

Original article

Cellular immune responses to ovalbumin and house dust mite in egg-allergic children

Background: Although IgE-mediated food (egg) allergy is typically lost with age the underlying immune mechanisms are not understood, particularly in relation to the development of persistent IgE-mediated aeroallergen sensitivity.

Methods: Lymphoproliferation and cytokine responses (IL-5, IL-10, IL-13 and IFN- γ) to house dust mite (HDM) allergen and egg ovalbumin (OVA) were assessed using peripheral blood mononuclear cells (PBMC) from children aged 6 months to 5 years ($n = 59$) with acute IgE-mediated egg allergy (urticaria and angioedema or anaphylaxis), as confirmed by positive skin prick testing (SPT). Of these 46 had positive SPT on the day of blood collection and 13 had outgrown egg allergy (negative SPT and successful egg challenge). Where possible, responses were compared with previous data from nonallergic children of similar ages ($n = 107$).

Results: Transient lymphoproliferative responses to OVA were seen in both egg-allergic and nonallergic children, but were more marked and more prolonged in egg-allergic children. Younger egg-allergic children (<18 months) showed a mixed Th0 cytokine response to OVA, with readily detectable IFN- γ , IL-5, IL-13 IL-10. Although IL-13 and IL-5 responses (OVA) correlated in younger egg-allergic children, there was a dissociation of these Th2 responses with age. Loss of clinical reactivity to egg was associated with almost complete loss of IL-5 responses and OVA-specific lymphoproliferation. Although IL-13 levels tended to be lower with age, this was not significant. Strong IFN- γ and IL-10 responses to OVA persisted in older children after loss of OVA-specific lymphoproliferation. Lymphoproliferative responses to HDM also developed earlier in egg-allergic children compared with nonallergic children. Th1 (IFN- γ) responses to HDM were largely below detection prior to 18 months of age, but increased significantly with age. In egg-allergic children Th2 (IL-5, IL-13) HDM responses also progressively increased with age. At 3 years of age almost all egg-allergic children had positive SPT to HDM and positive lymphoproliferative responses to HDM, with strong Th1 and Th2 (Th0) cytokine production.

Conclusions: IL-5 responses (rather than IL-13) responses most closely reflected clinical food allergy, with dissociation of IL-5 and IL-13 responses in older and egg-tolerant children. In this population, food and aeroallergen sensitivity was not associated with inability to produce IFN- γ , but rather with mixed Th2 and Th1 (Th0) responses. Strong IL-10 and IFN- γ responses were associated with the development of tolerance, suggesting persistent 'regulatory' populations of OVA-specific T cells, rather than clonal deletion of OVA responsive T-cells.

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Despite increasing morbidity and mortality from IgE-mediated allergic diseases, the contributing factors and underlying mechanisms are still poorly understood. Dysfunctional early immune development in allergic infants appears to result in inappropriate Type 2 T helper cell (Th2) responses to allergens, rather than the Type 1 (Th1) responses seen in nonatopic infants. Th2 responses to food allergens are frequently the first manifestation of allergic disease (1). Although these

responses are usually also short-lived, they can be debilitating or even life-threatening. Moreover, these children appear at much greater risk of developing subsequent, persistent allergic Th2-sensitization to aeroallergens (2–9). The underlying T cell cytokine responses which are associated with food allergen disease and its subsequent resolution are not well documented, particularly in relation to developing aeroallergen responses. This study examines the cyto-

kine responses to both food and inhalant allergens at different ages in young children with IgE-mediated food (egg) allergy.

Egg-allergic infants are of particular interest because this is the most frequent allergic hypersensitivity in young children (10), usually evident in the first year of life (8). There is a paucity of studies describing the cytokine response in these symptomatic infants, and results are conflicting (11–13). Most affected children become tolerant to egg in the preschool period, but the cytokine responses associated with loss of clinical disease are not well documented. Previous studies by our group (14) have shown that most children (regardless of atopic status) lose their *in vitro* lymphoproliferative responses to egg allergens in the first years of life. However, many of these children continued to show T cell cytokine responses to egg ovalbumin (OVA) *in vitro*, suggesting that food-responsive T cells may not be 'clonally deleted' as previously thought. Examining food allergen-specific cytokine responses in IgE-mediated food allergic children who have outgrown their allergy was therefore also of prime interest in the present study. Furthermore, as egg-allergic children are at very high risk of subsequent sensitivity to perennial inhalant allergens, this population also offers the opportunity to further study evolving presymptomatic cellular responses to house dust mite (HDM) allergen.

