

Increased Th1 and Th2 allergen-induced cytokine responses in children with atopic disease

J. M. Smart and A. S. Kemp

Department of Immunology, Murdoch Children's Research Institute, Royal Children's Hospital, Parkville, Australia

Summary

Background Polyclonal cytokine responses following stimulation of T cells with mitogens or superantigens provides information on cytokine production from a wide range of T cells. Alternatively allergen-induced T cell responses can provide information on cytokine production by allergen-reactive T cells. While there is evidence of increased Th2 and reduced Th1 cytokine production following T cell stimulation with non-specific mitogens and superantigens, the evidence that Th1 cytokine production to allergens is decreased in line with a postulated imbalance in Th1/Th2 responses is unclear, with studies finding decreased, no difference or increased IFN- γ responses to allergens in atopic subjects.

Objective To examine childhood polyclonal and allergen-induced cytokine responses in parallel to evaluate cytokine imbalances in childhood atopic disease.

Methods PBMC cytokine responses were examined in response to a polyclonal stimulus, staphylococcal superantigen (SEB), in parallel with two inhalant allergens, house dust mite (HDM) and rye grass pollen (RYE), and an ingested allergen, ovalbumin (OVA), in (a) 35 healthy children (non-atopic) and (b) 36 children with atopic disease (asthma, eczema and/or rhinitis) (atopic).

Results Atopic children had significantly reduced IFN- γ and increased IL-4 and IL-5 but not IL-13 production to SEB superantigen stimulation when compared with non-atopic children. HDM and RYE allergens stimulated significantly increased IFN- γ , IL-5 and IL-13, while OVA stimulated significantly increased IFN- γ production in atopic children.

Conclusion We show that a polyclonal stimulus induces a reduced Th1 (IFN- γ) and increased Th2 (IL-4 and IL-5) cytokine pattern. In contrast, the allergen-induced cytokine responses in atopic children were associated with both increased Th1 (IFN- γ) and Th2 (IL-5 and IL-13) cytokine production. The increased Th1 response to allergen is likely to reflect prior sensitization and indicates that increases in both Th1 and Th2 cytokine production to allergens exists concomitantly with a decreased Th1 response to a polyclonal stimulus in atopic children.

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

Submitted 14 April 2001; revised 2 July 2001; accepted 7 December 2001

Introduction

There are two approaches to the examination of cytokine imbalance in childhood atopic disease. The stimulation of T cells by non-specific mitogens such as phytohaemagglutinin (PHA) [1–4] or superantigens such as staphylococcal enterotoxin B (SEB) [5, 6] examines the underlying predisposition of cytokine production. Here the production of cytokines from a wide range of T cells is studied. This approach, which has identified a defect in IFN- γ secretion following polyclonal stimulation that is detectable at birth and precedes the development of atopic disease [7, 8], provides evidence that a cytokine imbalance with reduced Th1 secretion may predispose to the

development of atopic disease. An alternative approach is to examine allergen/antigen-stimulated T cell responses, which provides information on cytokine production by allergen-reactive cells influenced by prior antigen exposure. Although atopic disease is widely regarded as being due to the deviation of immune responses to a Th2-type [9, 10], the evidence that allergen-induced Th1 cytokine production is down-regulated is less clear. Some studies of allergen responses in childhood have reported increased IFN- γ [11–13], whilst others document reduced [14] or no difference [15] in IFN- γ production as compared with non-atopic subjects.

It is therefore possible there is a dichotomy with regard to cytokine imbalance, with a defect in IFN- γ secretion that predisposes to atopic disease being revealed by polyclonal stimulation while there is increased allergen-induced IFN- γ secretion resulting from prior sensitization and expansion of an allergen-specific T cell population. To explore this further we have evaluated non-specific cytokine responses using a polyclonal

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

stimulus, the superantigen staphylococcal enterotoxin B (SEB) in parallel with allergen-induced cytokine responses to inhalant (house dust mite and rye grass pollen) and ingested (ovalbumin) allergens.

Materials and methods

Reagents

The following reagents were used: AIM-V medium (GIBCO, 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) anti- $\text{INF-}\gamma$, biotinylated anti- $\text{INF-}\gamma$, anti-IL-4, biotinylated anti-IL-4, anti-IL-5, biotinylated anti-IL-5, anti-IL-13, biotinylated anti-IL-13, recombinant human (rh)- $\text{INF-}\gamma$, rh-IL-4, rh-IL-5, rh-IL-13 (Pharmingen, San Diego, CA, USA).

Patients

We selected study subjects who were aged 2 to 16 years as investigation of the ontogeny of cytokine responses found that children under 2 years produced significantly less $\text{INF-}\gamma$, IL-4, IL-5 and IL-13 in response to SEB than older children [16].

