

Ontogeny of T-helper 1 and T-helper 2 cytokine production in childhood

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Compared to adults, infants and young children demonstrate differences in their immune response, indicating that there is maturation or change over time and it is probable that this may be reflected in cytokine production. Cytokine responses have been demonstrated to be different in atopic and non-atopic individuals. In this study, we examined T-helper 1 (Th1) (interferon- γ [IFN- γ]) and T-helper 2 (Th2) (interleukin [IL]-4, IL-5, and IL-13) cytokine release from atopic and non-atopic children in response to the staphylococcal superantigen, staphylococcal enterotoxin B (SEB). In non-atopic and atopic children, IFN- γ , IL-4, and IL-5 release was significantly related to age. Non-atopic children younger than 2 years of age were found to have significantly reduced Th2 (IL-4, IL-5, and IL-13) responses when compared with older, non-atopic children. Atopic children had a reduced IFN- γ response when compared with non-atopics in early childhood; however, the decreased IFN- γ response seen in early childhood did not persist after 10 years. These age-related changes in cytokine production provide further support for the concept that cytokine deviations may determine the natural history of atopic disease during early childhood. In addition, the present study indicates the necessity of age-matched controls when examining children for both Th1 (IFN- γ) and Th2 (IL-4) cytokine release.

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Infants and young children demonstrate a reduced capacity for some antibody (1,2) and T-cell (2,3) responses when compared with older children and adults. Therefore, a discrepancy exists between the proliferative response of neonatal T cells to mitogens and superantigens, which appears largely intact, and the production of cytokines, which is significantly reduced (4–6). Such differences in the immune response indicate that there is a maturation or change over time and that it is probable that this may be reflected in cytokine production. It has been considered that the balance of T-helper 1 (Th1) (interferon- γ [IFN- γ]) and T-helper 2 (Th2) (interleukin [IL]-4, IL-5, and IL-13) cytokines may determine the development of atopic disease in childhood (7–9). The manifestations of atopic disease frequently change throughout childhood, evolving from early infantile eczema to the development of asthma, and such critical changes have been entitled *The Atopic March*. It is therefore

of considerable interest to determine if there are age-related changes in cytokine release throughout childhood in order to understand the ontogeny of immune responses in normal children and to determine whether this differs in atopic children. If there are age-related differences in cytokine release, this is of obvious importance in interpretation of studies in atopic children and may indicate the need for age-matched control subjects.

A number of defects in cytokine production in the neonate have been described. These defects appear to be selective, as cytokines produced predominantly by monocytes, such as interleukin-1 (10), interleukin-6 (11), and tumor necrosis factor- α (TNF- α) (10), are unaffected in the term neonate. In contrast, production of T-cell cytokines (IL-3, IL-4, IL-10, and IFN- γ) are significantly diminished in the neonate (4–6,12–14), and the production of IL-2 is affected to a lesser degree (4,14,15). Studies of T-cell cytokine release have

generally used phytohemagglutinin (PHA) (6,12–14,16,17), phorbol 12-myristate 13-acetate (PMA) (4,5), concanavalin A (ConA) (4,12), T-cell monoclonal antibodies (mAbs) (6,12,15) and, to a lesser extent, superantigens (4,12,18), as a polyclonal stimulus. The production of IFN- γ has been shown to increase rapidly throughout early infancy and reach adult levels by 1 year of age in one study (13) or by 3 years of age in another study (16). IL-4 release was found to be significantly reduced in neonates and children under 10 years of age, as compared to adults, and was observed to increase progressively with age (17). In contrast, PHA-induced IL-13 release in neonates was similar to that of adults (19). However, limited data are available on the ontogeny of most of the T-cell cytokines and further study of cytokine release, in relation to age, is indicated.

In this study, we examined Th1 (IFN- γ) and Th2 (IL-4, IL-5, IL-13) cytokine release from atopic and non-atopic children in response to the staphylococcal superantigen, staphylococcal enterotoxin B (SEB). SEB may provide a pathologically relevant stimulus because SEB has been implicated in the inflammatory response of atopic dermatitis (AD) and is commonly present on the skin of children with AD. Previous studies have demonstrated reduced IFN- γ secretion following SEB stimulation of PBMC from children with AD (18). This suggests that the superantigen SEB is likely to be a useful stimulus for examining the questions of cytokine response and cytokine imbalance in childhood atopic disease.

