

Cytokines, allergy, and asthma

Ly P. Ngoc^{a,b}, Diane R. Gold^{a,c}, Arthur O. Tzianabos^{a,c},
Scott T. Weiss^{a,c} and Juan C. Celedón^{a,c,d}

Purpose of review

This review examines recent articles on the relationship of cytokines to allergy and asthma with particular emphasis on immune mechanisms involved in disease development in early life.

Recent findings

It was previously proposed that reduced microbial exposure in early life is responsible for a shift of the Th1/Th2 balance in the immune system towards the proallergenic Th2 response. This Th1/Th2 imbalance results in the clinical expression of allergy and/or asthma. In recent years, accumulating data from mice and humans have identified Th2 cytokines [interleukin (IL)-4, IL-13, and IL-5] as major contributors to allergy and asthma. Interestingly, the Th1 cytokine interferon- γ has recently been shown to act concurrently with Th2 cytokines in maintaining the chronic inflammatory response in allergic diseases, particularly in asthmatic airways. Most recently, evidence suggests that suppression of T-regulatory cells may contribute to the underlying immune mechanisms involved in allergy and asthma.

Summary

An enhanced Th2 immune response and the elaboration of cytokines such as IL-4, IL-13, and IL-5 contribute to the induction of allergy and asthma. Interferon- γ , a Th1 cytokine, acts in conjunction with Th2 (IL-4, IL-13, and IL-5) in maintaining chronic allergic inflammation. The mechanisms leading to an enhanced Th2 response are still controversial. Th2-dominated immune responses may result from immune suppression of T-regulatory cells as well as Th1 cells. Understanding early-life immune mechanisms responsible for atopic diseases, specifically how cytokines of T-regulatory cells act to balance the Th1 and Th2 immune response, continues to be a fruitful area of research.

Keywords

asthma, allergy, cytokines, Th1, Th2, T-reg

Abbreviations

IL	interleukin
IL-4RA	IL-4 receptor α
SNP	single nucleotide polymorphism
TGF-β	transforming growth factor- β

© 2005 Lippincott Williams & Wilkins
1528-4050

Introduction

Allergic diseases, such as allergic rhinitis, allergic conjunctivitis, food allergy, atopic dermatitis, and asthma are complex genetic diseases with major environmental influences that occur in a developmental context. It is still unclear why allergy and the subsequent development of allergic diseases occur in some children but not others. The increased prevalence of allergic diseases in recent years [1–4], especially in industrialized countries, suggests the potential influence of improved hygiene and better infection control on the development of allergy and indirectly on atopic asthma. Supporting evidence for the ‘hygiene’ hypothesis suggests that reduced microbial burden during childhood as a result of a Westernized lifestyle [5–8] contributes to the increased prevalence of allergic diseases, particularly allergic rhinitis, atopic dermatitis, and to a lesser extent asthma.

It has been proposed that reduced microbial exposures in early life leads to the polarization of allergen-specific T-cell memory towards the Th2 instead of the Th1 immune response. Whether reduced microbial exposures are the only environmental stimuli influencing this immune effect is unclear, but this particular environmental exposure has received the most attention. Since the delineation of type 1 (Th1) and type 2 (Th2) CD4⁺ T-cells in mice (1989) [9] and in humans (1994) [10], evidence has largely shown that activated Th2 lymphocytes and the elaboration of certain cytokines such as interleukin (IL)-4, IL-13, and IL-5 are responsible for the cascade of eosinophil activation and IgE production necessary for allergic inflammation [11–13].

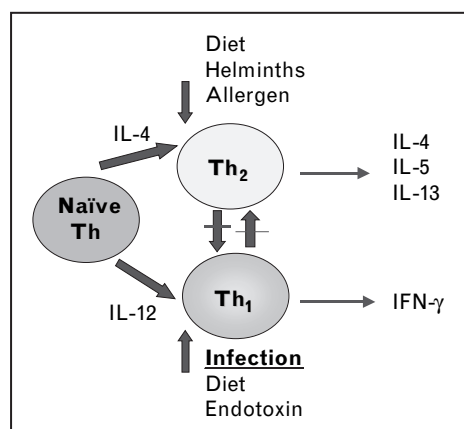
Th1 and Th2 subsets develop from the same precursor cells, which are naïve CD4⁺ T lymphocytes, and the pattern of differentiation is determined by environmental stimuli present early during immune responses (Fig. 1). Further consideration of these environmental exposures is beyond the scope of this review. Th2 differentiation occurs in response to environmental allergens and

Curr Opin Allergy Clin Immunol 5:161–166. © 2005 Lippincott Williams & Wilkins.

