucosal or other peripheral lymph nodes, but it interesting to note that allergens which enter via the mucosal immune system and cause a vigorous IgG response predict the development of sensitisation and allergy to airborne allergens in some individuals (10).

Early studies on the mechanism of venom immunotherapy, which is highly effective, led to the suggestion that the generation of specific IgG was important for protection (11,12). However subsequent studies showed untreated bee keepers with

anaphylaxis to bee stings had high venom-IgG levels, and a lack of correlation between the level of venom–specific IgG and protection from stings (13). As these patients were frequently stung, it seemed the IgG level related more to immunisation and allergen dose (13). Similarly no correlation was found between ratios of venom-specific IgG/IgE, IgG1/IgE or IgG4/IgE and protection (13-17).

However immunotherapy clearly leads to generation of specific IgG, particularly IgG4, and a gradual fall in specific IgE, with a rise in specific G: E ratio (mainly in G4 to E). This is a consequence of the switch from a TH2-dominant (allergy-skewed lymphocyte responses) to a TH-1 dominant (or non-allergy skewed) immune response (17) and generation of IL-10 seems an essential early step (18,19). Thus an increase in the specific IgG/ IgE ratio seems to be an important factor in resolution of allergy, although this is not always apparent from absolute serum levels.

This may be because other factors are also important in determining mast cell degranulation on exposure to allergen, such as the amount of IgE bound to mast cells or signalling through FcγR II receptors (20). Other factors including cytokines have been shown to influence whether ‘tolerance’ is maintained (IL-12) or allergy recurs (IL-15) after immunotherapy.

Allergy, sensitisation, tolerance and resolution

The difference between allergy, sensitisation and tolerance is of interest. This is not simply related to specific IgE, which may be present in all three categories. For many years allergists have known that a proportion of patients have specific IgE but are not clinically allergic, i.e. are sensitised. In allergy and sensitisation specific IgE is present but only the former group has symptoms on exposure to allergen. We have previously studied this phenomenon in peanut allergy and identified a certain size of skin prick test, above which patients always react clinically (2). In addition we showed there is a ‘grey area’ where specific IgE is positive but patients may be either allergic or tolerant (2). The reasons for this difference have not been investigated, but the balance between specific IgG and IgE may be a factor. Separately we have showed that in allergic patients there is no correlation between

the absolute level of nut-specific IgE and severity across a wide range, from mild localised urticaria through to anaphylaxis (2).

An attractive role for IgG would be as a specific 'blocking' antibody, either neutralising specific IgE or via downstream effects on the IgG surface receptor. For example in chronic helminth infection the hypo-responsive form of the infection is characterised by prevalence of IgG4, compared to IgE which dominates in the aggressive form (21). However, IgG4 switching has also been described as a marker of Th2 immune deviation with IgE and IgG4 classes sharing regulation by IL-4 in *in vitro* experiments (22). In other situations where allergen tolerance is induced, uncoupling of IgE and IgG4 occurs e.g. during insect venom immunotherapy where IgG4 levels rise as IgE falls. IL-10 is secreted from regulatory T cell subsets and acts to suppress inflammation. IL-10 also stimulates IgG4 production (23), suggesting an anti-inflammatory role for IgG4.
For IgG to effectively block IgE binding, both should share epitopes. Previous studies looked at IgG and IgE epitope for food allergens, in human cow's milk allergy and in the Brown Norway rat model against b-lactoglobulin (24,25). In these studies, whilst IgG and IgE epitopes were generally on the same protein sequence some IgE epitopes did not bind significant amounts of IgG e.g. allergen sites of bovine a-lactalbumin and b-lactoglobulin did not always correspond with antigenic determinants. Large inter-individual variation between epitopes bound by IgG and IgE was shown.

Why study peanut and egg allergy?

Egg and peanut allergy are appropriate food allergies to study for a number of reasons. First they are two of the commonest foods to cause allergy, both causing onset in young children. Egg allergy resolves in about 90% of children by age 5 years (26), but in about 50% by age 3 years. In contrast peanut allergy occurs in older children and is thought to be more persistent and has been shown to resolve in 22%, but only mild cases were enrolled in this study (27). In addition, there are few studies on the development and role of peanut IgG.