Materials and methods

Reagents

Reagents used were: AIM-V medium (Gibco, Grand Island, NY, USA) supplemented with 5×10^{-5} M β -mercaptoethanol (β -ME; ICN, Costa Mesa, CA, USA); SEB, avidin-peroxidase (Sigma Chemical Co., St Louis, MO, USA); Ficoll-Hypaque (Pharmacia, Uppsala, Sweden); and the following mAbs: anti-IFN- γ , biotinylated anti-IFN- γ , anti-IL-4, biotinylated anti-IL-4, anti-IL-5, biotinylated anti-IL-5, anti-IL-13, biotinylated anti-IL-13, recombinant human (rh)-IFN- γ , rhIL-4, rhIL-5, and rhIL-13 (Pharmingen, San Diego, CA, USA).

Patients

Heparinized venous blood samples were obtained from the two groups of patients described below:

1 Fifty healthy children (non-atopic) who were admitted for elective surgery at the Royal Children's Hospital (circumcisions, herniotomies,

orchidopexies, squint repairs or orthopedic procedures), and who had no history of atopy and plasma immunoglobulin E (IgE) levels within the normal range for age. Blood was obtained immediately on anesthetic induction. For comparison with atopic children, these children were divided into four age-groups: 0–2 years ($n=15$; IgE: mean 12, median 6); 2–5 years ($n=7$; IgE: mean 52, median 39); 5–10 years ($n=20$; IgE: mean 39, median 37); and 10–16 years ($n=8$, IgE: mean 24, median 17).

2 Seventy-three children with atopic disease, asthma, eczema, and/or rhinitis (atopic), with elevated IgE levels for age, who were also divided into four age-groups: 0–2 years ($n=12$; IgE: mean 638, median 392); 2–5 years ($n=14$; IgE: mean 13,158, median 940); 5–10 years ($n=35$; IgE: mean 2,337, median 940); and 10–16 years ($n=12$; IgE: mean 3,887, median 1,676).

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.

Plasma IgE

Plasma was stored at 4°C until processed for evaluation of IgE levels, determined by using the UniCAP total IgE fluoroenzyme immunoassay (Pharmacia & Upjohn Diagnostics Uppsala, Sweden). Levels of IgE are expressed in kU/l with the normal ranges for age as follows: 0–0.2 years, 0–6 kU/l; 0.2–0.5 years, 0–15 kU/l; 0.5–0.7 years, 0–20 kU/l; 0.7–1 year, 0–25 kU/l; 1–2 years, 0–35 kU/l; 2–4 years, 0–130 kU/l; 4–7 years, 0–150 kU/l; >7 years, 0–200 kU/l.

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 phosphate-buffered saline (PBS). PBMC were cultured at a concentration of 1×10^6 cells/ml in AIM-V with β -ME at 37°C in a humidified atmosphere with 5% CO₂. Cultures were stimulated with superantigen (1 μ g/ml of SEB) or left unstimulated for 48 h, as this time-period was previously been shown to be optimal for cytokine production (18).

Cell proliferation

PBMCs were cultured in triplicate for 48 h, with or without 1 μ g/ml of the SEB superantigen. Sixteen hours before harvesting onto glass-fibre filter paper, the PBMCs were pulsed with 0.5 μ Ci of