^aChanning Laboratory, Department of Medicine, Brigham and Women's Hospital, Boston, MA, USA, ^bPediatric Pulmonary Medicine, Massachusetts General Hospital for Children and Harvard Medical School, Boston, MA, USA, ^cDepartment of Medicine, Harvard Medical School, Boston, MA, USA and ^dDivision of Pulmonary/Critical Care Medicine, Beth Israel Deaconess Med. Ctr, Boston, MA, USA

Correspondence to Juan C. Celedón, Channing Laboratory, 181 Longwood Ave, 4th Floor, Boston, MA 02115, USA
Tel: +1 617 525 0964; fax: +1 617 525 0958;
e-mail: rejcc@channing.harvard.edu

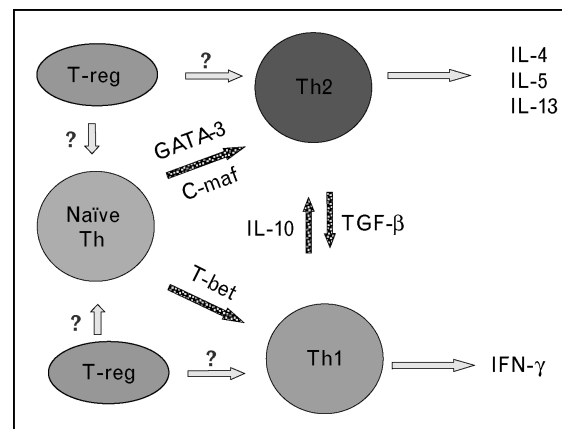
Current Opinion in Allergy and Clinical Immunology 2005, 5:161–166

Fig. 1. T-helper lymphocyte differentiation to Th1 or Th2

Th2 differentiation occurs in response to environmental allergens and helminths via activated antigen-presenting cells under the influence of IL-4. Activated Th2 cells produce interleukins IL-4, IL-13, and IL-5, which are responsible for IgE production by B-cells, eosinophil activation and recruitment, and mucus production. Th1 cells differentiate in response to microbial activation of antigen-presenting cells under the influence of IL-12. Activated Th1 cells produce interferon (IFN)- γ , which is important in intracellular destruction of phagocytosed microbes. Interferon- γ produced by Th1 cells and IL-4 produced by Th2 cells counter-regulate each other.

helminths via activated antigen-presenting cells under the influence of IL-4 [10,14]. Activated Th2 lymphocytes produce IL-4, IL-13, and IL-5, which are responsible for IgE production by B cells, eosinophil activation and recruitment, and mucus production [10,14]. In contrast, Th1 cells differentiate from naïve CD4⁺ cells in response to microbial activation of antigen-presenting cells under the influence of IL-12. Differentiated Th1 cells secrete interferon- γ , which is important in intracellular destruction of phagocytosed microbes. Furthermore, interferon- γ produced by Th1 cells and IL-4 produced by Th2 counter-regulate each other [15].

The initial immune model of the Th1/Th2 imbalance associated with the 'hygiene' hypothesis has recently been questioned. New studies indicate that the immune mechanisms involved in allergy and asthma are likely to be more complex and cannot be explained by a simple failure of a shift from the Th2 to Th1 immune responses. In fact, if reduced microbial exposure impairs the immune deviation from Th2 to Th1, we would not expect to see an increased prevalence of both autoimmune disease (Th1-dominant immune responses) such as multiple sclerosis, type I diabetes, and inflammatory bowel diseases, and allergic diseases (Th2-dominant immune responses) [16]. An additional piece of evidence against the Th1/Th2 dichotomy is provided by studies demonstrating a generally attenuated allergen-stimulated lymphocyte proliferation as well as attenuated Th1 and Th2 cytokine production at birth [17^{••}–19^{••}]. These globally attenuated