Heparinized venous blood samples were obtained from:

- Thirty-five healthy children (non-atopic) (median age 6.4 years) who were admitted for elective surgery (circumcisions, herniotomies, orchidopexies, squint repairs or orthopaedic procedures) at the Royal Children's Hospital, Melbourne, Australia. These children had no history of atopy and plasma IgE levels within the normal range for age (median IgE 29 kU/L). Blood was obtained immediately on anaesthetic induction.
- Thirty-six children with atopic disease (atopic) (median age 7.2 years), elevated IgE levels for age (median IgE 1300 kU/L) and a positive radioallergen sorbent test (RAST) and skin prick test (SPT) to one or more of three allergens tested (egg white, house dust mite, rye grass pollen). A positive SPT was defined as a weal diameter 3 mm or greater than the saline control. Allergen sensitization was defined as a positive RAST and SPT to house dust mite (HDM) ($n = 32$), rye grass pollen (RYE) ($n = 26$) and/or egg white ($n = 7$). These patients had eczema ($n = 27$), asthma ($n = 28$) and/or rhinitis ($n = 26$).

Informed consent was obtained from a parent/guardian of all subjects. This study was approved by the ethics committee of the Royal Children's Hospital, Melbourne.

Allergen preparation

Crude dried house dust mite (*Dermatophagoides pteronyssinus*; CSL, Parkville, Australia) was homogenized in PBS, clarified by centrifugation, dialysed in 0.05 M ammonium bicarbonate, freeze dried, then stored at -70°C . Samples were then resuspended in PBS, filter sterilized (pore size 2 μm) and stored at -70°C .

Grade II ovalbumin (Sigma Chemical Co.) was dissolved in PBS, clarified by centrifugation, filter sterilized (pore size 2 μm) then stored at -70°C .

Rye grass pollen extract (*Lolium perenne*, Greer Laboratories, Lenoir, NC, USA) was prepared by C. Suphioglu (Melbourne, Australia) according to previously published methods [17].

Plasma IgE and RAST

Plasma was analysed for total IgE and allergen-specific IgE levels using the 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 ranges for age: 2–4 years 0–130 kU/L, 4–7 years 0–150 kU/L, > 7 years 0–200 kU/L. Allergen-specific IgE levels above 0.7 kU/L were 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 (1×10^6 cells/mL) were cultured in AIM-V serum free media with B-ME at 37°C in a humidified atmosphere with 5% CO_2 . Cultures were stimulated with SEB (1 $\mu\text{g/mL}$), purified allergen (grade II ovalbumin 100 $\mu\text{g/mL}$, HDM 10 $\mu\text{g/mL}$, rye grass 20 $\mu\text{g/mL}$) or media alone.

Cell proliferation

PBMC (1×10^5 cells) were cultured in a volume of 100 μL 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 μCi 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 calculated by the ratio of counts per minute of mean of unstimulated to stimulated sample.

Cytokine production

For cytokine production PBMC (1×10^6 cells) were cultured in volumes of 1000 μL . Preliminary time course studies revealed SEB induced maximal IL-4, IL-5, IL-13 and $\text{INF-}\gamma$ production at 48 h. Allergens induced maximal IL-5, IL-13 and $\text{INF-}\gamma$ production at 6 days. Supernatants were collected at 48 h (SEB- and allergen-stimulated cultures) and day 6 (allergen-stimulated cultures) and stored at -70°C . $\text{INF-}\gamma$, IL-4, IL-5 and IL-13 were assayed by sandwich ELISA as previously described [6, 16]. Briefly, high binding ELISA plates were coated with purified capture MoAb (anti- $\text{INF-}\gamma$, anti-IL-4, anti-IL-5 or anti-IL-13) (1 $\mu\text{g/mL}$) 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 were incubated at room temperature for a further 4 h. After washing, biotinylated MoAb (anti- $\text{INF-}\gamma$, anti-IL-4, anti-IL-5 or anti-IL-13) (0.5 $\mu\text{g/mL}$) 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, Gaithersburg, MD, USA) was added and 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 data were analysed using Biomek 1000 data reduction software (Beckman Fullarton, CA, USA). Minimum detection levels were $\text{INF-}\gamma$ 10 pg/mL, IL-4 2 pg/mL, IL-5 10 pg/mL and IL-13 5 pg/mL.