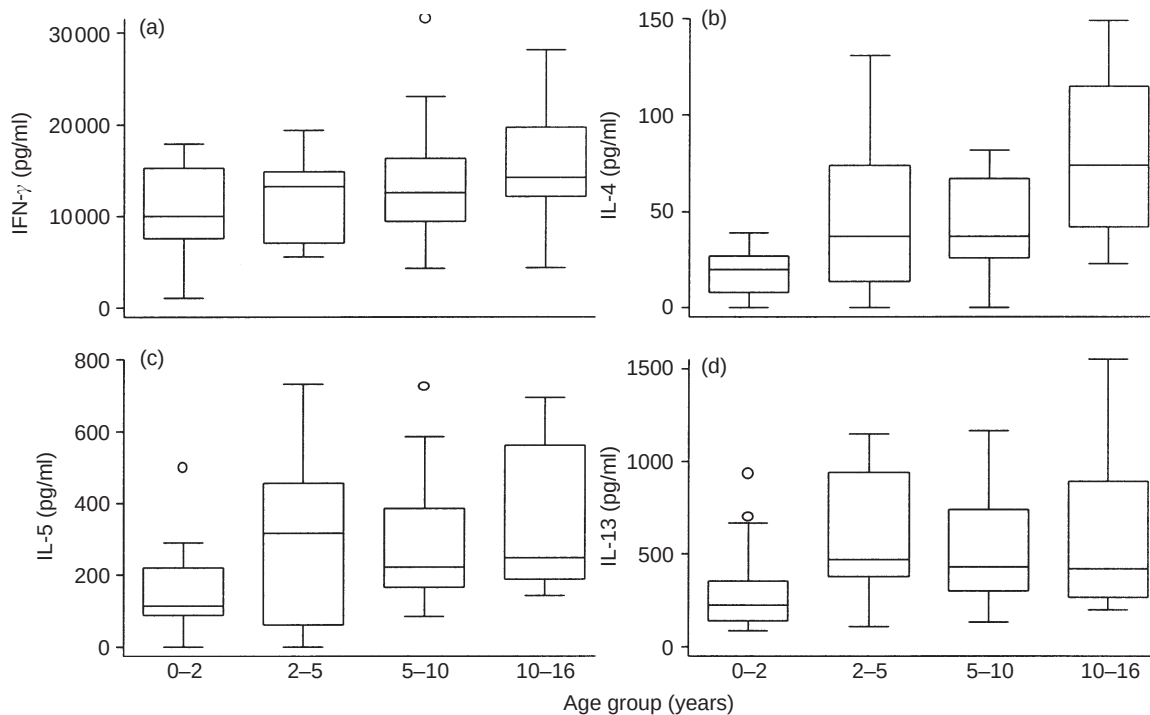


Fig. 1. Staphylococcal enterotoxin B (SEB)-induced cytokine production from peripheral blood mononuclear cells (PBMCs) from non-atopic children according to age-group. (a) Interferon- γ (IFN- γ); (b) interleukin-4 (IL-4); (c) interleukin-5 (IL-5); and (d) interleukin-13 (IL-13). Data are presented as box plots showing the median and extending to the 25th and 75th percentiles. The lines emerging from the box extend to upper and lower adjacent values. Outliers are represented as single points.

tritiated thymidine per 1×10^5 cells. Radioactivity was measured by liquid scintillation (in a β -counter) and proliferation was expressed as a stimulation index (SI), which is calculated as the ratio of counts per minute of the mean of the unstimulated to the mean of the stimulated sample.

Cytokine production

Cell cultures were centrifuged and cell-free supernatants were collected at 48 h and stored at -70°C until cytokine measurements were performed. SEB induced maximal IL-4, IL-5, IL-13, and IFN- γ production at 48 h. IFN- γ , IL-4, IL-5, and IL-13 were assayed by sandwich enzyme-linked immunosorbent assay (ELISA). Briefly, high-binding ELISA plates were coated with purified capture mAb (IFN- γ , IL-4, IL-5 or IL-13) at a concentration of $1 \mu\text{g/ml}$ 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 mAb (IFN- γ , IL-4, IL-5 or IL-13) at $0.5 \mu\text{g/ml}$ was added and incubated at room temperature for 45 min, after which avidin-peroxidase (1:400 of a 1 mg/ml solution) was added. After the final washing, 3,3',5,5'-tetramethylbenzidine substrate (KPL, Gaithersburg, MD, USA) 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 data were analyzed using Biomek 1000 data-reduction software (Beckman Fullarton, CA, USA). Minimum detection levels were calculated by using Biomek 1000 software and were as follows: IFN- γ , 10 pg/ml; IL-4, 2 pg/ml; IL-5, 10 pg/ml; and IL-13, 5 pg/ml.

Statistical analysis

Comparisons between non-atopic and atopic subjects were analyzed using the Mann-Whitney non-parametric *U*-test. A *p*-value of <0.05 was considered significant, except where multiple comparisons were made where a *p*-value of <0.02 was taken as significant when comparing cytokine responses of children younger than 2 years of age with those of older children. Data were analyzed using Stata statistical software, release 5.0 (Stata-Corp 1997, Stata Press, College Station, Texas).