Fig. 2. A more complex model of Th1 and Th2 differentiation

The transcription factors GATA-3 and C-maf are necessary for Th2 responses. The T-bet transcription factor is important for Th1 responses. In this model, the potential influence of the immunosuppressive T-regulatory (T-reg) cells on the Th1/Th2 imbalance is also depicted. These T-regulatory cells abolish specific allergen-stimulated lymphocyte proliferation and suppress Th1 and Th2 cytokine production. It has been proposed that immune suppression of the T-regulatory cells may be involved in a Th2-skewed immune response among atopic individuals.

immune responses suggest the presence of other control mechanisms of T-cell regulation (Fig. 2). There is strong evidence that peripheral T-cell regulation plays a crucial role in the control of harmful T-cell responses. These peripheral T-cells, recognized as T-regulatory (or T-reg) cells, act to abolish specific allergen-stimulated lymphocyte proliferation and suppress Th1 and Th2 cytokine production [14]. It has been proposed that an immune suppression of the T-regulatory cells may be involved in a Th2-skewed immune response among atopic individuals [16,20,21]. The relationship of environmental exposures to T-regulatory cell function in immune-system ontogeny in early life remains a high research priority.

Although there is epidemiological evidence to support the so-called hygiene hypothesis, its immunological basis for this hypothesis remains controversial. The reasons for some of the inconsistencies seen with the results of immunological studies can partly be explained by the fact that human studies are limited to in-vitro analysis. Examining immune response in peripheral blood gives limited information about the complex nature of the human immune system. Other factors that could contribute to conflicting results include cohorts with small sample sizes, different age groups, and different baseline risk characteristics for allergy and allergic diseases, and variation in laboratory techniques. For example, some researchers use allergen instead of mitogen stimulation and measure mRNA instead of protein. Despite these

inconsistencies, we have gained valuable information about the early immune responses involved in atopic diseases. It appears that genetic and environmental influences in early life are critical in defining the patterns of immune response involved in allergy and asthma outcomes, and we are just beginning to unravel the complex network of regulatory mechanisms at work.

Th2 cytokines, allergy, and asthma

Despite variation in sample sizes, laboratory techniques, and age or risk factors of the cohort examined, results from cross-sectional and longitudinal studies have consistently demonstrated a strong association between an upregulated Th2 immune response and atopic diseases. Studies have shown that cord-blood IL-13 in response to dust mite (*Der p 1*) and phytohemagglutinin were associated with atopic dermatitis at age 3 years [22]. In a group of 175 children with a high genetic risk for atopy based on family history, staphylococcal enterotoxin B-induced IL-13 responses in cord blood were shown to be the strongest independent predictor of allergy development as defined by positive skin-prick test at age 2 years [23••]. However, the heightened Th2 immune response to allergens or mitogens associated with allergy or atopic diseases is more consistently observed in peripheral blood obtained from children early in postnatal life rather than at birth. For example, a study in which investigators measured unstimulated cord-blood cytokine levels reported an association between lower concentrations of IL-4 and interferon- γ at birth and wheeze at 6 years [19••]. In another study, it was demonstrated that children who had a positive skin-prick test at age 6 years had lower Th2 (IL-13 and IL-6) cytokine responses at birth. However, a positive skin-prick test to house dust mite at 6 years was associated with higher IL-13 response to house dust mite at 1 year; clinical atopic disease at 6 years was associated with higher IL-5 mRNA responses to house dust mite at 1 year [17••]. Similarly, Neville and his group demonstrated that, although there were no associations between neonatal phytohemagglutinin-stimulated Th2 cytokines and atopic markers of allergy (i.e. absolute eosinophil count and total IgE) at age 1 year, there were associations between increased levels of IL-5 and IL-13 (Th2 polarization) and atopic markers of allergy at age 1 year [18••]. These two studies demonstrated that Th2 cytokines, although low at birth, increase significantly from birth to age 1 year [18••] and from birth to age 2 years [17••]. One study showed an association of increased IL-4 at 18 months and atopic disease at age 6 years [24]. In cross-sectional analysis of an older group of children ages 2–3 years, it was shown that allergen-stimulated IL-13 was associated with allergic sensitization and clinical allergy or wheeze [25•]. Th2 cytokine responses have been demonstrated in peripheral blood of atopic or asthmatic patients as well as at target sites of inflammation such as asthmatic airways [26,27••,28].