Material and methods

Overview

This study is a cross-sectional assessment of cellular immune responses to egg and HDM responses in symptomatic egg-allergic children at different ages. Children who had outgrown previous food allergy were assessed separately for comparison.

Egg-allergic subjects

Children (between 6 months and 5 years) were recruited from Immunology Outpatient Clinics at Princess Margaret Hospital, Perth, Western Australia. All eligible subjects ($n = 59$) had a history highly suggestive of egg hypersensitivity with acute urticaria and angioedema or anaphylaxis within minutes of egg ingestion, and egg-specific IgE measured by either skin prick testing (SPT) or RAST. A wheal size of more than 3 mm was considered positive on SPT. Because most children had life-threatening reactions to traces of egg protein it was considered too dangerous and unethical to perform oral challenges. Although this may be considered a limitation of the study, in all cases there was virtually no doubt about the clinical diagnosis because of the severity of symptoms that were clearly related to egg ingestion. There were 46 children with evidence of sensitization at the time of blood collection (SPT +), and 13 who had outgrown their allergy to egg (successful graded oral challenge and SPT –). The food challenge was only performed in children who had become skin test negative with age (because of their previous potentially life-threatening reactions). This consisted of serial administration of decreasing dilutions of raw whole egg in water (1 : 1000, 1 : 100, 1 : 10, neat egg).

The SPT + children were divided up in the following age groups for

comparison: SPT positive children <18 months (group 1, $n = 16$), 18 months to 3 years (group 2, $n = 12$), and >3 years of age (group 3, $n = 18$).

Subjects were excluded from the study if there was a history of immunodeficiency or if they were on medications that affect immune function. A detailed allergic history and physical examination was performed at time of enrolment. The diagnosis of asthma was based on a history of doctor-diagnosed disease with clinical response to bronchodilators (and steroids in some cases). Atopic eczema/dermatitis syndrome (AEDS) (15) was also based on diagnosis by a pediatrician or pediatric immunologist, and a history of response to topical steroids. Blood samples were collected the same day as SPT were performed. Approximately 5–10 ml of blood was collected by venipuncture into heparinized RPMI culture medium for transport at room temperature.

This study was approved by the Princess Margaret Hospital Ethics Committee. It was not considered appropriate to perform oral challenges for research purposes, because of the high risk of life-threatening reactions (anaphylaxis).

Non-allergic control subjects

Data previously collected from nonallergic children ($n = 106$) in other studies (14, 16–18) were used for comparison (also aged 6 months to 5 years). These children had no evidence of clinical allergy (IgE associated AEDS, asthma, or allergic rhinitis) and were all SPT negative. They were divided into the same age groups as the egg-allergic children, thus: <18 months ($n = 36$), 18 months to 3 years ($n = 31$), and >3 years of age ($n = 39$). These children previously underwent lymphoproliferative studies under identical conditions (below) as the egg-allergic children. This group also had cytokine studies, but under different conditions (supernatants collected at 24 h rather than 48 h). The main cytokines measured included IL-13, IFN- γ , IL-10, IL-5 mRNA and IL-4 mRNA. These studies were all performed in the last 3–5 years and used similar technology and reagents. Although cytokine data cannot be used in direct comparisons (between the populations), they have been used here to compare the age-related trends of each population.

Cell separation

Peripheral blood mononuclear cells (PBMCs) were isolated by gradient centrifugation in Ficoll-Hypaque (Nycomed Pharma, Norway) density gradient. Using established techniques (17) which have been shown not to significantly alter cell function, isolated PBMCs were cryopreserved in 7.5% dimethyl sulfoxide (DMSO) (AnalaR, Merck, Australia) supplemented with 2% fetal calf serum (MultiSer, Biosciences, Australia) and stored in liquid nitrogen.

Preparation and stimulation of PBMCs

PBMCs were thawed, washed and viable cells counted using Trypan Blue exclusion. The cells were washed and resuspended at 1×10^6 cells/ml in AIM V (Gibco, Life Technology, UK). A total of 2×10^5 cells/well were cultured in triplicate in microwells either alone or with: 100 μ g/ml ovalbumin (Sigma Chemicals, Castle Hill, Australia), unfractionated 10 μ g/ml HDM extract (CSL, Australia), 1 μ g/ml PHA (phytohaemagglutinin) (HA16, Murex, Biotech Ltd, UK), 100 μ g/ml or 1000 μ g/ml ovomucoid (Sigma). Cultures were incubated for 6 days at 37°C and 5% CO₂. PHA stimulated cells were incubated for 3 days. DNA synthesis was measured as incorporation of ³H-thymidine, after pulse labelling 16 h prior to harvest. Data were expressed as delta d.p.m. per culture, designating levels of incorporation above background (unstimulated) control cultures. Responses which were more than twice that of background level and >1000 d.p.m. above backgrounds were considered positive (19).