Results

Comparison by age

The Th1 (IFN- γ) and Th2 (IL-4, IL-5, and IL-13) cytokine responses to a polyclonal stimulus SEB were examined (Fig. 1, Fig. 2). Regression analysis of age vs. SEB-induced cytokine production

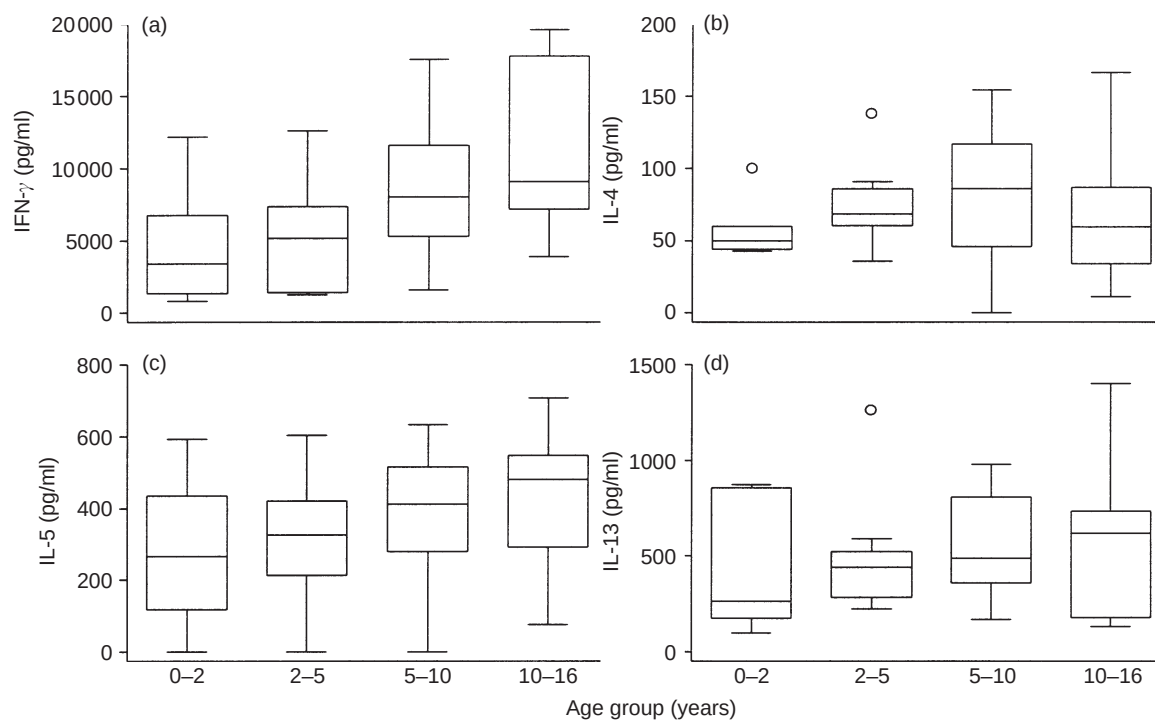


Fig. 2. Staphylococcal enterotoxin B (SEB)-induced cytokine production from peripheral blood mononuclear cells (PBMCs) from atopic children according to age-group. (a) Interferon- γ (IFN- γ); (b) interleukin-4 (IL-4); (c) interleukin-5 (IL-5); and (d) interleukin-13 (IL-13). Data are presented as box plots showing the median and extending to the 25th and 75th percentiles. The lines emerging from the box extend to upper and lower adjacent values. Outliers are represented as single points.

identified a significant relationship between age and cytokine production in non-atopics for IFN- γ ($p = 0.009$; $r = 0.36$), IL-4 ($p = 0.0002$; $r = 0.5$), and IL-5 ($p = 0.03$; $r = 0.31$), but not for IL-13 ($p = 0.06$; $r = 0.27$).

Comparison of the non-atopic children in the 0–2 year age-group with older age-groups revealed that there were reduced levels of the Th2 cytokines (IL-4, IL-5 and IL-13) in the 0–2 year age-group when compared with the 5–10 year ($p = 0.002$, $p = 0.006$, $p = 0.005$) and the 10–16-year ($p = 0.0005$, $p = 0.01$, $p = 0.03$) age-groups (Fig. 1).

Regression analysis of age and cytokine production in atopic children showed a relationship with age, similar to that seen in non-atopics, for IFN- γ ($p = 0.002$, $r = 0.36$), IL-4 ($p = 0.02$, $r = 0.29$), and IL-5 ($p = 0.02$, $r = 0.27$), but not for IL-13 ($p = 0.23$, $r = 0.14$). Comparison of the 0–2-year-old atopic children with the older atopic children revealed a significant reduction in IFN- γ ($p = 0.01$), but not in IL-4, IL-5 or IL-13, when compared with the 10–16-year age-group (Fig. 2).