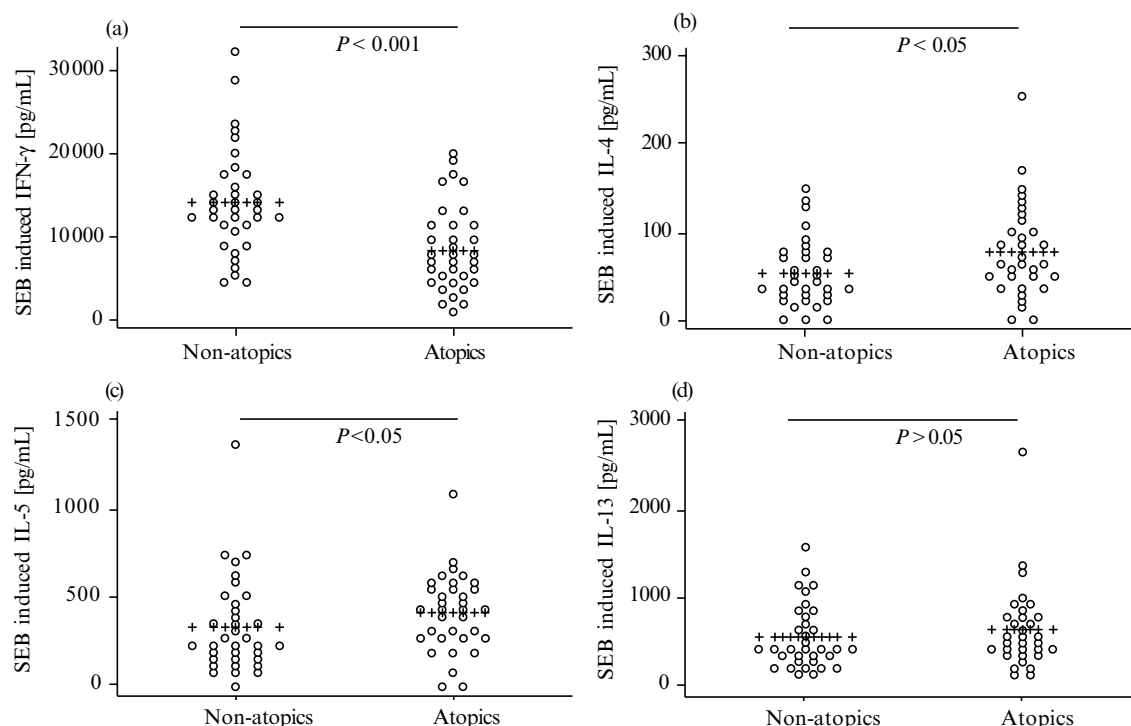


Fig. 1. SEB induced cytokine responses in non-atopic ($n = 35$) and atopic ($n = 36$) children. (a) IFN- γ ; (b) IL-4; (c) IL-5; (d) IL-13. Results are shown with mean and P -value for comparison of non-atopic and atopic groups.

Statistical analysis

Allergen-induced cytokine levels are presented as means and ranges. For the purpose of statistical analysis, if the level of cytokine production was below minimum detectable levels, a level of 1 pg/mL was arbitrarily allocated. As allergen-induced cytokine levels were not normally distributed the significance of the difference in the data was examined by the Mann–Whitney non-parametric U -test using Stata statistical software, release 5 (StataCorp 1997, College Station, TX, USA).

Results

Superantigen-induced cytokine responses

The SEB-induced cytokine response in atopic children revealed significantly reduced IFN- γ (atopic: mean = 8327 pg/mL; non-atopic: mean = 13858 pg/mL; $P < 0.001$) (Fig. 1a) and increased IL-4 (atopic: mean = 77 pg/mL; non-atopic: mean = 52 pg/mL; $P < 0.05$) (Fig. 1b) and IL-5 (atopic: mean = 421 pg/mL; non-atopic: mean = 335 pg/mL; $P < 0.05$) (Fig. 1c) production when compared with non-atopic children. There was no difference in SEB-induced IL-13 production between atopic and non-atopic children (Fig. 1d).

Allergen-induced cytokine responses

Cytokine responses were examined to house dust mite (HDM), rye grass pollen (RYE) and ovalbumin (OVA). Inhalant allergens induced more cytokine secretion than the ingested allergen. All three allergens stimulated increased IFN- γ production in atopic children. HDM: atopic, mean = 314 pg/mL; non-atopic, mean = 110 pg/mL ($P < 0.05$) (Fig. 2a). RYE: atopic,

mean = 186 pg/mL; non-atopic, mean = 60 pg/mL ($P < 0.05$) (Fig. 2b). OVA: atopic, mean = 76 pg/mL; non-atopic, mean = 23 pg/mL ($P < 0.05$) (Fig. 2c). Atopic children produced increased IL-5 (HDM: atopic, mean = 936 pg/mL; non-atopic, mean = 104 pg/mL; $P < 0.0001$) (Fig. 2d); RYE: atopic, mean = 295 pg/mL; non-atopic, mean = 31 pg/mL; $P < 0.0001$ (Fig. 2e) and IL-13 (HDM: atopic, mean = 1554 pg/mL; non-atopic, mean = 535 pg/mL; $P < 0.001$ (Fig. 2g); RYE: atopic, mean = 306 pg/mL; non-atopic, mean = 105 pg/mL; $P < 0.0001$) (Fig. 2h) to the two inhalant allergens; however, IL-5 and IL-13 production in response to OVA was not significantly different (IL-5 $P > 0.05$) (Fig. 2f) (IL-13 $P > 0.05$) (Fig. 2i) from non-atopic children. Insufficient IL-4 was produced in response to allergens to be detected by the ELISA method used.