For analysis of cytokine responses PBMCs were suspended at

2×10^6 cells/ml in AIM V for allergen stimulation or 1×10^6 cells/ml for PHA stimulation. Cells were cultured for 48 h alone or supplemented with: ovalbumin (OVA) (100 µg/ml), HDM (10 µg/ml), ovomucoid (OM) (100 µg/ml) or PHA (1 µg/ml). Supernatants were assayed for the presence of IL-5, IL-10, IL-13 or IFN- γ using commercial ELISA kits (Pharmingen, Becton-Dickenson, Australia). This time-point (48 h) for the cultures was chosen as optimal for the measurement of most cytokine proteins (our previous studies measured cytokine protein but principally focused on cytokine mRNA). The sensitivities of the ELISAs were 4 pg/ml (IL-5), 5 pg/ml (IL-10), 2 pg/ml (IL-13) and 5 pg/ml (IFN- γ). IL-4 was not measured because allergen-induced responses are largely below detection in children of this age, even with effort to optimize the assays. IL-13 was selected as it has similar biological activities as IL-4 and is more easily detected.

Choice of allergens

Although egg white comprises 23 different glycoproteins (20), the two most often implicated in allergic responses are OVA and OM (21, 22). OVA has been the most extensively used in research as a model allergen. However, there has been conflicting information in the literature about the relative allergenicity of OVA vs OM (21, 23–26). There is also ongoing debate about which epitopes on the ovalbumin molecule are recognized (27, 28). Most studies have described the existence of at least several epitopes that elicit a response (11). Where there were available cells, we assessed the responses to both OM and OVA. In this population of egg-allergic children, OVA responses were seen more consistently (see Results), and therefore this allergen was selected for use when PBMC numbers prevented the stimulation with both OVA and OM allergens.

Data analysis

The data were analyzed using the Mann–Whitney nonparametric *U*-test for comparison of groups. The chi-squared test was used for comparisons involving frequency data. Nonparametric correlation coefficients were calculated using the Spearman test. A *P*-value < 0.05 was considered significant. THE STATISTICAL PACKAGE FOR SOCIAL SCIENCES (Version 10 for Macintosh) and INSTAT computer programs were used.

Results

Clinical characteristics of sample population

As indicated above, the egg-allergic population comprised 46 children with current egg allergy (SPT+) and 13 children who had outgrown documented IgE-mediated egg allergy on the basis of a negative SPT to whole egg and a successful egg challenge. All children had a history of acute urticaria with angioedema or anaphylaxis and a positive SPT at diagnosis. The children with a positive SPT to egg at the time of sample collection, were divided into three age groups for comparison: < 18 months (group 1, $n = 16$), 18 months to 3 years (group 2, $n = 12$), and > 3 years of age (group 3, $n = 18$). Of the children who had outgrown their egg allergy, four were under the age of 3, and nine were 3 years or older. Because of limitation in cell numbers, not all assays were performed on each sample.

These egg-allergic patients showed an age-related increase in both allergic asthma (6% in group 1, 25% in group 2, 70% in group 3) and atopic eczema/dermatitis syndrome (56% in group 1, 73% in group 2, 88% in

group 3). The frequency of SPT positivity to HDM also increased steadily with age (16% in group 1, 57% in group 2, 93% in group 3). HDM skin prick wheal size also increased significantly with age.

By definition, the nonallergic population were selected because they had no clinical disease and where all SPT negative at the time of blood collection. As indicated previously this included the following groups: < 18 months ($n = 36$), 18 months to 3 years ($n = 31$), and > 3 years of age ($n = 39$).