Egg allergy will provide a large group of patients in whom allergy has resolved at a relatively early age. The reasons for resolution of egg allergy have never been studied, but it is known by clinicians that most children retain egg-specific IgE in the early years after resolution. In our clinical database of 1,500 peanut and nut allergy patients, about 10% also have a history of egg allergy, so that the role of IgG responses to two important foods can be studied in one group of patients.
A fundamental problem with previous studies of IgG is that the majority used assays which measured antibody in arbitrary units of optical density. The difficulty than is that results for different allergens cannot be compared and further, IgG results for the same allergens cannot be compared between different research groups. The aim of this study was to examine levels of specific IgG, IgG1 and IgG4 in children with peanut allergy, egg allergy, resolved egg allergy and non-allergic controls using an enzyme-linked immunosorbent assay (ELISA) optimised for quantifying antibody levels in μg/ml. This would allow comparisons to be made between antibodies of different subclasses and allergen specificities.

4. Method

Study populations

Peanut allergic population: peanut allergy was diagnosed by (a) a recent convincing history of clinical reaction to peanut with typical symptoms and (b) supported by the 95% predictive value for SPT weal diameter >/= 8mm to peanut (ALK-Abello, Horsholm, Denmark) or peanut-specific IgE >/= 15kU/l (ImmunoCap FEIA, Phadia, Uppsala, Sweden) on study entry. Challenges were not performed.

Egg-allergic and egg-resolved populations: only children with a confirmed history of a typical type-1 hypersensitivity reaction to egg and a SPT weal diameter >/= 3mm to egg egg (Allergy Therapeutics, West Sussex, UK) at time of diagnosis were enrolled for open oral challenge to determine their current clinical reactivity to egg. Anti-histamines were stopped 72h before challenge. Incremental doses of cooked or uncooked egg (cumulative doses 12g and 21.5g respectively) were given at 10min intervals. The challenge was stopped when either all doses were tolerated or an objective reaction (development of two or more of erythema, urticaria (distant to the mouth) or angioedema, rhinoconjunctivitis, wheeze, abdominal pain or vomiting) occurred. Assessments were made by two clinical observers experienced in allergy. Families were instructed to report any late onset symptoms to the study team by telephone. SPT and egg-specific IgE measurements were repeated on day of challenge. Children who reacted to *either* cooked or uncooked egg were classified as egg-allergic; children who tolerated *both* cooked and uncooked egg had resolved egg allergy.

Non-allergic population: these were recruited from children attending hospital for orthopaedic surgery. Parental questionnaires reported no history of allergy-related illness and that children were not actively avoiding egg or peanut. There were no symptoms suggestive of food intolerance. Egg and peanut-specific IgE were not detected (<0.35kU/l) in these controls. We further identified children with no history of sensitisation to peanut (specific IgE <0.35kU/L) but who were allergic to egg to examine the influence of another food allergy. The study was approved by the local Research Ethics Committee, and informed consent was obtained.

Preparation of crude peanut extract and ovalbumin proteins

Crude peanut extract (CPE) was prepared in house. 50g crushed peanuts were defatted by hexane-extraction for 2h at 4°C and air-drying the supernatant overnight. The defatted extract was dialysed into 50mM ammonium bicarbonate and freeze-dried for 48h before reconstitution in PBS at 10mg/ml. Aliquots were stored at -20°C until use. Ovalbumin (OVA) of grade V purity (Sigma, Poole, UK) was reconstituted in PBS at 10mg/ml. CPE and OVA concentrations were measured by the bichinchoninic acid assay (Pierce Biotechnology, Rockford, USA).

Quantification of specific antibody titres in reference anti-sera

Sera was pooled from 27 peanut allergic and 23 egg allergic subjects with high specific IgE titres against each food allergen (specific IgE >5.0 kU/l). Specific IgG, IgG1 and IgG4 titres in each reference pool were quantified by a competitive ELISA method modified from Rieben & Blaser [28, 29]. Multibind microtitre plates (Greiner Bio-one, Gloucester, UK) were coated for 2h at room temperature (RT) with CPE (2μg/ml) or OVA (5μg/ml) in 100μl carbonate/bicarbonate buffer (pH 9.6). Plates were washed three times in PBS containing 0.1% Tween 20 (PBST) and blocked with 5% heat-inactivated foetal calf serum (Sigma) in PBST for 1h at RT. One dilution of reference anti-sera was added to all wells and incubated overnight at 4°C. Serial two-fold dilutions of the monoclonal anti-human IgG, anti-IgG1 or anti-IgG4 (WHO/IUIS clones HP6064/8a4, HP6012/NL16 or HP6011/RJ4, respectively) antibodies were added in triplicate overnight at 4°C. Plates were washed three times in PBST, and the bound monoclonal antibodies detected by alkaline phosphatase-conjugated goat anti-mouse IgG (Sigma, 1:1000) incubated for 3h at RT. After two PBST washes and a final wash in distilled water, 100μl of p-nitrophenyl phosphate (Sigma, 1mg/ml in diethanolamine buffer pH 9.8) was added. Optical densities at 405nm were read (BioRad Model 550, Bio-Rad, Hemel Hempstead, UK). An optimal concentration of the monoclonal was selected based on the titration curve, and the assay repeated in the presence of pre-titrated amounts of inhibiting purified human IgG (Calbiochem, San Diego, USA), IgG1 kappa (Sigma) or IgG4 kappa (Sigma). The CPE- or OVA-specific IgG titres of each reference pool were calculated from the derived inhibition curves. Five independent measurements were made for each