Allergen-induced cytokine responses according to sensitization status

Allergen-induced cytokine responses were evaluated in atopic children according to their sensitization status as defined as a positive SPT and RAST to the relevant allergen (Table 1) and compared with the non-atopic control children. When compared with non-atopic children, HDM-sensitive children had significantly elevated IFN- γ ($P < 0.01$), IL-5 ($P < 0.0001$) and IL-13 ($P < 0.001$) in response to HDM. RYE-induced IL-5 and IL-13 production in both RYE sensitive (IL-5 $P < 0.0001$, IL-13 $P < 0.001$) and non-sensitive (IL-5 $P < 0.01$, IL-13 $P < 0.01$) children was significantly elevated compared with non-atopic children. Ovalbumin-sensitive children had significantly elevated OVA-induced IFN- γ ($P < 0.01$) production when compared with non-atopic children but no difference in OVA-induced IL-5 and IL-13 production.

Table 1. Allergen-induced cytokine responses according to sensitization status

	IFN- γ mean(range)	IL-5 mean(range)	IL-13 mean(range)
HDM			
SPT+ ($n = 32$)	379 (0–1936)*	1018 (0–2818)***	1614 (125–4970)**
SPT– ($n = 4$)	217 (0–613)	158 (0–631)	974 (241–2265)
Control ($n = 35$)	110 (0–771)	104 (0–629)	535 (0–3076)
RYE			
SPT+ ($n = 26$)	190 (0–1409)	289 (0–1204)***	250 (0–992)**
SPT– ($n = 10$)	163 (0–610)	304 (0–1029)*	421 (0–1507)*
Control ($n = 35$)	60 (0–552)	31 (0–372)	105 (0–258)
OVA			
SPT+ ($n = 7$)	162 (0–412)*	47 (0–328)	134 (0–604)
SPT– ($n = 29$)	56 (0–366)	75 (0–561)	140 (0–1012)
Control ($n = 35$)	23 (0–349)	13 (0–217)	58 (0–417)

Compared with control * $P < 0.01$, ** $P < 0.001$, *** $P < 0.0001$.