Th1 cytokines, allergy, and asthma

The relationship between Th1-mediated immune response and atopic diseases is more controversial. As mentioned previously, the discrepancies between studies may be related to subtle differences in laboratory methodologies or study design. Alternatively, these differences may mean that the immune mechanisms involved in defining allergy or asthma are more complex than those defined by a simple Th1/Th2 dichotomy.

Some studies have found no association between interferon- γ levels at birth and atopic dermatitis at 3 years [22], absolute eosinophil counts, or total IgE at 1 year [18••]. A study of children at genetic risk (positive family history) for allergy showed that these children have weaker polyclonal Th1 interferon- γ responses than children at low genetic risk from allergy. In that study, children with allergy at age 6 years tended to have weaker neonatal interferon- γ responses than those without allergic symptoms [17••]. Similarly, detectable cord blood interferon- γ levels have been associated with lower risks for asthma and allergy at age 6 years, and maternal smoking has been found to be associated with both reduced interferon- γ at birth and subsequent wheeze at 6 years [19••]. Furthermore, reduced interferon- γ at age 3 months has been associated with recurrent wheezing at age 1 year [29•], and interferon- γ in response to dust mite (*Der f 1*) and cockroach (*Bla g 2*) has been shown to be reduced among children with atopic disease at age 2 years [25•].

Conceptually, Th1 interferon- γ is known to counter-regulate Th2 immune response and Th2 cytokines are known to activate a cascade of events that are necessary for inflammation. However, there is recent evidence that interferon- γ secreted from both CD4⁺ and CD8⁺ lymphocytes may act concurrently with Th2 cytokines (IL-13, IL-5, and IL-4) in maintaining allergic inflammatory response at affected sites. In a recent publication, the authors demonstrated that production of IL-4, IL-5, and interferon- γ by unstimulated sputum CD4⁺ and CD8⁺ T-cells was increased in asthmatics and related to disease severity—more for CD8⁺ than for CD4 T-cells [27••]. Another group found that induced sputum T-cell cytokine production indicates a basic Th2 bias in asthma. This was accompanied during symptomatic periods by a Th1-like activation [26]. Rowe *et al.* evaluated 175 high-risk children, comparing their cord-blood allergen- and mitogen-stimulated cytokine levels to allergy as defined by skin prick test at age 2 years. They showed that among these high-risk children, allergic sensitization at 2 years was strongly associated with polyclonal interferon- γ responses to both phytohemagglutinin and staphylococcal enterotoxin B and that interferon- γ was produced predominantly by CD8⁺ cells [23••]. Furthermore, serum

interferon- γ was found to be associated with a decline in lung function among adult asthmatics [30].

Together these findings demonstrate that, although interferon- γ produced by Th1 cells and IL-4 produced by Th2 counter-regulate each other, interferon- γ also contributes to chronic inflammation.

T-regulatory cells, allergy, and asthma

Failure of an immune deviation from an allergen-specific Th2 response to a Th1 immune response has been proposed as the mechanism responsible for the increased allergic disease prevalence associated with reduced microbial exposures in early life. Recent evidence suggests that the dysregulated immune system involved in allergy and asthma cannot be explained simply by the Th1/Th2 dichotomy. Another mechanism may involve T-regulatory/suppressor cells. It has been shown that maternal T-regulatory cells act to suppress autoimmune responses and create an immune homeostasis in the feto-maternal relationship [31,32]. The presence of T-regulatory cells may explain the lower lymphocyte proliferation and mitogen- or allergen-stimulated cytokine production observed in neonatal cord blood as compared to those of adults [31,33]. As previously mentioned, some studies have found reduction in both Th1 and Th2 cytokines at birth [17^{••}–19^{••}]. In those studies, it was not until early postnatal life that a clearly heightened Th2 cytokine production was noted among children with allergy or allergic disease. Many types of T-regulatory cell have been identified, and most are recognized for their production of IL-10 and transforming growth factor- β (TGF- β). In in-vivo animal studies, T-regulatory cells suppress naïve memory Th1 and Th2 responses through the release of IL-10 and TGF- β [14,34[•],35[•]]. A recent study demonstrated that T-regulatory cells respond directly to proinflammatory bacterial products, a mechanism that likely contributes to the control of inflammatory responses [36^{••}]. Furthermore, decreased antigenic stimulation resulting from a decreased frequency of childhood infections has been associated with decreased levels of T-regulatory cytokines (IL-10 but also TGF- β) [37]. It has been proposed that an immune suppression of the T-regulatory cells may be involved in a Th2-skewed immune response among atopic individuals [16,20,21].