pool. The antibody titres (mean ± standard deviation in μg/ml) of the CPE-specific pool were 96.66 ± 2.13 (IgG), 19.93 ± 1.49 (IgG1) and 35.58 ± 1.68 (IgG4). Sensitivity limits (ng/ml) were 2.5 (IgG), 4.0 (IgG1) and 7.0 (IgG4). Inter-assay coefficients of variation (CV) were 3.8% (IgG), 6.2% (IgG1) and 9.4% (IgG4). The antibody titres (μg/ml) of the OVA-specific pool were 92.18 ± 0.69 (IgG), 7.47 ± 0.33 (IgG1) and 83.6 ± 4.14 (IgG4). Sensitivity limits (ng/ml) were 4.0 (IgG), 6.0 (IgG1) and 4.0 (IgG4). Inter-assay CV were 6.0% (IgG), 8.8% (IgG1) and 9.1% (IgG4).

Quantification of specific antibody titres in patient sera

Serial dilutions of reference anti-sera and at least three dilutions of patient sera (1:20 to 1:1600) were incubated with CPE or OVA-coated plates in duplicate overnight at 4°C. Each subclass of bound anti-sera was detected with monoclonal anti-human IgG (8a4), anti-IgG1 (NL16) or anti-IgG4 (RJ4) used at previously selected concentrations, overnight at 4°C. Bound monoclonal antibodies were detected with alkaline phosphatase-conjugated goat anti-mouse IgG (Sigma, 1:1000) for 3h at RT, followed by p-nitrophenyl phosphate substrate addition and reading of optical densities at 405nm. Washes and incubation conditions were performed as for quantification of reference anti-sera pools. Specific antibody titres in μg/ml were extrapolated from the standard curve of reference anti-sera.

Statistical Analysis

IgG values were not normally distributed so non-parametric tests were applied. The Mann-Whitney U test was used to compare medians between groups. The Kruskal Wallis test was used to compared medians in three groups. The Spearman rank test was used to assess the significance of correlations (rho) between different parameters measured, e.g. age, antibody titres, SPT weal diameters. All tests were performed using SPSS (version 15.0, SPSS Inc., Chicago, IL). Graphs were generated in Graphpad Prism (version 3.02, GraphPad Software Inc., San Diego, CA).

5. Results

Egg-specific IgE and OVA-specific IgG responses in children

Oral challenges with cooked or uncooked egg were performed on 63 children who all had a confirmed history of egg allergy. Children who could tolerate cooked egg were challenged with uncooked egg. 23 children passed the challenge with uncooked egg and have resolved egg allergy. Children who reacted to either cooked egg (n=17) or uncooked egg (n=23) were still allergic (total n=40). Age, total and specific IgE, and SPT weal diameters of children who were challenged and of 18 age-matched non-allergic controls are presented in Table 1. Total IgE was higher in children with active and resolved egg allergy compared to controls ($p<0.01$) with no significant differences between the allergic and resolved groups. In contrast, egg-specific IgE and SPT weal diameters were significantly higher in the allergic group compared to the resolved group ($p<0.05$). Egg-specific IgE titres were positively correlated to total IgE (rho = 0.58; $p<0.01$) but not to SPT (rho = 0.3; $p = 0.9$). Age was significantly correlated to total IgE (but not specific IgE) in egg allergic (rho = 0.63; $p<0.01$) and resolved (rho = 0.79; $p<0.01$) children but not controls.