Preliminary studies to select most appropriate egg allergen

In a subgroup ($n = 30$) of children with positive SPT to egg, PBMC responses to OVA and OM were compared. These subjects responded less frequently to OM compared to OVA stimulation. Significant proliferation occurred in only 9/30 (30%) samples stimulated with OM at 100 µg/ml and 9/17 (53%) at 1000 µg/ml, compared to 31/43 (72%) cultured with OVA (100 µg/ml). Cytokine responses of egg-allergic subjects to OM at varying concentrations were also weaker than OVA responses. The only cytokines detected in response to OM were IL-10 and IL-13 in children between the ages of 18 months to 3 years. Accordingly, OVA was selected for use in the remainder of the samples which had limited cell numbers.

Responses to OVA

As anticipated there was a strong relationship between positive SPT to egg and positive lymphoproliferative responses to OVA. Positive proliferation was seen in 72% (31/43) of egg-allergic children with a positive SPT to egg, compared with 12.5% (1/8) children who had outgrown egg allergy. Positive lymphoproliferative responses in the egg-SPT+ groups were more frequent at younger ages, with 12/14 (86%) positive responses in group 1, 8/11 (73%) in group 2 and 11/18 (61%) in group 3 (Fig. 1). The same age-related trend was seen in the nonallergic infants, but the frequency of positive responses was significantly lower at all ages (compared with egg-allergic children), and very few nonallergic infants showed positive responses after 18 months of age (Fig. 1).

In egg-allergic (SPT+) infants, IL-5 responses to OVA (Fig. 2) were significantly higher in younger infants (groups 1 and 2) compared to those over 3 years (group 3). Most patients in group 3 (13/16) had no detectable IL-5 responses to OVA despite persistently positive SPT. In children who had outgrown egg allergy IL-5 responses to OVA were not measurable (< 4 pg/ml). Although IL-13 response tended to be lower with age, this was not significant and responses were still detectable in children outgrowing their allergy (Fig. 2). Thus although OVA IL-13 and IL-5 responses closely correlated in younger infants (< 18 months) around the time of presentation ($R = 0.6$, $P = 0.01$), there

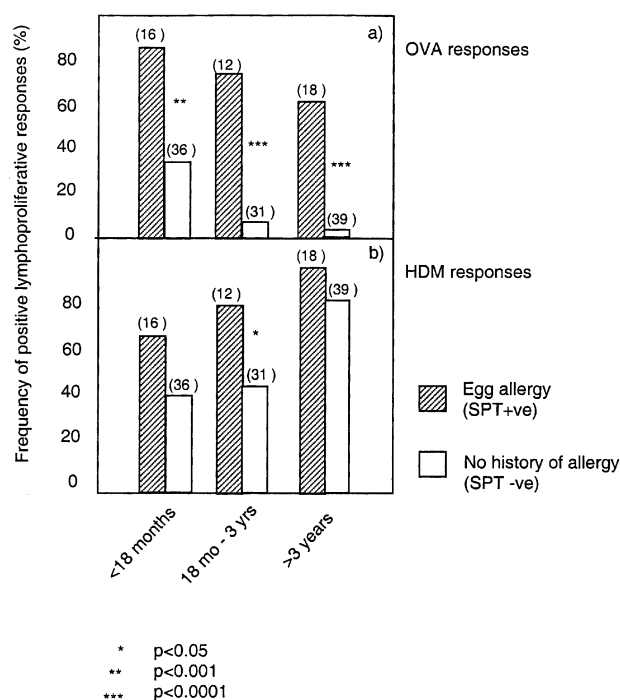


Figure 1. Lymphoproliferative responses to OVA and HDM in egg-allergic and nonallergic children. The frequency of positive lymphoproliferative responses (see Material and methods) in each age group is shown for nonallergic (clear bars) and allergic (shaded bars). Positive proliferative responses were defined as those in which ^3H -thymidine incorporation was greater than twice background level and > 1000 d.p.m. above background (unstimulated) cultures. Egg-allergic and nonallergic groups were compared using the Mann-Whitney *U*-test (significance levels as shown). The numbers of children in each group are also shown (in brackets).

was no correlation over the age of 18 months, and loss of IL-5 rather than IL-13 responses were associated with development of tolerance.

Both IL-10 and IFN- γ responses to OVA remained strong in ($>90\%$) children who had outgrown egg allergy. Of note, OVA-specific IFN- γ responses were readily measured in young infants (6–18 months) with egg allergy, and levels were not significantly different with age. Levels of IL-10 produced in response to OVA stimulation were also similar at all ages (Fig. 2).

