

Age-related T cell responses to allergens in childhood

J. M. Smart, C. Suphioglu* and A. S. Kemp

Department of Immunology and Murdoch Children's Research Institute, Royal Children's Hospital, Melbourne and *Department of Allergy, Asthma and Clinical Immunology, Monash Medical School, The Alfred Hospital, Prahran, Victoria, Australia

Summary

Background T cell priming, as determined by allergen-induced proliferative responses, is believed to occur principally in early childhood in both atopic and non-atopic infants under the influence of multiple factors including environmental allergen exposure. It is considered that T cell priming with expansion of Th2 cells is a crucial factor in the development of atopic disease.

Objective To examine T cell priming to commonly encountered allergens in childhood in relation to age.

Methods In a cross-sectional study T cell proliferation in relation to age was examined for three common allergens, ovalbumin (OVA), house dust mite (HDM) and rye grass pollen (RYE), in atopic and non-atopic children. The effect of age on Th1 (IFN- γ) and Th2 (IL-5 and IL-13) cytokine production in response to these allergens was investigated to examine the possibility of immune deviation with time.

Results A significant increase in T cell proliferation with age was observed with RYE among atopic children only. However, the same was not observed with the two other allergens studied (i.e. OVA and HDM). In addition, RYE-induced (but not HDM or OVA) cytokine production showed an increased Th2 deviation with age as reflected in the increasing IL-5/IFN- γ and IL-13/IFN- γ ratios only among the atopic subjects with rye grass pollen sensitivity.

Conclusion These findings suggest that grass pollen sensitivity in childhood is accompanied by a progressive accumulation of allergen-primed T cells and progressive deviation of the allergen-induced cytokine response towards a Th2 response in atopic subjects throughout childhood.

Keywords allergens, childhood atopy, cytokines, house dust mite, interferon- γ , interleukins-4/-5/-13, rye grass pollen

Submitted 29 October 2001; revised 13 September 2002; accepted 26 September 2002

Introduction

T lymphocytes are critical for the initiation and maintenance of the allergic inflammatory responses that develop following cellular activation leading to a cascade of responses, including cytokine and IgE production and inflammation [1]. T cell priming giving rise to an allergic phenotype is believed to occur in early childhood under the influence of multiple factors including genetic predisposition and environmental determinants such as allergen exposure. T cell priming to allergens occurs in both atopic and non-atopic infants [2–6]. A principal way of determining T cell priming is by measurement of the proliferative response to allergens. This methodology has suggested that priming of T cells can commence *in utero* as shown by low levels of allergen-induced proliferation in cord blood [7]. During childhood, it has been postulated that consolidation of the immune response with expansion of either Th1 or Th2 cells occurs and that this expansion of allergen-reactive T cells will

be reflected in an increasing proliferative response to allergen [3]. T cell priming to inhaled allergens has been considered to occur principally in early childhood and adult patterns to be established within the first 5 years of life [4, 8]. These conclusions have been reached utilizing house dust mite (HDM) [8] and apart from a few studies conducted in infants and pre-school children [3, 7], there are few data on the development of allergen reactive T cell populations to grass pollen allergens throughout childhood.

The investigation of changes in T cell reactivity to rye grass pollen (RYE) allergens in childhood is of great interest as, unlike sensitivity to common food and other allergens, such as HDM, which generally develops in the first years of life, sensitization to rye grass pollen occurs in later childhood [9, 10]. In addition, the principal clinical manifestation of this rye grass pollen sensitivity (i.e. hayfever) develops progressively throughout the childhood and teenage years [11]. It is likely that the natural history of grass pollen allergen sensitization, as compared with that of foods and HDM, is related to the seasonal nature of allergen exposure to grass pollen. It may also be possible that the differences in sensitization to HDM and RYE in childhood would be reflected in differing patterns of T cell proliferation, with a progressive increase occurring to

Correspondence: Andrew S. Kemp, Immunology Department, Royal Children's Hospital, Flemington Road, Parkville, Victoria, Australia 3052.
E-mail: kempa@crylic.rch.unimelb.edu.au

RYE throughout childhood. The intermittent nature of grass pollen exposure contrasts with the more constant exposure to food and HDM allergens that are continuously present in the diet and environment and thus provide more opportunity for allergen presentation and priming of the T cell repertoire in early childhood. In this study we examined allergen-induced T cell proliferation in relation to age for three common allergens, namely ovalbumin (OVA), HDM and RYE. In addition, the effect of age on Th1 (IFN- γ) and Th2 (IL-5 & IL-13) cytokine production in response to these allergens was investigated to determine evidence for immune deviation with time.

Materials and methods

Reagents

Reagents used were: AIM-V medium (Gibco-BRL, Grand Island, NY, USA) supplemented with 5×10^{-5} M B-mercaptoethanol (B-ME; ICN, Costa Mesa, CA, USA); staphylococcal enterotoxin B (SEB) (Sigma Chemical Co., St Louis, MO, USA); avidin-peroxidase (Sigma Chemical Co.); Ficoll-Hypaque (Pharmacia, Uppsala, Sweden); monoclonal antibodies (MoAbs) (*clone number*) (Pharmingen, San Diego, CA, USA): anti-IFN- γ (NIB42), biotinylated anti-IFN- γ (4S.B3), anti-IL-5 (TRFK5), biotinylated anti-IL-5 (JES1-5A10), anti-IL-13 (JES10-5A2), biotinylated anti-IL-13 (B69-2), anti-IL-4 (8D4-8), biotinylated anti-IL-4 (MP4-25D2); recombinant human (rh)-IFN- γ , rh-IL-5, rh-IL-13, rh-IL-4 (Pharmingen San Diego, CA, USA) were used as standards; [6- ^{3} H] thymidine (Amersham Life Science, Buckinghamshire, UK); TMB (3,3',5,5'-tetramethylbenzidine) substrate (KPL, Gaithersburg, MD, USA).