OVA-specific IgG, IgG1 and IgG4 levels are presented in Fig 1(a) to 1(c). Age was not correlated to IgG subclass. Significant levels of specific IgG, IgG1 and IgG4 were detected in all subjects. There were no significant differences in specific IgG, IgG1 and IgG4 levels between the non-allergic controls (n=18), egg-allergic (n=40) or egg-resolved (n=23) groups. The ratios of specific IgG4:IgG1 were also not significantly different between these three groups. In subjects with egg-specific IgE >0.35kU/l, positive correlations existed between specific IgE and specific IgG (rho = 0.54; $p<0.01$), IgG1 (rho = 0.49; $p<0.01$) and IgG4 (rho = 0.38; $p<0.05$).

The IgG4 subclass titres were spread over a wider range (0.03 to 488μg/ml) compared to IgG1 (0.2 to 17μg/ml) and IgG4 was the predominant subclass in 78% (14/18) of controls, 47% (19/40) of egg-allergic and 57% (13/23) of egg-resolved subjects. IgG1 and IgG4 were both strongly correlated (rho > 0.6; $p<0.01$) to total

specific IgG in all groups, but correlations between the two subclasses were seen only in the control (rho = 0.60; p<0.01) and egg-allergic (rho = 0.42; p<0.01) groups.

Peanut-specific IgE and IgG responses

CPE-specific antibody levels were measured in 92 children. The populations compared included the same non-allergic controls (n=18), children with peanut allergy (n=59), and 22 children who were not sensitised to peanuts but were egg-allergic (NPS). Specific IgE was correlated to total IgE (rho = 0.54 p<0.01) but not to SPT wheal size. These three groups were age-matched and there were no significant correlations of age with total IgE, peanut-specific IgE or CPE-specific IgG subclasses in any of the groups (table 2).

CPE-specific IgG, IgG1 and IgG4 levels are presented in Fig 2(a) to 2(c). CPE-specific IgG1 was detected in all subjects in this study, including all non-allergic controls and all NPS subjects. CPE-specific IgG4 was detected in all but three subjects (two controls and one NPS). CPE-specific IgG, IgG1 and IgG4 levels were significantly higher in children with peanut allergy (p<0.01) compared to controls and NPS subjects. CPE-specific IgG, IgG1 and IgG4 levels of NPS subjects were not significantly different from control levels. The ratio of specific IgG4:IgG1 did not differ between the three groups. In children who had detectable peanut-specific IgE, significant positive correlations existed between specific IgE and specific IgG (rho = 0.72; p<0.01), IgG1 (rho = 0.67; p<0.01) and IgG4 (rho = 0.49; p<0.01).

In the 18 non-allergic controls, both CPE- and OVA-specific IgG levels were measured. OVA-specific IgG levels were significantly higher than CPE-specific IgG (OVA-specific vs. CPE-specific median antibody titres in μg/ml = 15.9 vs. 2.2 (IgG), 1.3 vs. 0.6 (IgG1), 7.9 vs. 0.7 (IgG4); p<0.01). In peanut allergy, CPE-specific IgG were increased (median levels in μg/ml = 17 (IgG), 3.3(IgG1), 5.2 (IgG4)) to levels which were comparable to OVA-specific IgG.

The IgG4 subclass titres were spread over a wider range (0-157µg/ml) than IgG1 (0.5-55µg/ml). IgG4 was the predominant subclass in 44% (8/18) controls, 69% (41/59) peanut allergic and 65% (17/26) of NPS subjects. Both subclasses were correlated to total specific IgG (rho = 0.75, $p<0.01$ for IgG1; rho = 0.61, $p<0.01$ for IgG4), and positive correlations existed between IgG1 and IgG4 (rho > 0.49; $p<0.05$) in all subject groups.

Sensitivity and specificity of specific total IgG for diagnosis of food allergy

For both egg and peanut specific IgG there was a large overlap in the range of values between allergic subjects and non-allergic controls (table 3). Taking several cut-off values for OVA and CPE total IgG, these tests had very poor sensitivity and specificity for predicting allergic status (table 4).

6. Discussion

We have developed a sensitive and robust ELISA system for measuring serum CPE- and OVA-specific IgG, IgG1 and IgG4 in absolute units (μg/ml). The subject groups were well characterised and included non-allergic controls, patients with active allergy, resolved allergy and those who were allergic to another food. By determination of specific antibody titres in μg/ml, we were able to reveal differences between IgG responses to two different food allergens. We also observed quantitative differences in IgG1 and IgG4 subclass expression.