Genes, allergy, and asthma

Several transcriptional factors have been identified that are important in Th1 and Th2 phenotypic expression. For example, the T-bet (T-box expressed in T-cells) transcription factor promotes Th1 differentiation and has been shown to enhance interferon- γ transcription [38]. Furthermore, T-bet-deficient mice demonstrated defective Th1 responses and reduced interferon- γ production with over-production of Th2 cytokines, resulting in

increased airway hyper-responsiveness to methacholine and airway remodeling similar to that observed in humans with chronic asthma [39,40]. In contrast, GATA-3 has been identified as a major Th2-regulatory factor necessary for generating the Th2 responses [41–43] and suppressing Th1 responses [44]. Increased *GATA-3* gene expression in association with IL-5 mRNA+ cells were found in asthmatic airways [45]. In addition to GATA-3, C-maf, a basic leucine-zipper transcription factor, has been shown to directly augment IL-4 [46[•]].

The IL-4 and IL-4 receptor α chain (*IL-4RA*) loci are in genomic regions linked to asthma phenotypes (5q31 for IL-4 and 16p12 for *IL-4RA*). A functional single nucleotide polymorphism (SNP) in the promoter of the *IL-4* gene has been associated with total serum IgE [47–49], asthma [47,48,50–52], rhinitis [51], asthma severity [51,53], and atopic dermatitis [54]. SNPs in exons of *IL-4RA* have been associated with asthma [55–59], total serum IgE [55,60–64], atopic dermatitis [65], and asthma severity [66]. The *IL-13* gene is on chromosome 5q31–q33, a region linked to asthma phenotypes. Functional SNPs in the promoter and coding regions of IL-13 have been associated with asthma [48,67,68], airway responsiveness [68], atopy [68–72], and total serum IgE [69,70,72]. The IL-12 cytokine is involved in Th1 cell development and Th2 suppression. Recently polymorphisms of the *IL-12B* gene were found to be associated with asthma and asthma-related phenotypes such as eosinophil and total IgE among families of Caucasian children with asthma in the Childhood Asthma Management Program (CAMP) [73]. Polymorphisms in the *IL-10* gene, which is located on chromosome 1q31–q32, are associated with asthma [74]. The *TGF- β 1* gene (*TGF β 1*) is on chromosome 19q, a genomic region linked to asthma phenotypes. Functional SNPs in *TGF β 1* have been associated with asthma [75], total serum IgE [76], asthma severity [77], and atopic dermatitis [78].

Conclusion

Epidemiological studies suggest that reduced microbial exposures resulting from a Westernized lifestyle is at least partly responsible for the increased prevalence of allergic diseases in recent decades. The immune mechanisms involved in the phenotypic expression of allergic diseases are being elucidated. It is well-documented that allergen-specific Th2 responses with the subsequent release of interleukins such as IL-4, IL-13, and IL-5 are responsible for the cascade of events necessary for allergic inflammation. The initial view is that reduced microbial stimulation of cells of innate immunity, as a result of improved hygiene, causes a reduction of Th1 polarization and therefore reduced interferon- γ . As a result, a Th2-dominant immune response is observed. However, recent evidence suggests that reduced microbial exposure also leads to reduced stimulation of

T-regulatory cells, resulting in increased Th2 responses. It is most likely that the combination of reduced Th1 cytokines (interferon- γ and IL-12) and reduced T-regulatory cytokines (IL-10 and TGF- β) secondary to reduced microbial burden in early life is responsible for the Th2-skewed immune response. Understanding early-life immune mechanisms responsible for atopic diseases continues to be an exciting area of research, but further work is needed to determine how cytokines of T-regulatory cells act to balance the Th1 and Th2 immune response.