Patients

Heparinized venous blood samples were obtained from:

- Fifty-three healthy children (non-atopic) with a median age of 5.7 years (range: 5 months to 15 years) who were admitted for elective surgery at the Royal Children's Hospital (i.e. circumcisions, herniotomies, orchidopexies, squint repairs or orthopaedic procedures). These children had no history of atopy on clinical questionnaire enquiring into past or present history of atopic disease and had plasma IgE levels within the normal range for their age (median 20.1 kU/L; range 4 to 128 kU/L) and negative allergen-specific IgE to the three allergens tested, i.e. egg white, house dust mite and rye grass pollen. Blood was obtained immediately on anaesthetic induction.
- Fifty-nine children (atopic) had a clinical diagnosis of atopic disease made by a consultant paediatric allergist: eczema ($n = 15$); asthma ($n = 2$); rhinitis ($n = 5$); eczema and asthma ($n = 11$); asthma and rhinitis ($n = 6$); eczema and rhinitis ($n = 4$); (eczema, asthma and rhinitis ($n = 16$)). The definitions of the conditions were consistent with those utilized in the ISAAC study [12]. Thus asthma was defined as wheezing or whistling in the chest in the last 12 months, allergic rhinitis as a problem with sneezing, or a runny, or a blocked nose when you did not have a cold or the flu in the last 12 months, and eczema as an itchy rash that was coming and going for at least 6 months and affected any of the following places: the folds of the elbows, behind the knees, in front of the ankles, under the

buttocks, or around the neck, ears or eyes. The median age was 5.9 years (range: 3 months to 16 years). All children had elevated IgE levels for their age (median IgE: 670 kU/L; range 25 to 118 000) and at least one positive allergen specific IgE (UniCAP) test: egg white (EW) ($n = 5$); house dust mite (HDM) ($n = 13$); rye grass pollen (RYE) ($n = 4$); HDM and RYE ($n = 22$); EW and HDM ($n = 2$); EW and RYE ($n = 1$); EW, HDM and RYE ($n = 12$). The age distribution for allergen-specific sensitization was as follows: HDM-sensitive patients, median age 6.75 years, range 1 to 14 years; RYE-sensitive patients, median age 6.75 years, range 10 months to 16.25 years; OVA-sensitive patients, median age 2.29 years, range 7 months to 12.9 years. Forty per cent of RYE-sensitive patients and 31% of RYE non-sensitive patients had blood collected during rye grass pollen season. Informed consent was obtained from a parent/guardian of all subjects and the study was approved by the ethics committee of the Royal Children's Hospital, Melbourne.

Allergen preparation

House dust mite (HDM) (*Dermatophagoides pteronyssinus*) (CSL, Melbourne, Australia) was prepared according to the method of Stewart et al. [13]. Briefly, crude HDM was homogenized in PBS, clarified by centrifugation, dialysed in 0.05 M ammonium bicarbonate overnight, freeze dried and stored in aliquots at -70°C . Aliquots were reconstituted in PBS; filter sterilized (pore size $2\mu\text{m}$) and stored at -70°C until required. Rye grass pollen (*Lolium perenne*) extract (RYE) (Greer Laboratories, Lenoir, NC, USA) was prepared according to previously published methods [14]. Aliquots were stored at -70°C until required. Grade II ovalbumin (OVA) (Sigma Chemical Co.) was dissolved in PBS, clarified by centrifugation, filter sterilized (pore size $2\mu\text{m}$) and then stored at -70°C until required.

Total and specific IgE

Total IgE and allergen-specific IgE levels were determined by UniCAP total IgE and specific IgE fluoroenzyme immunoassays (Pharmacia & Upjohn Diagnostics Uppsala, Sweden). Levels of total IgE are expressed in kU/L with the normal range according to age: 0.5 to 0.7 years, 0 to 20 kU/L; 0.7 to 1 year, 0 to 25 kU/L; 1 to 2 years, 0 to 35 kU/L; 2 to 4 years, 0 to 130 kU/L; 4 to 7 years, 0 to 150 kU/L; > 7 years, 0 to 200 kU/L [15]. Allergen-specific IgE levels are expressed as kU/L, with levels above 0.7 kU/L considered positive.

Cell cultures

Peripheral blood mononuclear cells (PBMC) were separated from heparinized blood by Ficoll-Hypaque density gradient centrifugation. The PBMC layer was washed three times with sterile PBS. PBMC were cultured at a concentration of 1×10^6 cells/mL in AIM-V with B-ME at 37°C in a humidified atmosphere with 5% CO_2 . Maximal proliferation was found to occur with the following concentrations: superantigen (1 $\mu\text{g/mL}$ SEB) and purified allergen (HDM 10 $\mu\text{g/mL}$, RYE 20 $\mu\text{g/mL}$, OVA 100 $\mu\text{g/mL}$). These concentrations were then chosen for use in cell culture for both proliferative responses and cytokine production. An unstimulated sample was included for each patient. SEB is a suitable polyclonal stimulus as it may have relevance to atopic disease [16].

Cell proliferation

PBMC (1×10^5 cells) were cultured in a volume of 100 μ L per well in triplicate for 48 h (SEB) or 7 days (allergen) in parallel with media alone. Sixteen hours before harvesting onto glassfibre filter paper they were pulsed with 0.5- μ Ci tritiated thymidine per 1×10^5 cells. Radioactivity was measured by liquid scintillation (beta counter) and proliferation was expressed as a stimulation index (SI), which is expressed as the ratio of counts per minute of the mean of unstimulated to stimulated samples.

Cytokine production

For cytokine production PBMC (1×10^6 cells) were cultured in volumes of 1000 μ L. Preliminary time course experiments revealed allergen-induced maximal IL-5, IL-13 and IFN- γ production at 6 days, at which time cell cultures were centrifuged and cell free supernatants were collected and stored at -70°C until cytokine measurements were performed. IFN- γ , IL-4, IL-5 and IL-13 were assayed by sandwich ELISA. Briefly, high binding ELISA plates were coated with purified capture MoAb (IFN- γ , IL-4, IL-5 or IL-13) at a concentration of 1 μ g/mL in carbonate buffer (pH 9.6) and incubated overnight at 4°C . Plates were blocked with PBS-Tween-10% fetal calf serum (FCS) for 2 h, washed and samples incubated at room temperature for a further 4 h. After washing, biotinylated MoAb (IFN- γ , IL-4, IL-5 or IL-13) at 0.5 μ g/mL was added and incubated at room temperature for 45 min, after which avidin-peroxidase (1:400 of 1 mg/mL) was added. After final washing, TMB substrate (KPL) was added and the reaction stopped by addition of H_2SO_4 . Plates were read at 450 nm on a spectrophotometer. All standards and samples were performed in duplicate, and the data were analysed using Biomek 1000 data reduction software (Beckman, Fullerton, CA, USA). Minimum detection levels were calculated by Biomek 1000 software and were IFN- γ 10 pg/mL, IL-4 2 pg/mL, IL-5 10 pg/mL and IL-13 5 pg/mL. Allergen-induced IL-4 production was very low or below the limit of detection. The IL-5/IFN- γ and IL-13/IFN- γ ratios were calculated as IL-5 or IL-13 in pg/mL divided by IFN- γ in pg/mL.