An interesting finding of this study was the distinct pattern of IgG responses to peanut and egg proteins in children. OVA-specific IgG levels were significantly higher across all subject groups than CPE-specific IgG in non-allergic subjects. Significant OVA-specific IgG responses were detected in all children including controls, with no differences between egg allergic and resolved subjects. In contrast, CPE-specific IgG, whilst also detected in all children, were at low levels in controls and non-peanut sensitised subjects. Increased IgG responses to peanut were specifically associated with peanut allergy, with CPE-specific IgG levels reaching that comparable to OVA-specific IgG levels.

The finding of significant IgG responses to egg in all groups, including non-allergics, supports the notion that IgG production can be a normal physiological response to frequently ingested proteins. Others have also reported the presence of IgG antibodies to dietary antigens, in particular egg and milk, in a large proportion of healthy subjects [30-32]. CPE-specific IgG was also detected in all subjects in the study.

The larger IgG response to egg compared to peanut (in controls) may be due to earlier introduction of egg and/or a more frequent consumption of larger amounts of egg in these children. Indeed, exposure to cow's milk during the first 3 months of life is associated with high levels of IgG to beta-lactoglobulin [33], and OVA-specific IgG levels can reflect dietary intake of egg in healthy adults over a 20-week period [34].

On the other hand, our findings also suggest that for other allergens (e.g. peanut) food-specific IgG levels are not solely related to dietary exposure. IgG responses to peanut were significantly elevated in children with peanut allergy, despite current peanut avoidance. Although detailed consumption records were not available, it is known that 80% of peanut allergic children report reactions to the first apparent peanut ingestion [30]. Therefore it is unlikely that they would have eaten substantial amounts of peanut prior to diagnosis. In this setting, CPE-specific IgG upregulation is associated with the allergic state rather than dietary exposure. This enhanced IgG response to peanut which is specific to peanut-allergics (and not found in NPS subjects) extends the findings of Kolopp-Sarda and colleagues, who reported higher peanut-specific IgG in peanut-allergic adults compared to controls [35].

Platts-Mills and colleagues proposed that high cat allergen exposure results in a protective response characterised by high levels of specific IgG and IgG4 and absence of specific IgE [36]. Sletten's findings that high beta-lactoglobulin specific IgG4 is associated with tolerance to milk also seems to support the protective roles for IgG4 in allergy [37]. However, our results suggest that this might not be true of peanut allergy, where the association of high IgG1 and IgG4 levels with clinical reactivity argues against protective or blocking functions for IgG1 and IgG4. Although the IgG1 subclass has been reported to make the largest contribution to total IgG and IgG4 the smallest [38], we found a wide range of IgG4:IgG1 ratios in all subject , which were not associated with allergic status. Therefore, increased food-specific IgG4 subclass is not necessarily associated with Th2-type (classically allergic-type) lymphocyte responses. OVA-specific and CPE-specific IgG4 predominated over IgG1 in two thirds of all subjects. Whilst it is not known if IgG4 predominance extends to all food allergens, or indeed to other allergens, preliminary findings in our laboratory showed that IgG4 to a systemic antigen (tetanus toxoid) predominates in only 25% of subjects. This suggests that the route of sensitisation may be associated with the predominance of an IgG1 or IgG4 immune response.

Commercial tests are available for detection of food-specific IgG in serum, which are promoted as diagnostic tools for food intolerance. We therefore tested the

assumption that detection of food-specific IgG is associated with allergy or intolerance. It is important to appreciate that in this project we have characterised our clinical groups precisely, so we can be confident that our allergic groups have egg or peanut allergy and that our non-allergic group does not have egg or peanut allergy or intolerance. Our findings demonstrate that all subjects with or without food allergy produce detectable levels of OVA and CPE IgG and the observed ranges of IgG levels in allergic and control subjects overlap significantly. Further, elevated levels of OVA IgG are found in egg allergic and non-allergic subjects alike. In the case of peanut, CPE IgG is detectable in non-allergic subjects and elevated levels are found in allergic subjects. However, even though the median specific IgG levels in each group are statistically different, there is a large overlap in the range of results. A useful and accepted method of measuring the effectiveness of a diagnostic test is to calculate its sensitivity and specificity (see definitions; section 2). Using various cut-off values of egg and peanut specific IgG has revealed uniformly poor values of sensitivity and specificity and therefore these tests are of no diagnostic use (table 4). Therefore our findings do not support the use of tests for specific IgG as a diagnostic tool in food intolerance and/or allergy.