IL-10 responses to OVA were also readily detectable for nonallergic children at all ages even when lymphoproliferation (or other cytokine responses) were not detectable (not shown). Unlike the egg-allergic patients, nonallergic children showed a significant ($P=0.005$) increase in IL-10 responses with age (Fig. 4). Nonallergic children had low level IFN- γ response to OVA which did not change with age (Fig. 4). Although direct comparisons with the egg-allergic group are not possible, the Th2 responses in the nonatopic children were very low or below detection at all ages (shown for IL-13 on Fig. 4).

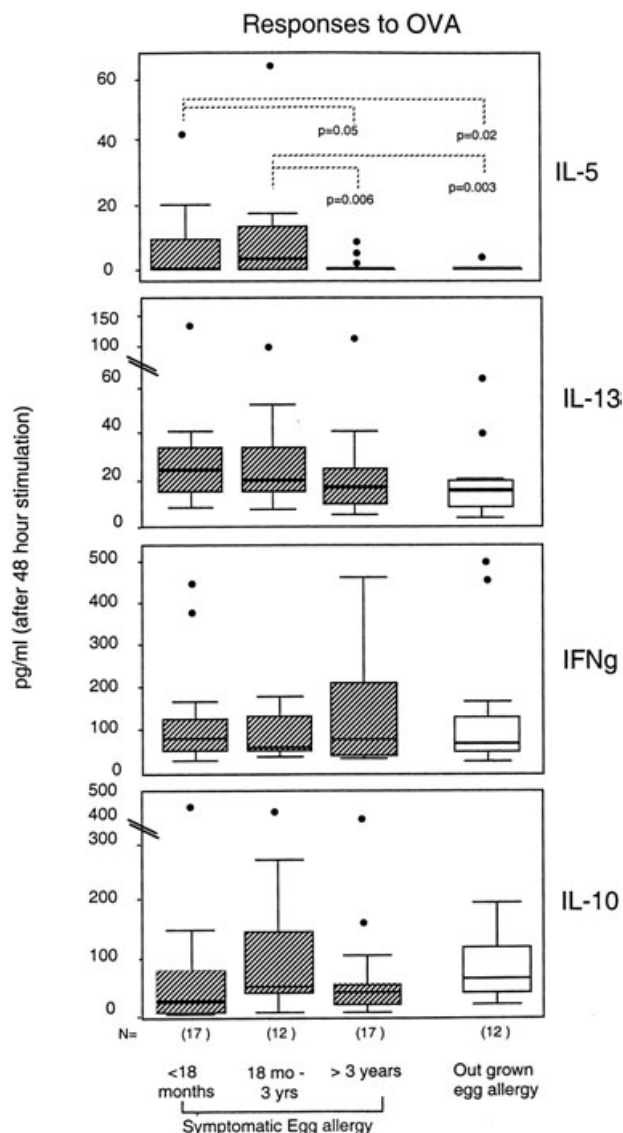


Figure 2. Cytokines levels (after 48 h) in response to OVA stimulation in egg-allergic children. Levels above unstimulated control cultures are shown for each age group (shaded bars), and compared with egg-allergic children who had outgrown their clinical disease (clear bars). Results are displayed as median, 5, 25, 75, 95 percentile ranges and outlying values. Groups were compared using nonparametric Mann-Whitney *U*-test (significance levels are as shown).

Responses to HDM

The frequency of positive lymphoproliferation to HDM increased with age, and all egg-allergic children had responses to HDM by age 3 (Fig. 1). Although the same pattern was observed in nonallergic children, positive lymphoproliferative responses to HDM were significantly less frequent (Fig. 1) than for the egg-allergic infants less aged than 3 years.

With increasing age egg-allergic children had significantly higher IL-5 and IL-13 responses to HDM stimulation (Fig. 3). Unlike the dissociation between IL-5 and IL-13 response to OVA with age, increasing