Statistical analysis

Proliferation was expressed as a stimulation index (SI), which is expressed as the ratio of counts per minute of the mean of unstimulated to stimulated samples. Regression analysis was used to evaluate the effect of age on allergen-induced T cell proliferation and cytokine production. A test of interaction was used to compare the age effect between atopics and controls. Comparison of the magnitude of proliferative responses among non-atopic vs. atopic children was performed by comparing the slopes of the two regression lines examining the term representing the interaction between status and age in the model. Data were analysed using Stata statistical software: release 5 (StataCorp 1997 College Station, Texas, USA).

Results

Proliferative responses

The proliferative responses to the superantigen, staphylococcal enterotoxin B (SEB), an ingested allergen, OVA and two

inhalant allergens, HDM and RYE, were examined in controls (Figs 1a–d) and patients (Figs 2a–d). Regression analysis of age vs. proliferative response revealed a significant relationship between age and proliferative response among atopic children to RYE ($P=0.0007$, $r=0.45$) (Fig. 2d) that was not apparent among the non-atopic controls ($P=0.3$, $r=0.2$) (Fig. 1d). There was no age-related effect on proliferation to SEB ($P=0.4$, $r=0.12$) (Fig. 1a), OVA ($P=0.1$, $r=0.2$) (Fig. 1b) or HDM ($P=0.1$, $r=0.2$) (Fig. 1c) among the non-atopic controls or atopic patients: SEB ($P=0.9$, $r=0.02$, Fig. 2a); OVA ($P=0.5$, $r=0.09$, Fig. 2b) or HDM ($P=0.8$, $r=0.02$, Fig. 2c). Further subgroup analysis of patients according to sensitization status to RYE allergen revealed a significant relationship between proliferation and age only among the patients sensitized to RYE ($P=0.005$, $r=0.48$) but not in those who were not sensitized to RYE ($P=0.5$, $r=0.1$) (data not shown). In addition the proliferative response to RYE was significantly related to age in atopic children with the symptom of rhinitis ($P=0.0173$, $r=0.45$). No significant relationship with age was observed in proliferative responses to OVA in egg-sensitized patients ($P=0.08$, $r=0.46$) (although there appeared to be a trend towards decrease in proliferative response with advancing age) or to HDM in HDM-sensitized patients ($P=0.7$, $r=0.04$). Comparison of the magnitude of proliferative responses among non-atopic vs. atopic children based on the comparison of the two regression lines revealed a significantly greater stimulation only to rye allergen ($P=0.0007$ for test of interaction).

Allergen-induced cytokine production

In order to determine whether the increased proliferation in response to RYE was related to expansion of Th1 or Th2 subsets we examined allergen-induced cytokine production in non-atopic controls and atopic groups. To examine the Th2/Th1 balance, we determined the IL-5/IFN- γ and IL-13/IFN- γ ratios with respect to age among control and atopic children. There was no significant relation between age and the cytokine ratio in non-atopic children for either HDM or RYE stimulation (data not shown). Similarly, there was no significant relation to age with HDM as a stimulus in the atopic group (IL-5/IFN- γ ratio, $P=0.7$, $r=0.06$; IL-13/IFN- γ ratio, $P=0.6$, $r=0.11$) (data not shown). However, there was a relation to age with RYE in the atopic group (IL-5/IFN- γ ratio, $P=0.0016$, $r=0.6$; IL-13/IFN- γ ratio, $P=0.07$, $r=0.36$). When the atopic group was examined according to rye sensitization, the association between IL-5/IFN- γ ratio and IL-13/IFN- γ ratio and age was observed only among the RYE-sensitive children (IL-5/IFN- γ ratio, $P=0.001$, $r=0.73$; IL-13/IFN- γ ratio, $P=0.014$, $r=0.616$) (Figs 3a, c) but not among the non RYE-sensitive atopic children (IL-5/IFN- γ ratio, $P=0.4$, $r=0.26$; IL-13/IFN- γ ratio $P=0.6$, $r=0.15$) (Figs 3b, d). In the subjects with rhinitis there was no association between age and the RYE-induced IL-5/IFN- γ ratio ($P=0.77$, $r=0.01$) or IL-13/IFN- γ ratio ($P=0.9$, $r=0.00$). There was a significant relation between RYE-induced IL-5 production and age in the atopics ($P=0.04$, $r=0.27$) that was not apparent in control ($P=0.6$, $r=0.08$) children. There was no significant relation between RYE-induced IFN- γ and IL-13 production and age in either the controls (IFN- γ , $P=0.3$, $r=0.1$; IL-13, $P=0.8$, $r=0.02$) or atopic (IFN- γ , $P=0.8$, $r=0.03$; IL-13, $P=0.6$, $r=0.07$) children (data not shown). There was no significant relationship