It is thus clear that different allergens, route, dosage and timing of exposure can induce varied magnitudes and distinct patterns of IgG responses. In this study, the patterns of IgG responses to two food allergens are shown to be very distinct. For egg allergens, IgG responses may reflect previous exposure and not indicative of disease; whereas for the peanut allergen, a higher IgG response is associated with clinical allergy, despite low exposure. Tests for specific IgG to food are not of value in diagnosing clinical allergy.

Possible future work

1. Examine the roles of IFNγ, IL-4 and IL-10 and their influence on IgG, G1 and G4 in the food allergy model

2. Examine the dominance of IgG subclasses in immune responses to several different foods, aero and systemically administered allergens/antigens in atopic and non-atopic individuals.

3. Prospective examination of OVA and CPE IgG and subclass levels in infants on and off avoidance diets.

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Tables and figures

Table 1. Characteristics of groups where OVA-specific IgG levels were compared (n=81).

	Controls	Egg-allergic	Egg-resolved
Total number (%male)	18 (16.8)	40 (37.4)	23 (21.5)
Age (years)	7.7 (8.5) 0.8 – 15.4	5.6 (6.8) 0.9 – 15.3	5.8 (7.5) 2.2 – 14.1
Total IgE (kU/l)	23 (86) 2 – 243	270[a] (796) 10 – 2828	394[a] (666) 2 – 2040
Egg-specific IgE (kU/l)	<0.35	2.4[a, b] (5.8) 0.35[f] – 39	0 – 4.4[a, c] 0.35 – 4.38
Egg SPT weal diameter (mm)	ND	4[a, b, e] (3.5) 0 – 12	0 [a, c] (2) 0 – 4

Data expressed as numbers (percentages) for age. For other parameters, data expressed as median (interquartile range) in top row, range in bottom row. [a]elevated compared to controls (Mann Whitney U test $p<0.05$); [b]elevated in egg-allergic compared to egg-resolved group (Mann Whitney U test $p<0.05$); [c]10/23 subjects had IgE>0.35kU/l; [d]6/23 subjects had SPT>0mm but </=4mm; [e]2/40 subjects has SPT=0 and 10/40 subjects had SPT<4mm; [f]1/40 subject had specific IgE<0.35kU/l. ND, not determined.

Table 2. Characteristics of groups where CPE-specific IgG levels were compared (n=103).

	Controls	Peanut-allergic	NPS
Total number (%male)	18 (17.5)	59 (57.3)	26 (25.2)
Age (years)	7.7 (8.5) 0.8 – 15.4	10.5 (5.8) 1.9 – 15.1	4.5 (3.0) 1.8 – 15.3
Total IgE (kU/l)	23 (86) 2 – 243	600[a] (1503) 22 - 6690	176[b] (343) 2 - 983
Peanut-specific IgE (kU/l)	<0.35	23.7[a] (80.4) 0.48 – 100	<0.35
Peanut SPT weal diameter (mm)	ND	10 [a] (4) 3 – 12	ND

Data expressed as numbers (percentages) for age. For other parameters, data expressed as median (interquartile range) in top row, range in bottom row. [a]elevated compared to controls and to NPS groups (Kruskal Wallis test for all 3 groups, $p<0.05$; Mann-Whitney U test for each paired group compared, $p<0.05$); [b]lower levels compared to peanut-allergic group (Mann-Whitney U test, $p<0.05$). ND, not determined. NPS, non-peanut-sensitised but egg-allergic.

Table 3a

Range, inter-quartile range (IQR) and median values of OVA specific total IgG for egg allergic and control subjects.

µg/ml	Range (IQR)	Median
Egg allergic	0.8-433 (5.5-22.1)	10.2
Control	0.7-914 (8.7-33.2)	16.0

Table 3b

Range, inter-quartile range (IQR) and median values of peanut specific total IgG for peanut allergic and control subjects.