Acknowledgements

N.P.L. is funded by grant HL07427.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
 - of outstanding interest
- 1 The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. *Lancet* 1998; 351:1225–1232.
 - 2 Aberg N, Hesselmar B, Aberg B, Eriksson B. Increase of asthma, allergic rhinitis and eczema in Swedish schoolchildren between 1979 and 1991. *Clin Exp Allergy* 1995; 25:815–819.
 - 3 Celedon JC, Soto-Quiros ME, Hanson LA, Weiss ST. The relationship among markers of allergy, asthma, allergic rhinitis, and eczema in Costa Rica. *Pediatr Allergy Immunol* 2002; 13:91–97.
 - 4 Ninan TK, Russell G. Respiratory symptoms and atopy in Aberdeen schoolchildren: evidence from two surveys 25 years apart. *BMJ* 1992; 304:873–875.
 - 5 Braun-Fahrlander C, Gassner M, Grize L, *et al.* Prevalence of hay fever and allergic sensitization in farmer's children and their peers living in the same rural community. SCARPOL team. Swiss Study on Childhood Allergy and Respiratory Symptoms with Respect to Air Pollution. *Clin Exp Allergy* 1999; 29:28–34.
 - 6 Stein RT, Sherrill D, Morgan WJ, *et al.* Respiratory syncytial virus in early life and risk of wheeze and allergy by age 13 years. *Lancet* 1999; 354:541–545.
 - 7 Sepp E, Julge K, Vasar M, *et al.* Intestinal microflora of Estonian and Swedish infants. *Acta Paediatr* 1997; 86:956–961.
 - 8 Ernst P, Cormier Y. Relative scarcity of asthma and atopy among rural adolescents raised on a farm. *Am J Respir Crit Care Med* 2000; 161:1563–1566.
 - 9 Mosmann TR, Coffman RL. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu Rev Immunol* 1989; 7:145–173.
 - 10 Romagnani S. Lymphokine production by human T cells in disease states. *Annu Rev Immunol* 1994; 12:227–257.
 - 11 Till S, Durham S, Dickason R, *et al.* IL-13 production by allergen-stimulated T cells is increased in allergic disease and associated with IL-5 but not IFN- γ expression. *Immunology* 1997; 91:53–57.
 - 12 Kay AB, Ying S, Varney V, *et al.* Messenger RNA expression of the cytokine gene cluster, interleukin 3 (IL-3), IL-4, IL-5, and granulocyte/macrophage colony-stimulating factor, in allergen-induced late-phase cutaneous reactions in atopic subjects. *J Exp Med* 1991; 173:775–778.
 - 13 Robinson DS, Hamid Q, Ying S, *et al.* Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med* 1992; 326:298–304.
 - 14 Akdis CA, Blaser K, Akdis M. Genes of tolerance. *Allergy* 2004; 59:897–913.
 - 15 de Vries JE, Carballido JM, Aversa G. Receptors and cytokines involved in allergic TH2 cell responses. *J Allergy Clin Immunol* 1999; 103:S492–S496.
 - 16 Bach JF. The effect of infections on susceptibility to autoimmune and allergic diseases. *N Engl J Med* 2002; 347:911–920.
 - 17 Prescott SL, King B, Strong TL, Holt PG. The value of perinatal immune responses in predicting allergic disease at 6 years of age. *Allergy* 2003; 58:1187–1194.
This paper demonstrates two important findings. First, it shows that in the neonatal period a global suppression of both Th1 and Th2 cytokines was observed. It then shows that by early postnatal life a reverse trend in Th2 was observed among children who later became sensitized to allergen or had allergic disease at 6 years.
 - 18 Neaville WA, Tisler C, Bhattacharya A, *et al.* Developmental cytokine response profiles and the clinical and immunologic expression of atopy during the first year of life. *J Allergy Clin Immunol* 2003; 112:740–746.
In this paper, the authors show an association of enhanced Th2 immune response and markers of allergy (eosinophil and total IgE) by 1 year not observed at birth. They also show a dramatic increase in IL-5 from birth to 1 year.
 - 19 Macaubas C, de Klerk NH, Holt BJ, *et al.* Association between antenatal cytokine production and the development of atopy and asthma at age 6 years. *Lancet* 2003; 362:1192–1197.