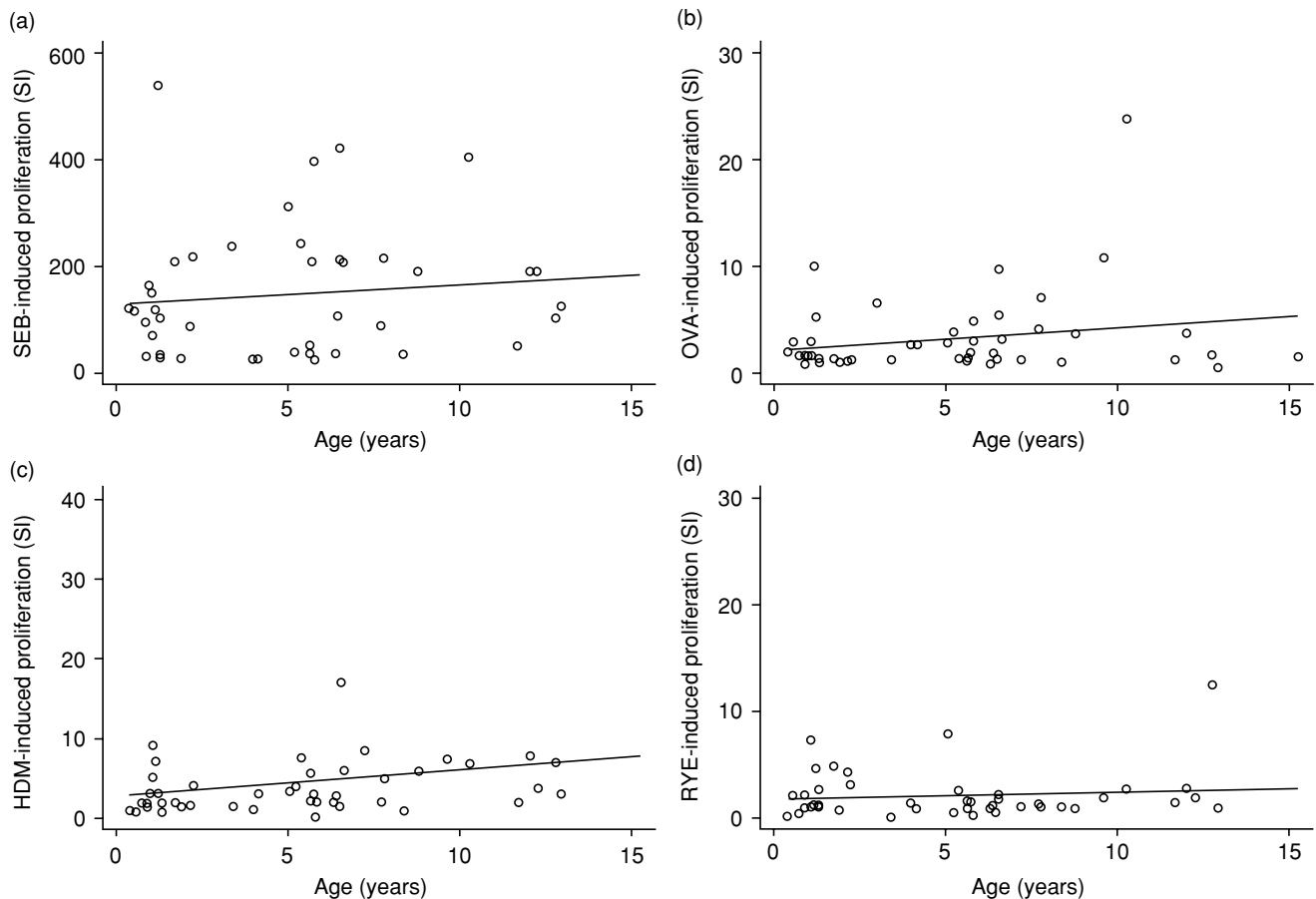


Fig. 1. Age-related proliferative responses in non-atopic children. PBMC proliferative responses were examined to (a) SEB, (b) OVA, (c) HDM and (d) RYE in non-atopic children ($n = 53$) according to age in years. Data are represented as a stimulation index (SI) (ratio of counts per minute of mean of unstimulated to stimulated sample). Regression analysis was used to evaluate the effect of age on allergen-induced T-cell proliferation. A regression line represents the relationship between proliferation and age.

with age for HDM-induced cytokine responses in the HDM-sensitized patients (IFN- γ , $P = 0.35$, $r = 0.14$; IL-5, $P = 0.186$, $r = 0.2$; IL-13, $P = 0.46$, $r = 0.11$), nor for OVA-induced cytokine responses in the OVA-sensitized patients (IFN- γ , $P = 0.47$, $r = 0.19$; IL-5, $P = 0.69$, $r = 0.1$; IL-13, $P = 0.77$, $r = 0.07$). Allergen-induced IL-4 production was very low or below the limit of detection.

Discussion

In this study we have investigated the association between proliferative responses to allergens and age to examine T-cell priming in childhood. We found significant increase of proliferative response with age in response to RYE allergens only among the atopic but not control children. However, the same was not observed with the two other allergens studied (i.e. OVA and HDM). There was no significant relation between age and stimulation by the polyclonal stimulus SEB, which indicates that the changes seen with RYE grass are not due to a generalized increase in proliferative capacity with age. In addition, examination of allergen-induced cytokine production revealed an increased Th2 deviation with age as shown by the increasing IL-5/IFN- γ and IL-13/IFN- γ ratio among RYE-sensitive but not HDM-sensitive atopic subjects. It was not possible to assess

the IL-4/IFN- γ ratio as only low levels of allergen-induced IL-4 could be detected and special measures such as blocking of cell-associated IL-4 receptors may be required to detect significant levels [17]. In a previous paper [18] we showed that both Th1 (IFN- γ) and Th2 cytokines were increased in response to allergen (RYE, HDM, OVA) stimulation in atopic children and concluded that both Th1 and Th2 responses to allergens contribute to the inflammatory responses that lead to atopic disease. However, in that study the relationship to age and the IL-5/IFN- γ and IL-13/IFN- γ ratios were not examined. The present findings, which have examined the relationship with age, indicate that despite the elevated rather than reduced allergen-induced IFN- γ the Th2/Th1 cytokine ratios increased with age, suggesting a progressive Th2 deviation with time in the response to RYE allergen. Furthermore the RYE-induced Th2 deviation was restricted to those with evidence of specific IgE antibodies to the allergen. The lack of a significant association between RYE-induced Th2 cytokine deviation and age in the group of children with symptoms of rhinitis may be a reflection of the median age of this group being 7.7 years and that perennial rhinitis, in which sensitization to allergens other than RYE, such as HDM, is likely to be more significant, is the predominant manifestation of rhinitis in early and middle childhood.