µg/ml	Range (IQR)	Median
Peanut allergic	2-272 (7.7-48)	17.6
Control	0.8-32 (0.9-6.9)	2.1

Table 4a

Number of egg allergic and control children with OVA IgG levels < or >/= 0.8µg/ml. Sensitivity=100%; specificity=6%

	Egg allergic	Control
OVA IgG >/=0.8µg/ml	40	17
OVA IgG <0.8µg/ml	0	1

Table 4b

Number of egg allergic and control children with OVA IgG levels < or >/= 914.5µg/ml. Sensitivity=0; specificity=100

	Egg allergic	Control
OVA IgG >/=914.5µg/ml	0	0
OVA IgG <914.5µg/ml	40	18

Table 4c

Number of peanut allergic and control children with CPE IgG levels < or >/= 2.0µg/ml. Sensitivity=100%; specificity=50%

	Peanut allergic	Control
CPE IgG >/=2.0µg/ml	59	9
CPE IgG <2.0µg/ml	0	9

Table 4d

Number of peanut allergic and control children with CPE IgG levels < or >/= 32.4µg/ml. Sensitivity=31%; specificity=100%

	Peanut allergic	Control
CPE IgG >/=32.4µg/ml	18	0
CPE IgG <32.4µg/ml	41	18

Figure 1. Ovalbumin-specific IgG (a), IgG1 (b) and IgG4 (c) levels in controls (n=18), egg-allergic (n=40) and egg-resolved (n=23) groups. Median bar and individual titres (dots) are indicated. Comparisons of medians between groups by Mann-Whitney U test marked by bars and p values. EA, egg-allergic; ER, egg-resolved' ns, not significant.

Figure 2. CPE-specific IgG (a), IgG1 (b) and IgG4 (c) levels in controls (n=18), peanut-allergic (n=59) and NPS (n=26) groups. Median bar and individual titres (dots) are indicated. Comparisons of medians between groups by Mann-Whitney U test marked by bars and p values. PA, peanut allergic; NPS, non-peanut-sensitised but egg-allergic; ns, not significant.

Figure 1 (a), 1 (b), 1(c).

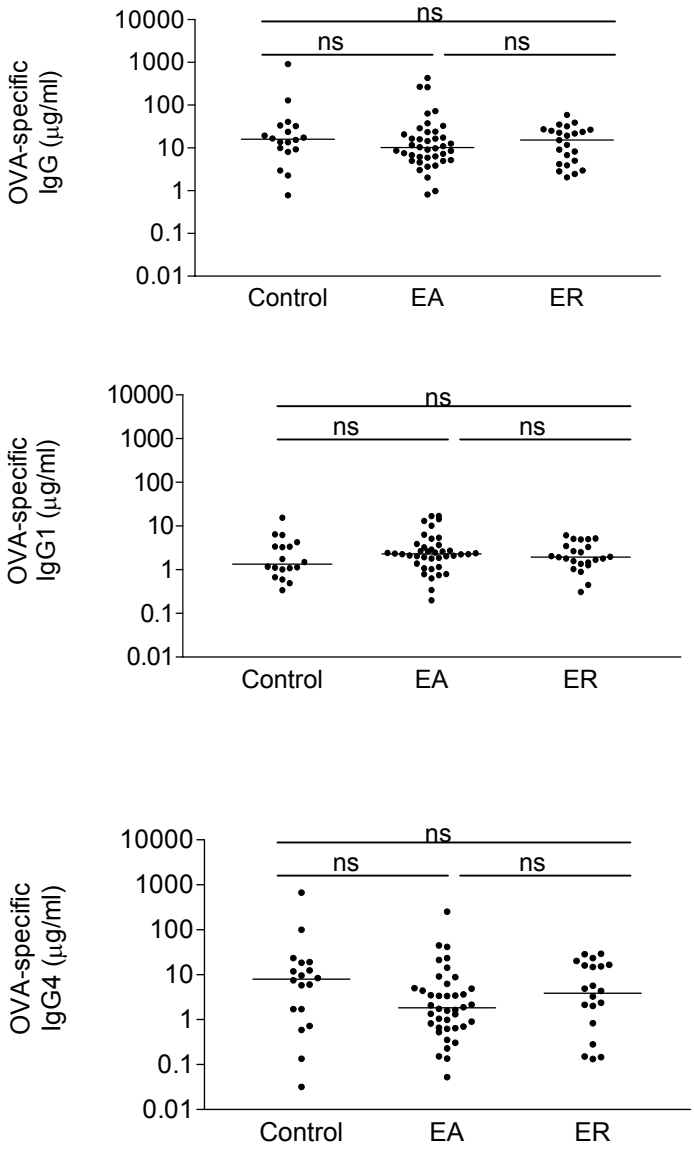

Figure 2(a), 2(b), 2(c)