Comparison of non-atopics and atopics

In addition to the examination of cytokine release from non-atopic and atopic children at different ages, we compared cytokine release of each of the age-groupings of non-atopic vs. atopic children. IFN- γ release in atopic children was reduced in the

0–2-year ($p = 0.02$), 2–5-year ($p = 0.008$), and 5–10-year ($p = 0.005$) age-groups when compared with non-atopic children. In children older than 10 years of age there was no statistically significant difference between the two groups of patients. IL-4 production was significantly elevated in atopic children in the 0–2-year ($p = 0.0002$) and 5–10-year ($p = 0.009$) age-groups. No significant difference was noted between atopics and non-atopics of any age-group in their IL-5 and IL-13 responses.

Proliferative responses to SEB were also studied and there was no difference in SEB-induced proliferation between atopics and non-atopics at any age (data not shown).

Discussion

There are two approaches to the study of cytokine release. The first approach is the stimulation of multiple T cells by mitogens or superantigens, which would be expected to reveal the predisposition to cytokine production not necessarily related to prior antigenic exposure. The second approach is to examine allergen/antigen-specific T-cell cytokine responses, which may reflect both the prior exposure and sensitization of the subject to that particular allergen or antigen and a possible skewing or deviation of T-cell responses. In order to examine the ontogeny of cytokine release, a more

global stimulus with activation of multiple T cells would appear to be appropriate as it is less dependent on the occurrence of specific sensitization to individual antigens. Furthermore, the use of SEB as a stimulus may be of particular relevance when studying cytokine release in atopic subjects, as superantigens such as SEB have been postulated to have an important role in the development of the inflammatory response in AD (18,20–25) and poorly controlled asthma (26). There are three principal findings utilizing this approach:

1 In non-atopic and atopic children, Th1 (IFN- γ) and Th2 (IL-4 and IL-5) cytokine responses were significantly related to age.

2 Atopic children have a reduced IFN- γ response, particularly in early childhood; however, this did not persist in children older than 10 years of age.

3 Production of the Th2 cytokine, IL-13, was not significantly related to age.

The reduced secretion of IFN- γ during the first years of life in normal children is consistent with previous findings where mitogen (PHA)-induced IFN- γ production was reduced in early life, with adult levels achieved in 1-year-old (13) or 3-year-old (16) children. Reduced PHA-induced IFN- γ production has also been reported in the neonatal period (6,14) with a progressive increase from birth to 12 months of age (14). In the present study, in non-atopic subjects there was a significant association of IFN- γ release with age up to 16 years of life, which is also paralleled by an age-related increase in the number of T cells, with intracellular IFN- γ following SEB stimulation (27). Atopic children also demonstrated an increase in IFN- γ secretion throughout childhood, with no significant difference between atopics and non-atopics being observed in the 10–16-year age-group. This finding suggests that the reduced IFN- γ production that has been observed at birth prior to the onset of atopic disease may not be permanent. This is also consistent with the observations of a number of studies that IFN- γ production in response to polyclonal stimuli is reduced in childhood AD (7,9,18,28) and asthma (29–31), but is not reduced in adult (32) or adolescent asthmatics (33). Such observations raise the possibility that maturation of the Th1/IFN- γ response may be a factor in the observed changes in atopic disease throughout childhood, with resolution of symptoms, such as AD and food allergy, being a common finding. The present results utilizing SEB extend our previous findings on the ontogeny of a Th2 cytokine – IL-4 – which found that PHA-induced IL-4 production in healthy children increased progressively throughout childhood until adult life (17). In non-atopic children, IL-4

was the Th2 cytokine that was most strongly related to age (in terms of production), with an *r*-value of 0.5. This association with age was less apparent in atopic subjects.

The most marked differences between non-atopic and atopic children with regard to Th2 cytokine production was seen with IL-4 in the 0–2-year age-group. This is consistent with the failure to demonstrate increased SEB-induced IL-4 secretion in children (mean age 4 years) with AD (18), our observation that PHA-induced IL-4 secretion was increased only in highly atopic children (mean age 5.5 years) and not in those with a lesser degree of atopy (mean age 5.2 years) (7), and the lack of differences in PHA- or ConA-induced IL-5 production in two studies of asthmatic children of mean age 8.9 years (30) and mean age 9.3 years (31). There is one report of polyclonally induced IL-13 production by cord blood cells that did not differ from polyclonally induced IL-13 production by adult cells (19), and another showed no significant difference in allergen-induced IL-13 secretion at 2 years of age between atopic and non-atopic children (34). Studies in adults showed no differences in the polyclonal IL-13 response in patients with allergic rhinitis (35) and those with asthma (36). Polymorphisms in the IL-13 gene region appear to be related to serum IgE levels (37); however, it is not clear if this is because of increased IL-13 release following stimulation. The study of Th2 cytokine secretion at birth has been restricted to cytokines such as IL-13 and IL-10, as IL-4 and IL-5 cytokine secretion is generally below the level of detection. These studies have suggested an up-regulation of Th2 cytokine activity, which was more pronounced in non-atopic rather than atopic individuals (19,34). These findings, however, are not necessarily inconsistent with the present findings as the increased cord blood cell IL-13 secretion in response to allergen in non-atopic subjects was not maintained and secretion at 2 years of age was in fact higher, but not significantly so, in atopic subjects owing to up-regulation of the IL-13 response (34). Similar observations have been made utilizing mRNA detection of IL-4 and IL-5 (34).