Although this is a cross-sectional and not a longitudinal study, the findings suggest that grass pollen sensitivity in

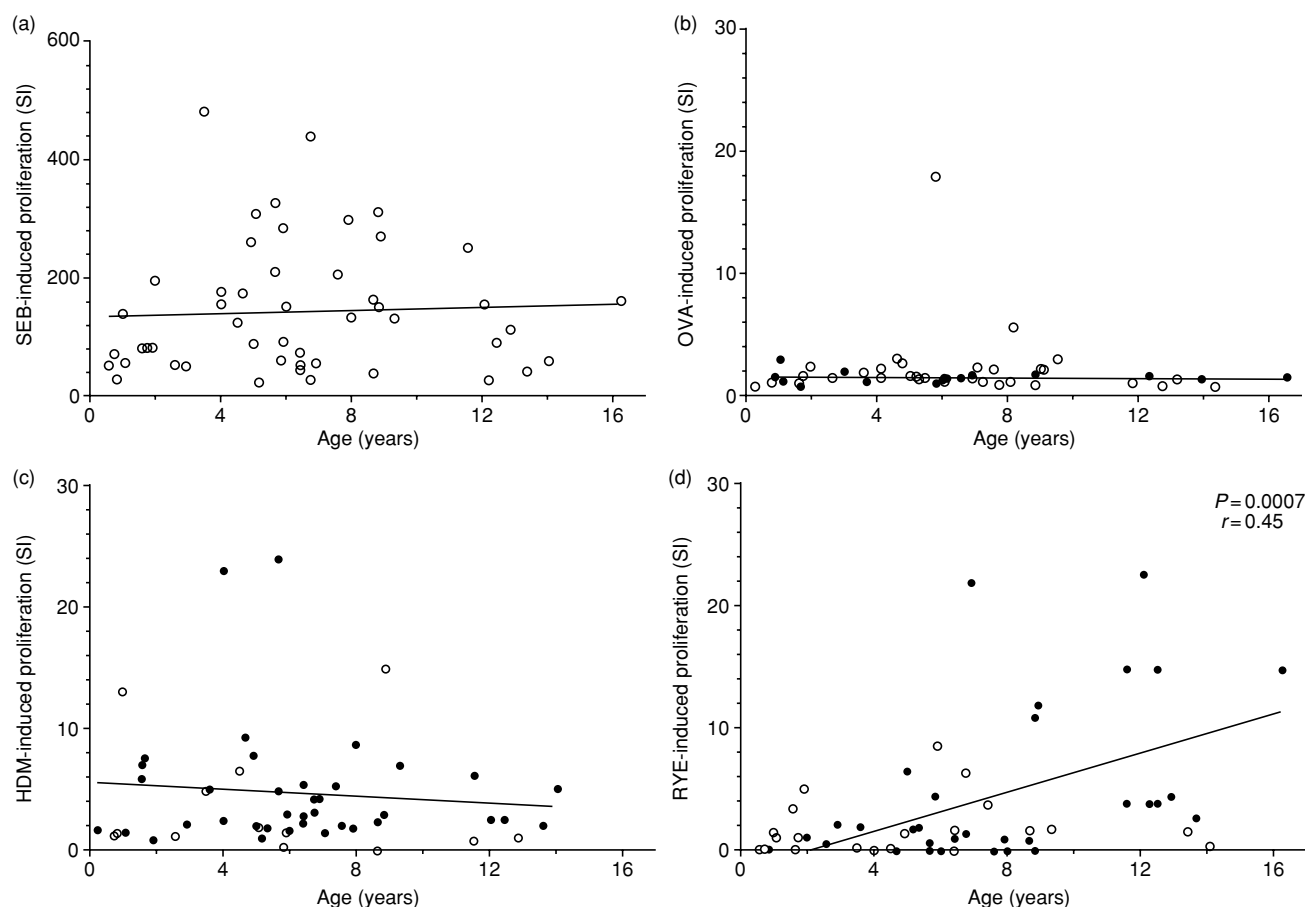


Fig. 2. Age-related proliferative responses in atopic children. PBMC proliferative responses were examined to (a) SEB, (b) OVA, (c) HDM and (d) RYE in atopic children ($n = 59$) according to age in years. Patients sensitized to the allergen under investigation are represented as shaded circles. Data are represented as a stimulation index (SI) (ratio of counts per minute of mean of unstimulated to stimulated sample). Regression analysis was used to evaluate the effect of age on allergen-induced T-cell proliferation. A regression line represents the relationship between proliferation and age.

childhood is accompanied by a progressive accumulation of allergen-primed T cells and progressive deviation of the allergen-induced cytokine response towards a Th2 response in atopic subjects. This phenomenon of increasing allergen-responsive T cells and Th2 deviation throughout childhood appears specific for RYE as it was not observed with HDM or OVA. Sensitization to rye grass is antecedent to the development of hayfever and develops progressively throughout childhood and the teenage years [9–11]. Hayfever is predominantly a disease of older children and adults. In one study the mean age of onset of seasonal allergic rhinitis has been reported as 10.6 years [19]. The prevalence of sensitization and seasonal allergic rhinitis symptoms were low during early childhood (< 2%) and increased steadily with age from 2.6% at 2 years to 15.3% by the age of 7 years in a prospective birth cohort study [11]. At least two seasons of pollen exposure were required for sensitization to pollen allergens, which developed prior to clinical manifestations in 56% of the patients [11]. While the reasons for this later onset of seasonal rhinitis, as compared to other atopic symptoms, are unclear, the present results indicate a possible immunological basis, with progressive Th2 deviation and expansion of allergen-reactive cells occurring throughout childhood. It is possible that disease manifestations will only occur once the Th2 immune response reaches a certain threshold. The present study suggests that the childhood response to

the inhalant allergen HDM and the ingested allergen OVA develop in a different manner. Perhaps maximum priming and Th2 deviation is reached in the first years of life and immunoregulatory feedback mechanisms then either maintain the immune response at a certain level or down-regulate the response as is frequently observed in the spontaneous remission of food sensitivity before 5 years of age.