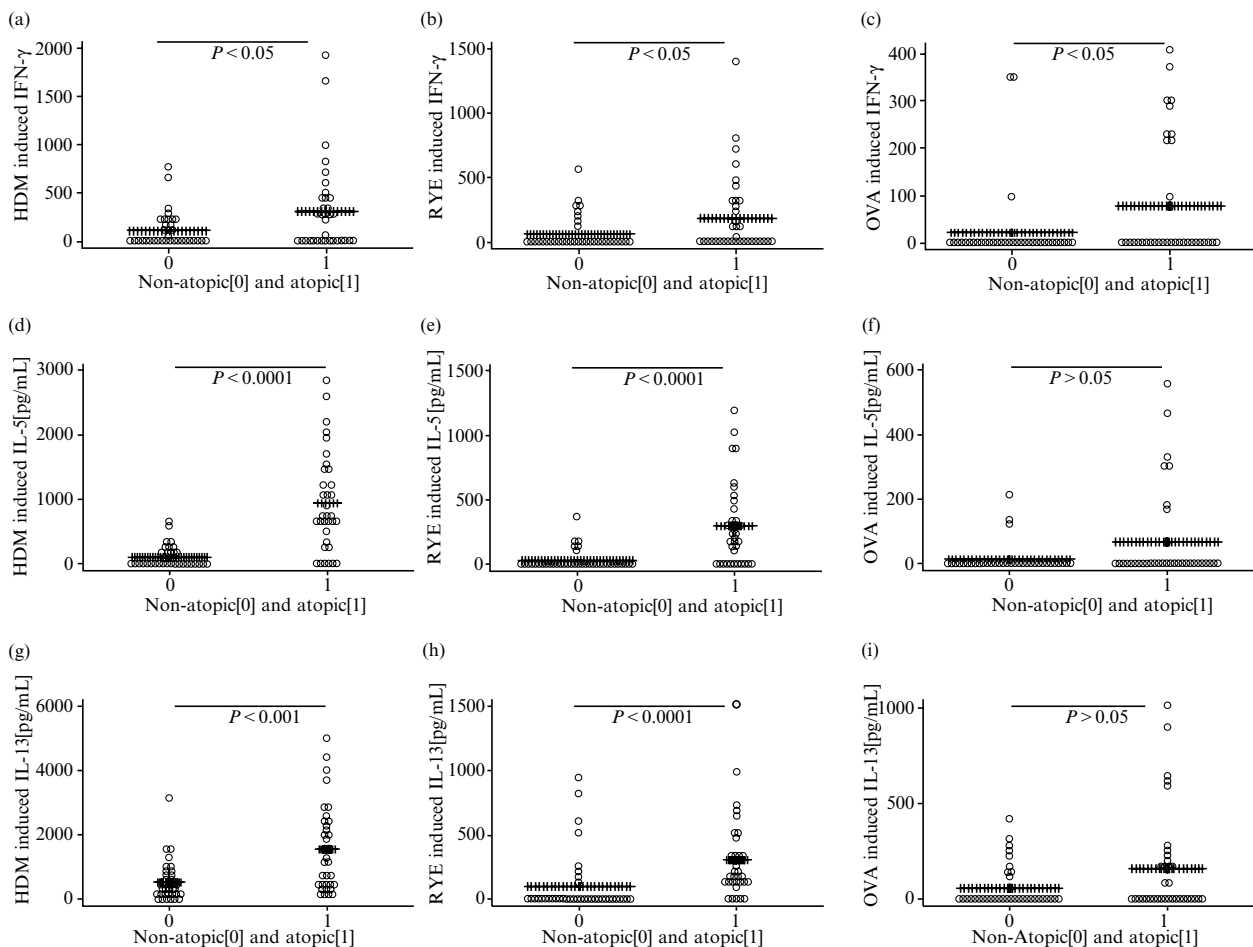


Fig. 2. Allergen-induced cytokine responses in non-atopic ($n = 35$) and atopic ($n = 36$) children. (a) HDM-induced IFN- γ ; (b) RYE-induced IFN- γ ; (c) OVA-induced IFN- γ ; (d) HDM-induced IL-5; (e) RYE-induced IL-5; (f) OVA-induced IL-5; (g) HDM-induced IL-13; (h) RYE-induced IL-13; (i) OVA-induced IL-13. Results are shown with mean and P -value for comparison of non-atopic and atopic groups.

The allergen-induced proliferative responses were also compared between atopic children who were both sensitive and non-sensitive to the relevant allergen and non-atopic

children. There was no significant difference in proliferative responses to the relevant allergens in each group (data not shown).

Discussion

The cytokine imbalance in childhood atopic disease has been examined in two principal ways. Stimulation of T cells by non-specific mitogens such as PHA [1–4] or superantigens such as staphylococcal enterotoxin B (SEB) [5, 6] examines the underlying predisposition of cytokine production. Here the production of cytokines from a wide range of T cells is studied. Using this approach and SEB as the stimulus we found the polyclonal cytokine responses of children over 2 years of age were consistent with our previous findings that PBMC from children with atopic disease showed a reduction in Th1 (IFN- γ) and an increase in Th2 (IL-4 and IL-5) cytokines [2, 3, 6]. This defect in IFN- γ secretion following polyclonal stimulation is detectable at birth and precedes the development of atopic disease [7, 8], which suggests that a cytokine imbalance towards a Th2 profile may predispose to the development of atopic disease. A second approach is to examine allergen/antigen-induced T cell responses, which provide information on cytokine production by sensitized T cells, which may be influenced by prior antigen exposure and does not necessarily provide the same information as a polyclonal stimulus. Neonates and infants considered to be at high risk for the development of atopic disease have been found to have delayed postnatal maturation of allergen-induced Th1 cell functions and a failure to deviate away from Th2 cell functions [10]. Such findings have suggested that the cytokine imbalance with increased Th2 and decreased Th1 secretion leads to the development of atopic disease in childhood [18]. In this study we demonstrate a significantly increased IFN- γ response to both inhaled and ingested allergens in atopic children concomitantly with an increased Th2 (IL-5 and IL-13) response to the same allergens. These observations indicate an enhancement of both Th1 and Th2 type allergen-induced responses rather than a down-regulation of Th1 responses as has been suggested to occur in the development of atopic disease.

Our findings are consistent with other workers who have described increased IFN- γ production in response to allergens in childhood [11–14, 19] and adult atopics [20, 21], although none have demonstrated the increased IFN- γ response to allergens and decreased response to a polyclonal stimulus simultaneously. The findings of increased IFN- γ to allergen but decreased IFN- γ to a polyclonal stimulus are not necessarily inconsistent. In contrast to mitogens and superantigens that stimulate large numbers of lymphocytes, allergens stimulate only those that are specifically sensitized to the antigen in question with a requirement for antigen presentation in combination with MHC class II receptor present on CD4⁺ T cells. As only a minority of cells in an atopic inflammatory lesion are allergen specific [22, 23], increased T cell sensitization as reflected in increased IFN- γ production following allergen stimulation could coexist with a decreased polyclonal response. The increased allergen-induced Th1 and Th2 cytokine production by atopic children is likely to reflect the expansion of a memory (CD45RO) T cell population, which has occurred to a greater degree in atopic than non-atopic children, as memory cells produce 10-fold more IFN- γ than do naïve T cells [24]. The decreased IFN- γ secretion in response to SEB reflects the propensity of both CD4⁺ and CD8⁺ T cells [25] and is consistent with the previous findings of ourselves [2, 16, 25] and others [4, 26] that atopic children exhibit a reduced IFN- γ response to polyclonal stimuli.