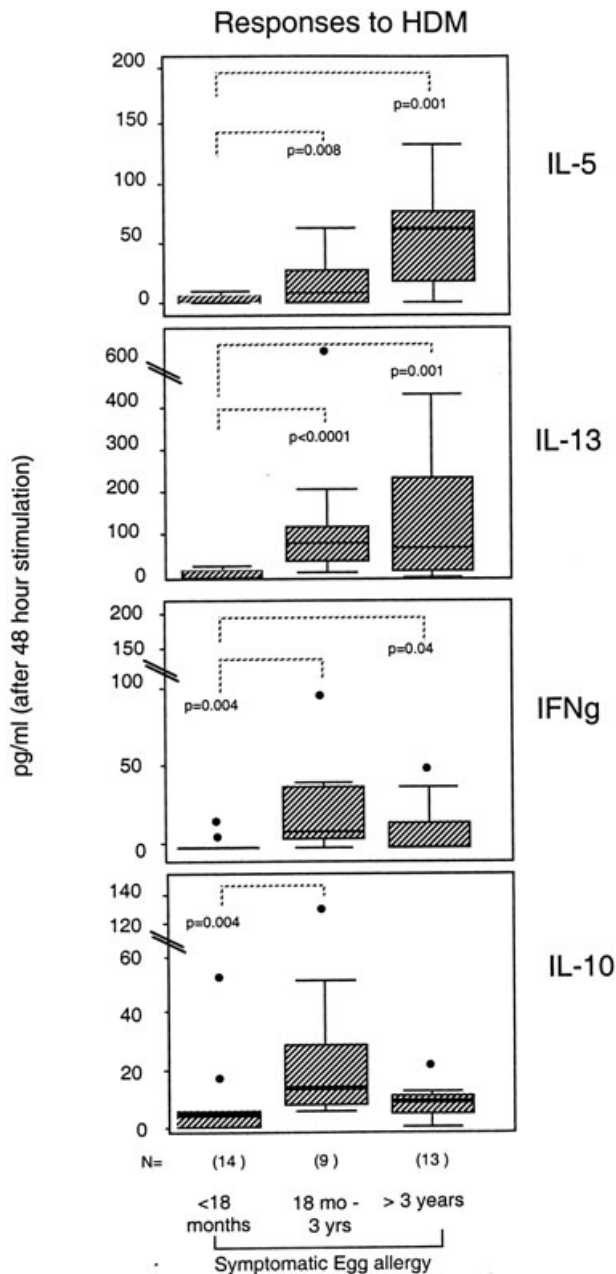


Figure 3. Cytokines levels (after 48 h) in response to HDM stimulation in egg-allergic children. Levels above unstimulated control cultures are shown for each age group. Results are displayed as median, 5, 25, 75, 95 percentile ranges and outlying values. Groups were compared using nonparametric Mann-Whitney *U*-test (significance values are as shown).

aeroallergen sensitivity with age was associated with stronger correlations between IL-5 and IL-13 responses to HDM ($R=0.6$, $P=0.03$ below 18 months of age, and $R=0.95$, $P<0.0001$ over 3 years of age).

HDM stimulated IFN- γ levels were undetectable until after 18 months of life, but were strong thereafter even in children with HDM sensitization (HDM SPT+) and asthma. Similarly, HDM IL-10 levels were low until after 18 months. HDM-specific cytokine responses of

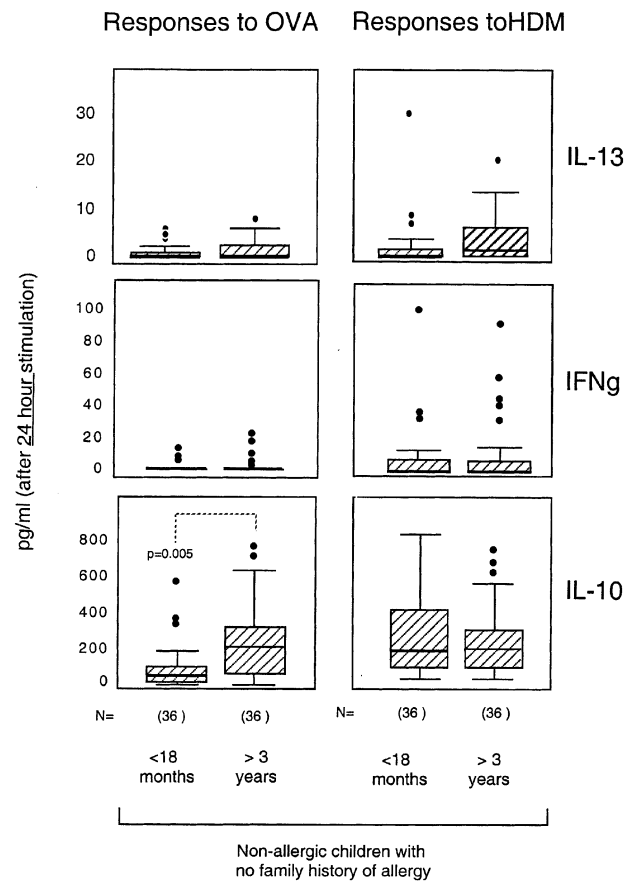


Figure 4. Cytokines levels (after 24 h) in response to OVA or HDM stimulation nonallergic children. Comparisons are made for children <18 months and >3 years of age. Levels above unstimulated control cultures are shown for each age group. Results are displayed as median, 5, 25, 75, 95 percentile ranges and outlying values. Groups were compared using nonparametric Mann-Whitney *U*-test (significance values are as shown).

children over the age of 3 who had outgrown egg allergy were not significantly different to similarly aged egg-SPT positive children.