There are few cross-sectional or longitudinal studies that examine cytokine responses to allergens in relation to age in childhood. In a cross sectional study Noma et al. [20] found that children growing out of egg allergy had decreased IL-4 and increased IFN- γ responses to egg allergen, suggesting a Th1 deviation in a situation where the atopic response was decreasing. Prescott et al. [21] examined cytokine responses to HDM allergen and found there was no difference in IL-4 and IFN- γ responses (as measured by mRNA) between 6 and 18 months and IL-13 secretion between 1 and 2 years of age in atopic children; however, a longer time frame may be required to observe an immune deviation as shown in the present study. van der Velden et al. [22] examined egg and dust mite cytokine responses at birth and at 12 months of age. Atopic children showed an increase in HDM-induced IFN- γ secretion but no difference in the response to ovalbumin. Interestingly, the IL-10 responses in atopic children to both allergens decreased rather than increased over 12 months, while ovalbumin-induced

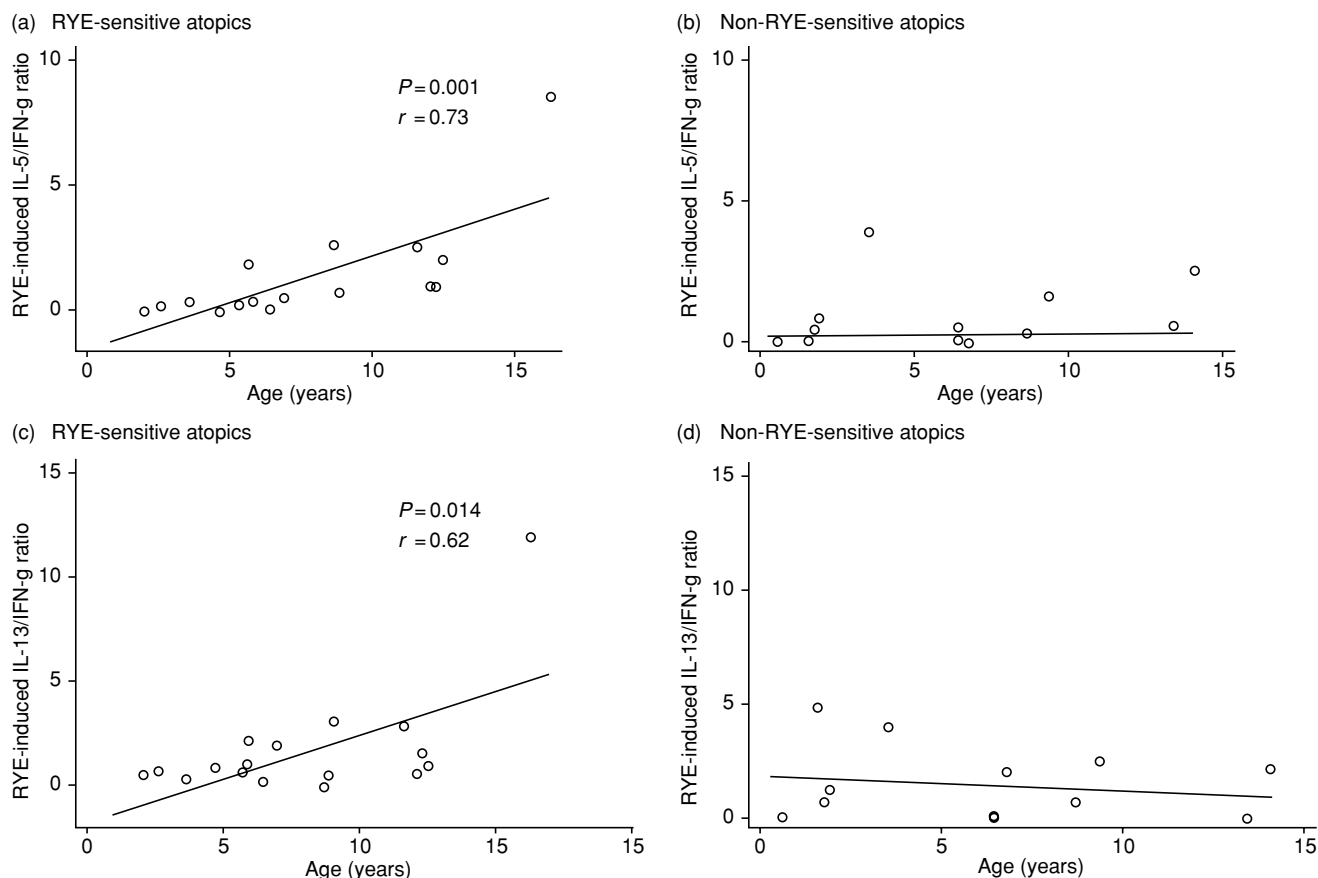


Fig. 3. Age-related, RYE-induced cytokine responses in atopic children. Rye-induced PBMC IL-5:IFN- γ (a and b) and IL-13:IFN- γ (c and d) cytokine ratios according to age in years were examined among atopic children who were (a and c) RYE sensitized or (b and d) RYE non-sensitized. A regression line represents the relationship between IL-5:IFN- γ and IL-13:IFN- γ cytokine ratios and age.

IL-13, which was not detectable at birth, was higher in the atopic children at 1 year of age. In view of the IL-13 results these data were interpreted as evidence of Th2 deviation over the first year of life; however, this could be questioned on the basis of the increasing IFN- γ and decreasing IL-10 response. As all these studies have examined the responses of infants and young children to ovalbumin and house dust mite the conclusions concerning the presence or absence of immune deviation in early life are not necessarily incompatible with the present observations, which indicate an immune deviation occurring in later childhood to a different allergen, RYE but not to HDM and OVA. As IgE antibodies to OVA and HDM develop earlier in life than those to RYE, it is possible that the critical shifts in cytokine balance, which determines the development of Th2 reactivity to these particular allergens, have already occurred prior to the principal time frame of our study, which is from 1 to 15 years.

In this study, there was a significant increase of RYE-induced proliferation among the atopics, in particular those with RYE-specific IgE as compared with non-atopics. In contrast, there was no difference in response to the other allergens (i.e. OVA and HDM) when atopics and non-atopics were compared. These findings are consistent with those of previous investigators who have found proliferative responses to HDM to be prominent at all ages, in both children and adults regardless of

their atopic status [23–25]. Furthermore, the magnitude of these T cell responses did not differ in most cases between subjects with and without specific IgE [26]. Most of the previous studies dealing with pollen-induced proliferation have been performed with adults [27–30] and some [27, 28] but not others [29, 30] have observed increased proliferation in symptomatic and or sensitized subjects, a finding that is consistent with our observation that greater stimulation was among the atopic children. Two previous studies have examined the proliferative responses in children. However, neither one of these studies included a non-atopic control population nor did they did examine the relationship between sensitization status and age throughout childhood. Holt et al. demonstrated that infants under 10 weeks of age and children from 2 to 13 had reduced T cell proliferation to both HDM and RYE when compared with adults. However, the changes in childhood were not examined in detail [3]. Miles et al. showed that T cell proliferation to rye grass pollen allergens increased at 6 and 12 months of age from that seen in cord blood in a group of allergy-prone children, suggesting an accumulation of allergen-reactive T cells in the first year of life [7]. Both of these studies do indeed indicate an increase in allergen-induced T cell reactivity with time, which the present study demonstrates to occur progressively throughout childhood. In this study we have utilized serum free media as described by Upham et al. [26], which results in significant improvement in

the sensitivity of allergen-specific T cell assays as compared with those performed in media supplemented with human or fetal calf serum.