Further evidence for a significant Th1 response in atopic disease comes from studies of cytokine expression and production from peripheral blood mononuclear cells and at sites of allergic inflammation. HDM-stimulated PBMC from HDM-sensitive adult asthmatics produced IFN- γ in amounts far greater than IL-4 and IL-5 [27]. IFN- γ mRNA-positive cells predominated over IL-4 mRNA-positive cells in the lesional skin of atopic dermatitis [28]. Punch biopsy specimens from patients with atopic dermatitis following application of HDM allergen to the skin showed that the predominant response after 48 h was IFN- γ -containing cells that were almost twice as frequent as IL-4-containing cells [29]. The majority (71%) of HDM-specific T-cell clones generated from biopsies of affected skin from patients with atopic dermatitis express IFN- γ mRNA and secrete IFN- γ protein [30]. Local allergen challenge in subjects with perennial rhinitis induced a marked increase in IFN- γ and IL-5 but no detectable IL-4 in nasal secretions [31] and HDM-stimulated bronchiolar lavage cells from adult atopic asthmatics produced IFN- γ in quantities that exceeded IL-5 [32]. Thus examination of cells from peripheral blood and at sites of the inflammatory reaction in atopic disease indicates a significant IFN- γ response in addition to a Th2 response and is consistent with the present findings of significant IFN- γ production by PBMC following allergen stimulation.

Increased Th2 cytokine production was not necessarily linked to the production of IgE, as we found Th2 cytokine production to RYE was also significantly increased in the atopic subjects who were not sensitized to rye grass pollen allergen. A similar observation of increased Th2 cytokine expression not necessarily associated with IgE production has been made in bronchial biopsies from subjects with non-atopic asthma [33]. Such an increase provides additional mechanisms for the development of tissue inflammation independent of IgE sensitization, such as eosinophil infiltration due to IL-5. It is also possible that the increased Th2 cytokine production to rye grass pollen allergen in the absence of specific IgE may precede the subsequent development of specific IgE antibodies later in life. In this context it is of interest that while HDM sensitization seems to be largely determined before 10 years of age, sensitization to RYE increases progressively throughout the first two decades of life. Thus, in the Australian environment, a study of atopic children with positive skin prick test responses found that by 2 to 4 years approximately 90% had positive reactions to HDM but only 20% at this age had positive reactions to RYE, increasing progressively to 45% by 12 to 14 years [34].

IL-13 appears to be required for optimal induction of IgE synthesis [35] and has been considered to be an important mediator of allergic disease and asthma [36, 37]. In the present study IL-13 secretion in response to the polyclonal stimulus was not increased in atopic subjects, in contrast to the other Th2 cytokines IL-4 and IL-5. IL-13 production in response to a polyclonal stimulus was not different in studies of atopic and non-atopic-older children [38] and adults [39, 40]. In contrast to the findings with the polyclonal stimulus we found a significant increase in IL-13 secretion in atopic children following stimulation with the inhalant allergens HDM and RYE, which is consistent with the findings of other workers [12, 41]. These findings indicate that the atopics do not necessarily have an intrinsic increased propensity to secrete IL-13, as demonstrated with a polyclonal stimulus; however, with age following

expansion of specific memory T cells by allergens the allergen-induced IL-13 response is enhanced.

It has been postulated that the failure of deviation of infant T cells from a universal neonatal Th2-type response to allergen to a Th1-type profile is the mechanism underlying the development of allergic disease [9, 10]. However, the allergen-induced secretion of cord blood Th1 cytokines, such as IFN- γ and Th2 cytokines IL-4 and IL-5, is very low to undetectable and thus the cytokine profile has been characterized on the basis of mRNA responses [10]. The detection of mRNA does not necessarily reflect cytokine secretion and as demonstrated both in the present study and in a study of cord blood [42] conclusions concerning the Th1/Th2 balance derived from cytokine responses to polyclonal stimuli and allergens may differ. Furthermore, the hypothesis of an environmentally based deviation of the immune response towards a Th2 and away from a Th1 response is hard to reconcile with the epidemiological evidence that the occurrence of Th1 diseases such as type 1 diabetes is positively rather than negatively associated with asthma [43] and that both Th1 and Th2 diseases have increased under the same environmental influences [44]. A conception of atopic disease in which both Th1 and Th2 responses may be enhanced, as demonstrated in the present study, accords better with these epidemiological observations.

From the present study it seems that the atopic state in childhood is characterized by both an increased Th1 response to common allergens as reflected by IFN- γ secretion as well as increased Th2 cytokine responses. These heightened responses are not necessarily associated with the development of sensitization as measured by specific IgE. Although it has been considered that Th1 and Th2 cell development may be reciprocally linked [18] in view of the capacity of IFN- γ to promote Th1 and suppress Th2 cell development, recent animal models challenge this view. Thus, transgenic mice with abnormal IFN- γ receptor expression resulting in impaired Th1 cell development did not exhibit enhanced Th2 development either *in vitro* or *in vivo* [45]. The precise events determining Th1 or Th2 development *in vivo* are unclear; however, it would appear from the present study and the results of others that increased allergen-induced Th1 and Th2 responses occur together in atopic children. In summary we view childhood atopic disease as being characterized by an innate reduction in Th1 cytokine responses, as revealed by cytokine production induced by polyclonal stimuli in association with increased Th1 and Th2 responses to allergens, which results in an inflammatory response that leads to specific organ disease.