Superantigens, such as SEB, activate T cells by binding to the T-cell receptor, which bears certain types of variable chain, and to major histocompatibility complex (MHC) class II chains outside the peptide-binding groove, which leads to vigorous T-cell activation and cytokine release (38). As a result of stimulation of a range of T cells, superantigens provide a useful indication of the underlying predisposition to cytokine production. The present findings probably reflect cytokine release

from both CD4⁺ and CD8⁺ T cells by SEB. Superantigens stimulate both CD4⁺ and CD8⁺ thymocytes from infants (39). Intracellular IFN- γ is found in both CD4⁺ and CD8⁺ T cells following SEB stimulation of PBMC from atopic and non-atopic children (27). In adults with AD, both CD4⁺ and CD8⁺ T-cell populations respond to SEB by proliferating and secreting a Th2 (IL-5 and IL-13)-dominated cytokine profile in an equivalent manner (40). Thus, the findings of the present study probably reflect the cytokine balance determined by both CD4⁺ and CD8⁺ T cells.

The acquisition of memory T cells with increasing age may contribute to the increase in cytokine production with age. In adults, $\approx$ 50–60% of circulating T cells are of the CD45RO memory-cell phenotype, and memory T cells have been shown to produce $\approx$ 10-fold more IFN- γ than naïve T cells (41). IL-4 and IL-5 appear to be produced exclusively by CD45RO T cells, while IL-13 is produced by both activated CD45RA naïve T cells and CD45RO memory T cells (42,43). In this context it is of interest that IL-13 is the cytokine that showed the weakest association with age in both non-atopic and atopic children. Neonatal T cells are almost exclusively of the naïve phenotype, accounting for 90% of neonatal CD4⁺ cells (44–46). Although not investigated as part of this current study, a relative deficiency of memory T cells in early childhood could lead to the decreased cytokine release following polyclonal stimulation. If this were so, it would imply a rapid expansion of cells secreting Th2 cytokines in atopic children and a more gradual expansion in non-atopic children. Such an expansion of IL-4-secreting cells may determine the progressive increase in serum IgE levels in both non-atopic and atopic subjects, and is consistent with the observed correlation between serum IgE levels and polyclonal IL-4 release in childhood (7).

An important issue in studies of cytokine release in childhood is the question of necessity for age-matched controls. The present study indicates that this is most pertinent when examining children for Th1 (IFN- γ) and Th2 (IL-4 and IL-5) cytokines, particularly in the 0–2-year age-group, as these cytokines were those most strongly associated with age.