Yabuhara et al. highlighted the differences in the development of immune responses to inhalant as compared with ingested allergens in childhood [8]. However, they have considered HDM as an archetypal inhalant allergen and concluded on the basis of a cross-sectional study that adult equivalent patterns of immunity to inhalant allergens are established within the first 5 years of life [4, 8]. While this may be true for HDM, and our data do not argue against this conclusion, the present findings indicate a clear difference in the time frame of the immune responses to HDM and RYE. Our findings are also consistent with the clinical observation that RYE sensitivity and RYE-induced allergic disease are not a major problem in pre-school children but develop progressively throughout the first two decades of life. Based on the results generated in this study, allergen priming and the development of Th2 responses during childhood should not be regarded as similar for all inhalant allergens and deserve careful analysis of the observed differences.

A possible reason for the observed differences between the childhood response to RYE and HDM allergens could lie in the intermittent timing of antigen presentation. In atopic subjects IgE responses to environmental allergens develop on a genetic background that favours a Th2 response. Exposure to HDM and OVA is likely to be continuous, as these allergens are continually present in the environment or the diet and thus may stimulate Th2 responses in individuals with the appropriate genetic predisposition. An additional factor may be the timing and persistence of allergen presentation. Thus exposure to RYE allergens occurs intermittently over 6 to 8 weeks/year [31], and depending on the time of birth, may not occur for a number of months after delivery. Animal models of allergic airways disease demonstrate that isolated or infrequent presentation of allergen to the respiratory tract results in sensitization and airways inflammation, while a more persistent challenge produces down-regulation of the allergic response with evidence of Th1 deviation of the immune response [32, 33]. The balance between Th1 and Th2 responses appears to be controlled by complex feedback mechanisms involving T lymphocyte and dendritic cell cytokines such as IFN- γ , IL-4 and IL-12 [34]. It could be postulated that in order to reach a steady state, a certain period of continuous allergen exposure is required. In the absence of an allergen source, such a steady state may not be reached and intermittent exposure results in increased T cell priming and deviation to a Th2 response. A more regular exposure to an inhalant allergen such as occurs in allergen immunotherapy, might allow a more normal balance between Th1 and Th2 responses to be achieved. This proposition is supported by the observations that during immunotherapy regular administration of RYE allergens by the nasal or parenteral route results in decreased allergen-induced proliferation [28, 35] and deviation of the allergen-induced cytokine secretion towards a Th1 response [35, 36].

In conclusion, do the present findings contribute to the current knowledge on the development of allergic disease in response to inhalant allergens during childhood, where allergic disease has been postulated to result from a failure to down-regulate infantile Th2 responses, with the subsequent consolidation of a Th2 response during infancy and early childhood [4].

Indeed, the results of the present study revealed a significant difference between the immune reactions to the perennial inhalant allergen HDM and a seasonal allergen, RYE. While this study provides evidence for Th2 deviation among children with RYE sensitivity, in contrast to HDM the deviation appears to increase throughout childhood, suggesting that the time frame of consolidation of Th2 responses to inhalant allergens varies according to allergen exposure. Moreover, the present study demonstrates a process of consolidation of Th2 polarized inhalant allergen specific cells, as envisaged by Holt et al. [3, 8]. However, our study shows that for RYE allergen this occurs progressively throughout childhood rather than being established in infancy and the pre-school years. It is pertinent to note that this pattern of development of the immune response to RYE also has biological plausibility, as it is consistent with the clinical expression of rye grass pollen allergy that develops progressively with age throughout childhood and early adult life.

Acknowledgement

J. M. Smart was supported by a National Health and Medical Research Council Medical Postgraduate Research Scholarship.

References

- 1 Donovan CE, Finn PW. Immune mechanisms of childhood asthma. *Thorax* 1999; 54:938–46.
- 2 Buckley RH, Seymour F, Sanal SO, Ownby DR, Becker WG. Lymphocyte responses to purified ragweed allergens in vitro. I. Proliferative responses in normal, newborn, agammaglobulinemic, and atopic subjects. *J Allergy Clin Immunol* 1977; 59:70–8.
- 3 Holt PG, O'Keeffe P, Holt BJ et al. T-cell 'priming' against environmental allergens in human neonates: sequential deletion of food antigen reactivity during infancy with concomitant expansion of responses to ubiquitous inhalant allergens. *Pediatr Allergy Immunol* 1995; 6:85–90.
- 4 Holt PG. Development of T-cell memory against inhalant allergens: risks for the future. *Clin Exp Allergy* 1999; 29 (Suppl. 2):8–13.
- 5 Kobayashi Y, Kondo N, Shinoda S, Agata H, Fukutomi O, Orii T. Predictive values of cord blood IgE and cord blood lymphocyte responses to food antigens in allergic disorders during infancy. *J Allergy Clin Immunol* 1994; 94:907–16.
- 6 Piastra M, Stabile A, Fioravanti G, Castagnola M, Pani G, Ria F. Cord blood mononuclear cell responsiveness to beta-lactoglobulin: T-cell activity in 'atopy-prone' and 'non-atopy-prone' newborns. *Int Arch Allergy Immunol* 1994; 104:358–65.
- 7 Miles EA, Warner JA, Jones AC, Colwell BM, Bryant TN, Warner JO. Peripheral blood mononuclear cell proliferative responses in the first year of life in babies born to allergic parents. *Clin Exp Allergy* 1996; 26:780–8.
- 8 Yabuhara A, Macaubas C, Prescott SL et al. TH2-polarized immunological memory to inhalant allergens in atopics is established during infancy and early childhood. *Clin Exp Allergy* 1997; 27:1261–9.
- 9 Niemeijer NR, de Monchy JG. Age-dependency of sensitization to aero-allergens in asthmatics. *Allergy* 1992; 47 (4, Part 2):431–5.
- 10 van Asperen PP, Mellis CM, South RT, Simpson SJ. Allergen skin-prick testing in asthmatic children. *Med J Aust* 1980; 2:266–8.
- 11 Kulig M, Klettke U, Wahn V, Forster J, Bauer CP, Wahn U. Development of seasonal allergic rhinitis during the first 7 years of life. *J Allergy Clin Immunol* 2000; 106:832–9.