This is an elegant analysis showing the impact of maternal smoking in the antenatal period on neonatal cytokine production (i.e. reduced IL-4 and interferon- γ) and wheeze in children by age 6 years.
 - 20 Yazdanbakhsh M, Kremsner PG, van Ree R. Allergy, parasites, and the hygiene hypothesis. *Science* 2002; 296:490–494.
 - 21 Romagnani S. Immunologic influences on allergy and the TH1/TH2 balance. *J Allergy Clin Immunol* 2004; 113:395–400.
 - 22 Lange J, Ngoumou G, Berkenheide S, *et al.* High interleukin-13 production by phytohaemagglutinin- and Der p 1-stimulated cord blood mononuclear cells is associated with the subsequent development of atopic dermatitis at the age of 3 years. *Clin Exp Allergy* 2003; 33:1537–1543.
 - 23 Rowe J, Heaton T, Kusel M, *et al.* High IFN- γ production by CD8+ T cells and early sensitization among infants at high risk of atopy. *J Allergy Clin Immunol* 2004; 113:710–716.
This is the first report that shows that increased interferon- γ production by CD8+ T-cells is concurrent with increased IL-13 production by Th2 cells in the cord blood of children who become allergic by 2 years.
 - 24 Borres MP, Bjorksten B. Peripheral blood eosinophils and IL-4 in infancy in relation to the appearance of allergic disease during the first 6 years of life. *Pediatr Allergy Immunol* 2004; 15:216–220.
 - 25 Contreras JP, Ly NP, Gold DR, *et al.* Allergen-induced cytokine production, atopic disease, IgE, and wheeze in children. *J Allergy Clin Immunol* 2003; 112:1072–1077.
Interesting cross-sectional study on the relationship between Th1/Th2 cytokines, IgE, and allergic disease in a group of children 2–3 years old with parental histories of allergy or asthma.
 - 26 Boniface S, Koscher V, Mamessier E, *et al.* Assessment of T lymphocyte cytokine production in induced sputum from asthmatics: a flow cytometry study. *Clin Exp Allergy* 2003; 33:1238–1243.
 - 27 Cho SH, Stanciu LA, Holgate ST, Johnston SL. Increased interleukin-4, -5 and interferon- γ in airway CD4+ and CD8+ T cells in atopic asthma. *Am J Respir Crit Care Med* 2004; Epub ahead of print.
Nicely demonstrates increased production of IL-4, IL-5, and interferon- γ by unstimulated CD4+ and CD8+ lymphocytes obtained from the sputum of asthmatic patients. They also show increased interferon- γ in in-vitro stimulation of cells obtained from the sputum of asthmatic patients.
 - 28 Robinson D, Hamid Q, Bentley A, *et al.* Activation of CD4+ T cells, increased TH2-type cytokine mRNA expression, and eosinophil recruitment in bronchoalveolar lavage after allergen inhalation challenge in patients with atopic asthma. *J Allergy Clin Immunol* 1993; 92:313–324.
 - 29 Guerra S, Lohman IC, Halonen M, *et al.* Reduced interferon gamma production and soluble CD14 levels in early life predict recurrent wheezing by 1 year of age. *Am J Respir Crit Care Med* 2004; 169:70–76.
Longitudinal analysis demonstrating a relationship between interferon- γ at age 3 months and recurrent wheeze in the first year of life.
 - 30 Litonjua AA, Sparrow D, Guevarra L, *et al.* Serum interferon-gamma is associated with longitudinal decline in lung function among asthmatic patients: the Normative Aging Study. *Ann Allergy Asthma Immunol* 2003; 90:422–428.
 - 31 Takahata Y, Nomura A, Takada H, *et al.* CD25+CD4+ T cells in human cord blood: an immunoregulatory subset with naive phenotype and specific expression of forkhead box p3 (Foxp3) gene. *Exp Hematol* 2004; 32:622–629.
 - 32 Aluvihare VR, Kallikourdis M, Betz AG. Regulatory T cells mediate maternal tolerance to the fetus. *Nat Immunol* 2004; 5:266–271.
 - 33 Williams TJ, Jones CA, Miles EA, *et al.* Fetal and neonatal IL-13 production during pregnancy and at birth and subsequent development of atopic symptoms. *J Allergy Clin Immunol* 2000; 105:951–959.