Again, although direct comparisons are not possible, HDM-stimulated Th2 responses in nonallergic children (IL-13) were largely below detection at all ages (most had levels <5 pg/ml as shown in Fig. 4). IL-10 responses to HDM did not change with age. IFN- γ responses were also similar with age, but at a low level (below detection in many children as seen in Fig. 4).

Responses to PHA

IFN- γ responses to PHA stimulation increased with age, particularly after the first 18 months of life ($P=0.032$). IL-5 response to PHA also increased with age, with significantly higher levels ($P=0.028$) in group 3 (>3 years) compared to group 1 (<18 months). Other cytokine responses to PHA did not significantly differ between age groups. PHA responses were not assessed in the nonallergic children.

Cytokine responses in relation to clinical symptoms

We assessed cytokine patterns in children with different associated allergic diseases. As atopic disease is clearly heterogeneous we wished to determine if different cytokine responses were associated with respiratory vs cutaneous manifestations. Infants (<3 years) with egg hypersensitivity who also had atopic dermatitis ($n = 14$) had significantly higher IFN- γ responses to HDM ($P = 0.006$) compared to those with no dermatitis ($n = 8$) (not shown). In egg-allergic (SPT+) subjects, IL-5 production to HDM was significantly higher in asthmatics ($n = 16$) compared to nonasthmatics ($n = 28$, $P = 0.041$). Differences in other cytokine responses to HDM were not significant between the groups. In response to OVA stimulation, IFN- γ levels were significantly lower ($P = 0.039$) in asthmatics. It is important to note that children with asthma tended to also be older than those that did not have a diagnosis of asthma. Because of the small numbers, it was not possible to examine each age group separately.

Discussion

Despite the high frequency, severity and substantial risk of inhalant allergy, food allergy is a relatively neglected area in immunological research. The early processes which lead to failed food allergen tolerance may give clues to developing aeroallergen hypersensitivity, and possibly future intervention strategies. Although the data presented here are based on a cross-sectional analysis this study provides useful information about the cytokine responses to OVA and HDM in egg-allergic children. The main limitations of the study are that it is not prospective, and that it was not possible to make direct comparisons with the nonatopic group.

Both ovomucoid (Gal d 1) and ovalbumin (Gal d 11) were used in preliminary assessments of egg allergen response in this population, on the basis of previous reports that egg-allergic individuals may recognize OM more commonly than OVA (23). However, this population demonstrated stronger proliferative responses to OVA, and these better reflect clinical status. This may reflect reported differences in individual reactivity to these two major egg allergens (21).

Atopic diseases arise from the failure of mechanisms that normally suppress the emergence of the Th2-responder T cells which induce IgE mediated disease. Although many children show early transient lymphoproliferation to food allergens (14), this is not usually associated with disease. This study indicates that transient egg-responsiveness is more prolonged and more exaggerated in egg-allergic children compared to nonallergic children (Fig. 1).

In egg-allergic infants OVA responses are associated with strong production of Th2 cytokines, particularly under 3 years of age. This is consistent with previous

reports that IL-4 responses are higher in egg-allergic children compared to nonallergic controls (29, 30). It was of interest, however, that in the present study IL-5 responses to OVA appeared to correlate most closely with clinical status (compared to IL-13 which often parallels IL-4 activity). IL-4 however, was not measured in the present study. Rather than a 'global' reduction of Th2 responses, loss of clinical reactivity was most clearly associated with loss of IL-5 responses, while IL-13 levels were not significantly lower with age. This dissociation of Th2 responses suggests functional heterogeneity in responsive cell populations. Other studies have also noted that IL-5 is produced more consistently (than other Th2 cytokines) by OVA-specific T cell clones derived from egg-allergic individuals (23) suggesting that this cytokine may play an important role in food sensitivity. It is of further interest that in the present study IL-5 responses were also lower in older children prior to the loss of SPT reactivity. These results suggest that changes in underlying cellular responses which lead to tolerance predate the loss of clinical and SPT reactivity by many months. The significance of the stronger HDM-specific IFN- γ responses in infants with atopic dermatitis is not clear. Kuwabara et al. (30) previously noted stronger IFN- γ (to OVA) in egg allergic children with atopic dermatitis compared to those with immediate hypersensitivity, whereas Campbell et al. (29) found lower IFN- γ responses (to PHA) in children with atopic dermatitis compared to controls and patients with acute hypersensitivity. While allergy-prone infants clearly have an early propensity for Th2/Th0 responses to food allergens, the different immunological mechanisms which govern phenotypic expression still need to be determined.