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References

1. ALLANSMITH M, MCCLELLAN BH, BUTTERWORTH M, MALONEY JR. The development of immunoglobulin levels in man. *J Pediatr* 1968; 72: 276–90.
2. HAYWARD AR, LAWTON AR. Induction of plasma cell differentiation of human fetal lymphocytes: Evidence for functional immaturity of T and B cells. *J Immunol* 1977; 119: 1213–7.
3. CLERICI M, DEPALMA L, ROILIDES E, BAKER R, SHEARER GM. Analysis of T helper and antigen-presenting cell functions in cord blood and peripheral blood leukocytes from healthy children of different ages. *J Clin Invest* 1993; 91: 2829–36.
4. WILSON CB, WESTALL J, JOHNSTON L, LEWIS DB, DOWER SK, ALPERT AR. Decreased production of interferon-gamma by human neonatal cells. Intrinsic and regulatory deficiencies. *J Clin Invest* 1986; 77: 860–7.
5. LEWIS DB, YU CC, MEYER J, ENGLISH BK, KAHN SJ, WILSON CB. Cellular and molecular mechanisms for reduced interleukin 4 and interferon-gamma production by neonatal T cells. *J Clin Invest* 1991; 87: 194–202.
6. PIRENNE-ANSART H, PAILLARD F, DE GROOTE D, et al. Defective cytokine expression but adult-type T-cell receptor, CD8, and p56lck modulation in CD3- or CD2-activated T cells from neonates. *Pediatr Res* 1995; 37: 64–9.
7. TANG M, KEMP A, VARIGOS G. IL-4 and interferon-gamma production in children with atopic disease. *Clin Exp Immunol* 1993; 92: 120–4.
8. TANG ML, KEMP AS, THORBURN J, HILL DJ. Reduced interferon-gamma secretion in neonates and subsequent atopy. *Lancet* 1994; 344: 983–5.
9. TANG M, KEMP A. Production and secretion of interferon-gamma (IFN-gamma) in children with atopic dermatitis. *Clin Exp Immunol* 1994; 95: 66–72.
10. WEATHERSTONE KB, RICH EA. Tumor necrosis factor/cachectin and interleukin-1 secretion by cord blood monocytes from premature and term neonates. *Pediatr Res* 1989; 25: 342–6.
11. YACHIE A, TAKANO N, YOKOI T, et al. The capability of neonatal leukocytes to produce IL-6 on stimulation assessed by whole blood culture. *Pediatr Res* 1990; 27: 227–33.
12. WAKASUGI N, VIRELIZIER JL. Defective IFN-gamma production in the human neonate. I. Dysregulation rather than intrinsic abnormality. *J Immunol* 1985; 134: 167–71.
13. FRENKEL L, BRYSON YJ. Ontogeny of phytohemagglutinin-induced gamma interferon by leukocytes of healthy infants and children: Evidence for decreased production in infants younger than 2 months of age. *J Pediatr* 1987; 111: 97–100.
14. VIGANO A, ESPOSITO S, ARIENTI D, et al. Differential development of type 1 and type 2 cytokines and beta-chemokines in the ontogeny of healthy newborns. *Biol Neonate* 1999; 75: 1–8.
15. HASSAN J, REEN DJ. Interleukin-1 augments the diminished interleukin-2 mRNA expression and proliferative response of neonatal T lymphocytes to anti-CD2 antibodies. *Scand J Immunol* 1994; 39: 597–601.
16. MIYAWAKI T, SEKI H, TAGA K, SATO H, TANIGUCHI N. Dissociated production of interleukin-2 and immune (gamma) interferon by phytohemagglutinin stimulated lymphocytes in healthy infants. *Clin Exp Immunol* 1985; 59: 505–11.