Acknowledgements

J. M. Smart was supported by a National Health & Medical Research Council Medical Postgraduate Research Scholarship. We are grateful to Cenk Suphioglu, who prepared and provided the rye grass pollen allergen for use in cell culture.

References

- Juho K, Renz H, Abe J, Gelfand EW, Leung DY. Decreased interferon gamma and increased interleukin-4 production in atopic dermatitis promotes IgE synthesis. *J Allergy Clin Immunol* 1992; 90:323–31.
- Tang M, Kemp A, Varigos G. IL-4 and interferon-gamma production in children with atopic disease. *Clin Exp Immunology* 1993; 92:120–4.
- Tang ML, Coleman J, Kemp AS. Interleukin-4 and interferon-gamma production in atopic and non-atopic children with asthma. *Clin Exp Allergy* 1995; 25:515–21.
- Nurse B, Haus M, Puterman AS, Weinberg EG, Potter PC. Reduced interferon-gamma but normal IL-4 and IL-5 release by peripheral blood mononuclear cells from Xhosa children with atopic asthma. *J Allergy Clin Immunol* 1997; 100:662–8.
- Konig B, Neuber K, Konig W. Responsiveness of peripheral blood mononuclear cells from normal and atopic donors to microbial superantigens. *Int Arch Allergy Immunol* 1995; 106:124–33.
- Campbell DE, Kemp AS. Proliferation and production of interferon-gamma (IFN-gamma) and IL-4 in response to *Staphylococcus aureus* and staphylococcal superantigen in childhood atopic dermatitis. *Clin Exp Immunology* 1997; 107:392–7.
- Tang ML, Kemp AS, Thorburn J, Hill DJ. Reduced interferon-gamma secretion in neonates and subsequent atopy. *Lancet* 1994; 344:983–5.
- Warner JA, Miles EA, Jones AC et al. Is deficiency of interferon gamma production by allergen triggered cord blood cells a predictor of atopic eczema? *Clin Exp Allergy* 1994; 24:423–30.
- Holt PG. Development of T-cell memory against inhalant allergens: risks for the future. *Clin Exp Allergy* 1999; 29:8–13.
- Prescott SL, Macaubas C, Smallacombe T et al. Development of allergen-specific T-cell memory in atopic and normal children. *Lancet* 1999; 353:196–200.
- Holt PG, Rudin A, Macaubas C et al. Development of immunologic memory against tetanus toxoid and pertactin antigens from the diphtheria-tetanus-pertussis vaccine in atopic versus nonatopic children. *J Allergy Clin Immunol* 2000; 105:1117–22.
- Macaubas C, Sly PD, Burton P et al. Regulation of T-helper cell responses to inhalant allergen during early childhood. *Clin Exp Allergy* 1999; 29:1223–31.
- Kimura M, Tsuruta S, Yoshida T. Unique profile of IL-4 and IFN-gamma production by peripheral blood mononuclear cells in infants with atopic dermatitis. *J Allergy Clin Immunol* 1998; 102:238–44.
- 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.
- Nurse B, Puterman AS, Haus M et al. PBMCs from both atopic asthmatic and nonatopic children show a TH2 cytokine response to house dust mite allergen. *J Allergy Clin Immunol* 2000; 106:84–91.
- Smart J, Kemp A. Ontogeny of TH1 and TH2 cytokine production in childhood. *Paed Allergy Immunol* 2001; 12:181–7.
- 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.
- Romagnani S. T-cell subsets (Th1 versus Th2). *Ann Allergy, Asthma Immunol* 2000; 85:9–18.
- Shinbara M, Kondo N, Agata H et al. Interferon-gamma and interleukin-4 production of ovalbumin-stimulated lymphocytes in egg-sensitive children. *Ann Allergy, Asthma Immunol* 1996; 77:60–6.
- Noma T, Yoshizawa I, Kou K et al. Pattern of cytokine production by T cells from adolescents with asthma in remission, after stimulation with *Dermatophagoides farinae* antigen. *Pediatric Res* 1995; 38:187–93.
- Laan MP, Baert MR, Vredendaal AE, Savelkoul HF. Differential mRNA expression and production of interleukin-4 and interferon-gamma in stimulated peripheral blood mononuclear cells of