Although atopy has been previously associated with an early *transient* functional Th1 immaturity (31), egg-allergy was not characterized by poor IFN- γ responses in this study. IFN- γ responses to both OVA and HDM were strong, and OVA-specific IFN- γ production was readily measured in egg-allergic infants as young as 6 months old. This supports our previous observations (14) that atopy in young children is associated with mixed Th1/Th2 (Th0) allergen-responses rather than a clear Th2 bias. As anticipated, loss of clinical (and SPT) reactivity to egg was associated with loss of lymphoproliferation to OVA. Although this decline in proliferation followed the same pattern of nonallergic children (Fig. 1), tolerance was clearly greatly delayed in egg-allergic children. IL-10 and IFN- γ cytokine responses remained detectable in virtually all children despite loss of proliferation to OVA. The nonallergic children, with no measurable lymphoproliferation responses to egg, also produced IL-10 and IFN- γ in response to OVA stimulation. In a smaller study, Noma et al. (13) also noted persistent IFN- γ response in children ($n = 7$) who had outgrown egg allergy.

Responses to HDM (Fig. 1) also appear much earlier

in the egg-allergic infants compared to the nonallergic infants. Both Th1 and Th2 responses to HDM increased with age, with low levels of all cytokine responses in most egg-allergic children under the age of 18 months. After 18 months IFN- γ responses to HDM were significantly higher and egg-allergic children had a mixed Th0 response at 18 months to 3 years of age. Older children (>3 year) showed further increases in Th2 responses to HDM, but not IFN- γ . Kimura et al. also noted an HDM-specific Th0 response in young infants (and a more Th2 profile at older ages) (32).

The present findings suggest that production of Th2 cytokines (particularly IL-5) is the defining feature of atopy, and that the Th1 cytokine component is common to atopic and nonatopic children. Non-allergic children appear to have more conservative cytokine responses to allergens in the early postnatal period. The contrasting generalized early 'hyperresponsiveness' in allergic children further suggests abnormal tolerance mechanisms. Vaccine studies also indicate that this 'mixed Th0 hyperresponsiveness' is not limited to allergens; infants (18 months of age) at high risk of atopy also show transiently stronger Th0 (Th1 and Th2) responses to vaccine antigens (tetanus toxoid) and allergens (HDM) than low-risk infants (33).

It is not known whether mixed cytokine responses reflect the activity of the same cells (i.e. Th0 cells) or mixed populations of Th1 and Th2 populations. Early (Th0) responses to food-allergens evolve into more Th1 skewed responses (dominated by both IFN- γ and IL-10) before the loss of clinical allergy. It is also of note that in the absence of any symptoms, nonallergic children

showed a significant increase in IL-10 responses to OVA with age (Fig. 4). Certainly these responses indicate that the previously proposed 'clonal deletion' of food allergen-specific cells is not responsible for food allergen tolerance. It may be speculated that these allergen-specific IFN- γ and IL-10 producing cells represent persistent 'regulatory' populations capable of suppressing Th2 responses. IL-10 particularly is known to play an important role in the induction and maintenance of antigen-specific T cell tolerance (34, 35). Therapeutic induction of tolerance (Th2 suppression) in atopic individuals (using immunotherapy) is also associated with increased IL-10 and IFN- γ responses [reviewed in (36)]. IFN- γ and IL-10 are also produced readily in nonallergic (SPT-negative) children in response to aeroallergen (16). Together, these observations suggest that this combination of cytokines (IFN- γ and IL-10) may be important for counter-regulating Th2 responses (reviewed in (37)). Thus, the role of IFN- γ and IL-10, and the apparent differential regulation of IL-5 and IL-13 in the development of food allergen tolerance, are important avenues for further investigation.

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