- 12 Anonymous. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema. ISAAC. The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. *Lancet* 1998; 351:1225–32.
- 13 Stewart GA, Thomas WR. In vitro translation of messenger RNA from the house dust mite *Dermatophagoides pteronyssinus*. *Int Arch Allergy Appl Immunol* 1987; 83:384–9.
- 14 Suphioglu C, Singh MB, Simpson RJ, Ward LD, Knox RB. Identification of canary grass (*Phalaris aquatica*) pollen allergens by immunoblotting. IgE and IgG antibody-binding studies. *Allergy* 1993; 48:273–81.
- 15 Bhalla R, Rappaport I, Filippi ID, Schwartz M. Serum IgE Levels in a Northeast United States Caucasian Population. New York: Marcel Dekker, 1982.
- 16 Smart JM, Kemp AS. Ontogeny of T-helper 1 and T-helper 2 cytokine production in childhood. *Pediatr Allergy Immunol* 2001; 12:181–7.
- 17 Bullens DM, Kasran A, Peng X, Lorre K, Ceuppens JL. Effects of anti-IL-4 receptor monoclonal antibody on in vitro T cell cytokine levels: IL-4 production by T cells from non-atopic donors. *Clin Exp Immunol* 1998; 113:320–6.
- 18 Smart JM, Kemp AS. Increased Th1 and Th2 allergen induced cytokine responses in children with atopic disease. *Clin Exp Allergy* 2002; 32:796–802.
- 19 Settipleane GA. Allergic rhinitis – update. *Otolaryngol Head Neck Surg* 1986; 94:470–5.
- 20 Noma T, Yoshizawa I, Aoki K, Yamaguchi K, Baba M. Cytokine production in children outgrowing hen egg allergy. *Clin Exp Allergy* 1996; 26:1298–307.
- 21 Prescott SL, Macaubas C, Smallacombe T, Holt BJ, Sly PD, Holt PG. Development of allergen-specific T-cell memory in atopic and normal children. *Lancet* 1999; 353:196–200.
- 22 van der Velden VH, Laan MP, Baert MR, de Waal Malefyt R, Neijens HJ, Savelkoul HF. Selective development of a strong Th2 cytokine profile in high-risk children who develop atopy: risk factors and regulatory role of IFN-gamma, IL-4 and IL-10. *Clin Exp Allergy* 2001; 31:997–1006.
- 23 Prescott SL, Macaubas C, Yabuhara A et al. Developing patterns of T cell memory to environmental allergens in the first two years of life. *Int Arch Allergy Immunol* 1997; 113:75–9.
- 24 Bjorksten B, Holt BJ, Baron-Hay MJ, Munir AK, Holt PG. Low-level exposure to house dust mites stimulates T-cell responses during early childhood independent of atopy. *Clin Exp Allergy* 1996; 26:775–9.
- 25 Hales BJ, Shen HD, Thomas WR. Cross-reactivity of T-cell responses to *Dermatophagoides pteronyssinus* and *D. farinae*. Studies with group 1 and 7 allergens. *Clin Exp Allergy* 2000; 30:927–33.
- 26 Upham JW, Holt BJ, Baron-Hay MJ et al. Inhalant allergen-specific T-cell reactivity is detectable in close to 100% of atopic and normal individuals: covert responses are unmasked by serum-free medium. *Clin Exp Allergy* 1995; 25:634–42.
- 27 Wurtzen PA, van Neerven RJ, Arnved J, Ipsen H, Sparholt SH. Dissection of the grass allergen-specific immune response in patients with allergies and control subjects: T-cell proliferation in patients does not correlate with specific serum IgE and skin reactivity. *J Allergy Clin Immunol* 1998; 101 (2, Part 1):241–9.
- 28 Baskar S, Hamilton RG, Norman PS, Ansari AA. Grass immunotherapy induces inhibition of allergen-specific human peripheral blood mononuclear cell proliferation. *Int Arch Allergy Immunol* 1997; 112:184–90.
- 29 Kahler H, Stuwe H, Cromwell O, Fiebig H. Reactivity of T cells with grass pollen allergen extract and allergoid. *Int Arch Allergy Immunol* 1999; 120:146–57.
- 30 Jepsen KF, Nielsen L, Olsen OT, Bendtzen K, Poulsen LK. Seasonal variations in T-lymphocyte response to grass pollen allergens from pollen-allergic patients and healthy controls. *Exp Clin Immunogenetics* 1998; 15:144–53.
- 31 Ong E, Singh M, Knox R. Grass pollen in the atmosphere of Melbourne: seasonal distribution over nine years. *Grana* 1995; 34:58–63.
- 32 Todt J, Sonstein J, Polak T, Seitzman GD, Hu B, Curtis JL. Repeated intratracheal challenge with particulate antigen modulates murine lung cytokines. *J Immunol* 2000; 164:4037–47.
- 33 Yiamouyiannis CA, Schramm CM, Puddington L et al. Shifts in lung lymphocyte profiles correlate with the sequential development of acute allergic and chronic tolerant stages in a murine asthma model. *Am J Pathol* 1999; 154:1911–21.
- 34 Hochrein H, O'Keeffe M, Luft T et al. Interleukin (IL)-4 is a major regulatory cytokine governing bioactive IL-12 production by mouse and human dendritic cells. *J Exp Med* 2000; 192:823–33.
- 35 Giannarini L, Maggi E. Decrease of allergen-specific T-cell response induced by local nasal immunotherapy. *Clin Exp Allergy* 1998; 28:404–12.
- 36 Benjaponpitak S, Oro A, Maguire P, Marinkovich V, DeKruyff RH, Umetsu DT. The kinetics of change in cytokine production by CD4 T cells during conventional allergen immunotherapy. *J Allergy Clin Immunol* 1999; 103 (3, Part 1):468–75.