- house-dust mite-allergic patients. *European Cytokine Network* 1998; 9:75–84.
- 22 Romagnani S. Induction of TH1 and TH2 responses: a key role for the 'natural' immune response? *Immunol Today* 1992; 13:379–81.
- 23 Dunbar PR, Ogg GS, Chen J et al. Direct isolation, phenotyping and cloning of low-frequency antigen-specific cytotoxic T lymphocytes from peripheral blood. *Current Biol* 1998; 8:413–6.
- 24 Wilson CB. The ontogeny of T lymphocyte maturation and function. *J Pediatrics* 1991; 118:S4–9.
- 25 Campbell DE, Fryga AS, Bol S, Kemp AS. Intracellular interferon-gamma (IFN-gamma) production in normal children and children with atopic dermatitis. *Clin Exp Immunology* 1999; 115:377–82.
- 26 Hoekstra MO, Hoekstra Y, De Reus D et al. Interleukin-4, interferon-gamma and interleukin-5 in peripheral blood of children with moderate atopic asthma. *Clin Exp Allergy* 1997; 27:1254–60.
- 27 Chang JH, Chan H, Quirce S et al. In vitro T-lymphocyte response and house dust mite-induced bronchoconstriction. *J Allergy Clin Immunol* 1996; 98:922–31.
- 28 Grewe M, Gyufko K, Schopf E, Krutmann J. Lesional expression of interferon-gamma in atopic eczema. *Lancet* 1994; 343:25–6.
- 29 Thepen T, Langeveld-Wildschut EG, Bihari IC et al. Biphasic response against aeroallergen in atopic dermatitis showing a switch from an initial TH2 response to a TH1 response in situ: an immunocytochemical study. *J Allergy Clin Immunol* 1996; 97:828–37.
- 30 Werfel T, Morita A, Grewe M et al. Allergen specificity of skin-infiltrating T cells is not restricted to a type-2 cytokine pattern in chronic skin lesions of atopic dermatitis. *J Invest Dermatology* 1996; 107:871–6.
- 31 Klimek L, Dormann D, Jarman ER et al. Short-term preseasonal birch pollen allergoid immunotherapy influences symptoms, specific nasal provocation and cytokine levels in nasal secretions, but not peripheral T-cell responses, in patients with allergic rhinitis. *Clin Exp Allergy* 1999; 29:1326–35.
- 32 Tang C, Rolland JM, Ward C, Quan B, Walters EH. IL-5 production by bronchoalveolar lavage and peripheral blood mononuclear cells in asthma and atopy. *European Respiratory J* 1997; 10:624–32.
- 33 Humbert M, Durham SR, Ying S et al. IL-4 and IL-5 mRNA and protein in bronchial biopsies from patients with atopic and nonatopic asthma: evidence against 'intrinsic' asthma being a distinct immunopathologic entity. *Am J Respiratory Crit Care Med* 1996; 154:1497–504.
- 34 van Asperen PP, Mellis CM, South RT, Simpson SJ. Allergen skin-prick testing in asthmatic children. *Med J Australia* 1980; 2:266–8.
- 35 de Vries JE. The role of IL-13 and its receptor in allergy and inflammatory responses. *J Allergy Clin Immunol* 1998; 102:165–9.
- 36 Wills-Karp M, Luyimbazi J, Xu X et al. Interleukin-13: central mediator of allergic asthma. *Science* 1998; 282:2258–61.
- 37 Grunig G, Warnock M, Wakil AE et al. Requirement for IL-13 independently of IL-4 in experimental asthma. *Science* 1998; 282:2261–3.
- 38 Koning H, Neijens HJ, Baert MR, Oranje AP, Savelkoul HF. T cell subsets and cytokines in allergic and non-allergic children. I. Analysis of IL-4, IFN-gamma and IL-13 mRNA expression and protein production. *Cytokine* 1997; 9:416–26.
- 39 Li Y, Simons FE, HayGlass KT. Environmental antigen-induced IL-13 responses are elevated among subjects with allergic rhinitis, are independent of IL-4, and are inhibited by endogenous IFN-gamma synthesis. *J Immunol* 1998; 161:7007–14.
- 40 Gabrielsson S, Soderlund A, Paulie S et al. Increased frequencies of allergen-induced interleukin-13-producing cells in atopic individuals during the pollen season. *Scand J Immunology* 1998; 48:429–35.
- 41 Kimura M, Tsuruta S, Yoshida T. IL-4 production by PBMCs on stimulation with mite allergen is correlated with the level of serum IgE antibody against mite in children with bronchial asthma. *J Allergy Clin Immunol* 2000; 105:327–32.
- 42 Devereux G, Hall AM, Barker RN. Measurement of T-helper cytokines secreted by cord blood mononuclear cells in response to allergens. *J Immunol Methods* 2000; 234:13–22.
- 43 Stene L, Nafstad P. Relation between occurrence of type 1 diabetes and asthma. *Lancet* 2001; 357:607–8.
- 44 Rook GA, Rosa-Brunet L, Hunt J. Cat allergen and sensitisation. *Lancet* 2001; 357:2137–8.
- 45 Tau GZ, von Der Weid T, Lu B et al. Interferon gamma signaling alters the function of T helper type 1 cells. *J Exp Medicine* 2000; 192:977–86.