17. TANG ML, KEMP AS. Ontogeny of IL-4 production. *Pediatr Allergy Immunol* 1995; 6: 11–9.
18. 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 Immunol* 1997; 107: 392–7.
19. WILLIAMS TJ, JONES CA, MILES EA, WARNER JO, WARNER JA. Fetal and neonatal IL-13 production during pregnancy and at birth and subsequent development of atopic symptoms. *J Allergy Clin Immunol* 2000; 105: 951–9.
20. HOEGER PH, LENZ W, BOUTONNIER A, FOURNIER JM. Staphylococcal skin colonization in children with atopic dermatitis: Prevalence, persistence, and transmission of toxigenic and nontoxigenic strains. *J Infect Dis* 1992; 165: 1064–8.
21. LEUNG DY, HARBECK R, BINA P, et al. Presence of IgE antibodies to staphylococcal exotoxins on the skin of patients with atopic dermatitis. Evidence for a new group of allergens. *J Clin Invest* 1993; 92: 1374–80.
22. HOEFER MF, HARBECK RJ, SCHLIEVERT PM, LEUNG DY. Staphylococcal toxins augment specific IgE responses by atopic patients exposed to allergen. *J Invest Dermatol* 1999; 112: 171–6.
23. LEUNG DY, HAU P, STRICKLAND I, TRAVERS JB, NORRIS DA. The role of superantigens in human diseases: Therapeutic implications for the treatment of skin diseases. *Br J Dermatol* 1998; 139 (Suppl. 53): 17–29.
24. STRANGE P, SKOV L, LISBY S, NIELSEN PL, BAADSGAARD O. Staphylococcal enterotoxin B applied on intact normal and intact atopic skin induces dermatitis. *Arch Dermatol* 1996; 132: 27–33.
25. SKOV L, OLSEN JV, GIORNO R, SCHLIEVERT PM, BAADSGAARD O, LEUNG DY. Application of Staphylococcal enterotoxin B on normal and atopic skin induces up-regulation of T cells by a superantigen-mediated mechanism. *J Allergy Clin Immunol* 2000; 105: 820–6.
26. HAU P, WENZEL SE, TRUMBLE AE, SZEFLER SJ, LEUNG DY. Increased T-cell receptor $\text{v}\beta 28^+$ T cells in bronchoalveolar lavage fluid of subjects with poorly controlled asthma: A potential role for microbial superantigens. *J Allergy Clin Immunol* 1999; 104: 37–45.
27. CAMPBELL DE, FRYGA AS, BOL S, KEMP AS. Intracellular interferon-gamma (IFN-gamma) production in normal children and children with atopic dermatitis. *Clin Exp Immunol* 1999; 115: 377–82.
28. CAMPBELL DE, HILL DJ, KEMP AS. Enhanced IL-4 but normal interferon-gamma production in children with isolated IgE mediated food hypersensitivity. *Pediatr Allergy Immunol* 1998; 9: 68–72.
29. 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.
30. 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.
31. HOEKSTRA MO, HOEKSTRA Y, DE REUS D, RUTGERS B, GERRITSEN J, KAUFFMAN HF. Interleukin-4, interferon-gamma and interleukin-5 in peripheral blood of children with moderate atopic asthma. *Clin Exp Allergy* 1997; 27: 1254–60.
32. TANG C, ROLLAND IM, WARD C, BISH R, THIEN F, WALTERS EH. Seasonal comparison of cytokine profiles in atopic asthmatics and atopic non-asthmatics. *Am J Respir Crit Care Med* 1996; 154: 1615–22.
33. 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. *Pediatr Res* 1995; 38: 187–93.
34. 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.
35. 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.
36. GABRIELSSON S, SODERLUND A, PAULIE S, RAK S, VAN DER POUW KRAAN TC, TROYE-BLOMBERG M. Increased frequencies of allergen-induced interleukin-13-producing cells in atopic individuals during the pollen season. *Scand J Immunol* 1998; 48: 429–35.
37. GRAVES PE, KABESCH M, HALONEN M, et al. A cluster of seven tightly linked polymorphisms in the IL-13 gene is associated with total serum IgE levels in three populations of white children. *J Allergy Clin Immunol* 2000; 105: 506–13.
38. SKOV L, BAADSGAARD O. Bacterial superantigens and inflammatory skin diseases. *Clin Exp Dermatol* 2000; 25: 57–61.
39. THULESEN S, JORGENSEN A, GERWIEN J, et al. Superantigens are presented by and activate thymocytes from infants. *Exp Clin Immunogenet* 1999; 16: 226–33.
40. AKDIS M, SIMON HU, WEIGL L, KREYDEN O, BLASER K, AKDIS CA. Skin homing (cutaneous lymphocyte-associated antigen-positive) $\text{CD}4^+$ T cells respond to superantigen and contribute to eosinophilia and IgE production in atopic dermatitis. *J Immunol* 1999; 163: 466–75.
41. WILSON CB. The ontogeny of T lymphocyte maturation and function. *J Pediatr* 1991; 118: S4–9.
42. BRINKMANN V, KRISTOFIC C. TCR-stimulated naive human $\text{CD}4^+ 45\text{RO}^-$ T cells develop into effector cells that secrete IL-13, IL-5, and IFN-gamma, but no IL-4, and help efficient IgE production by B cells. *J Immunol* 1995; 154: 3078–87.
43. JUNG T, WIJDENES J, NEUMANN C, DE VRIES JE, YSSEL H. Interleukin-13 is produced by activated human $\text{CD}45\text{RA}^+$ and $\text{CD}45\text{RO}^+$ T cells: Modulation by interleukin-4 and interleukin-12. *Eur J Immunol* 1996; 26: 571–7.
44. CLEMENT LT, YAMASHITA N, MARTIN AM. The functionally distinct subpopulations of human $\text{CD}4^+$ helper/inducer T lymphocytes defined by anti- $\text{CD}45\text{R}$ antibodies derive sequentially from a differentiation pathway that is regulated by activation-dependent post-thymic differentiation. *J Immunol* 1988; 141: 1464–70.
45. CLEMENT LT, VINK PE, BRADLEY GE. Novel immunoregulatory functions of phenotypically distinct subpopulations of $\text{CD}4^+$ cells in the human neonate. *J Immunol* 1990; 145: 102–8.
46. JANEWAY CA JR. The T cell receptor as a multicomponent signalling machine: $\text{CD}4/\text{CD}8$ coreceptors and $\text{CD}45$ in T cell activation. *Annu Rev Immunol* 1992; 10: 645–74.