- 34 Chen W, Jin W, Hardegen N, *et al.* Conversion of peripheral CD4⁺CD25⁻ naive T cells to CD4⁺CD25⁺ regulatory T cells by TGF- β induction of transcription factor Foxp3. *J Exp Med* 2003; 198:1875–1886.
The authors eloquently demonstrate the ability to suppress T-cell proliferation and Th1 and Th2 cytokine production *in vitro* and TGF- β 1's ability to inhibit antigen-driven CD4⁺ T-cell expansion and to prevent inflammation *in vivo*.
- 35 Akdis M, Verhagen J, Taylor A, *et al.* Immune responses in healthy and allergic individuals are characterized by a fine balance between allergen-specific T regulatory 1 and T helper 2 cells. *J Exp Med* 2004; 199:1567–1575.
A comprehensive review of T-regulatory cells and their ability to suppress T-cell proliferation and Th1 and Th2 cytokines.
- 36 Caramalho I, Lopes-Carvalho T, Ostler D, *et al.* Regulatory T cells selectively express toll-like receptors and are activated by lipopolysaccharide. *J Exp Med* 2003; 197:403–411.
This is the first study to examine the effect of TLR-4 ligand lipopolysaccharide, a bacterial product on T-regulatory cell activation.
- 37 Weiss ST. Eat dirt—the hygiene hypothesis and allergic diseases. *N Engl J Med* 2002; 347:930–931.
- 38 Szabo SJ, Kim ST, Costa GL, *et al.* A novel transcription factor, T-bet, directs Th1 lineage commitment. *Cell* 2000; 100:655–669.
- 39 Finotto S, Neurath MF, Glickman JN, *et al.* Development of spontaneous airway changes consistent with human asthma in mice lacking T-bet. *Science* 2002; 295:336–338.
- 40 Neurath MF, Weigmann B, Finotto S, *et al.* The transcription factor T-bet regulates mucosal T cell activation in experimental colitis and Crohn's disease. *J Exp Med* 2002; 195:1129–1143.
- 41 Zheng W, Flavell RA. The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. *Cell* 1997; 89:587–596.
- 42 Zhang DH, Cohn L, Ray P, *et al.* Transcription factor GATA-3 is differentially expressed in murine Th1 and Th2 cells and controls Th2-specific expression of the interleukin-5 gene. *J Biol Chem* 1997; 272:21597–21603.
- 43 Bour-Jordan H, Grogan JL, Tang Q, *et al.* CTLA-4 regulates the requirement for cytokine-induced signals in T(H)2 lineage commitment. *Nat Immunol* 2003; 4:182–188.
- 44 Ferber IA, Lee HJ, Zonin F, *et al.* GATA-3 significantly downregulates IFN- γ production from developing Th1 cells in addition to inducing IL-4 and IL-5 levels. *Clin Immunol* 1999; 91:134–144.
- 45 Nakamura Y, Ghaffar O, Olivenstein R, *et al.* Gene expression of the GATA-3 transcription factor is increased in atopic asthma. *J Allergy Clin Immunol* 1999; 103:215–222.
- 46 Finotto S, Glimcher L. T cell directives for transcriptional regulation in asthma. • Springer Semin Immunopathol 2004; 25:281–294.
Excellent and extensive review of identified transcription factors that are involved in Th1 and Th2 pathways.
- 47 Rosenwasser LJ, Klemm DJ, Dresback JK, *et al.* Promoter polymorphisms in the chromosome 5 gene cluster in asthma and atopy. *Clin Exp Allergy* 1995; 25 (Suppl 2):74–78; discussion 95–96.
- 48 Allen M, Heinzmann A, Noguchi E, *et al.* Positional cloning of a novel gene influencing asthma from chromosome 2q14. *Nat Genet* 2003; 35:258–263.
- 49 Tanaka K, Sugiura H, Uehara M, *et al.* Lack of association between atopic eczema and the genetic variants of interleukin-4 and the interleukin-4 receptor alpha chain gene: heterogeneity of genetic backgrounds on immunoglobulin E production in atopic eczema patients. *Clin Exp Allergy* 2001; 31:1522–1527.
- 50 Noguchi E, Shibasaki M, Arinami T, *et al.* Association of asthma and the interleukin-4 promoter gene in Japanese. *Clin Exp Allergy* 1998; 28:449–453.
- 51 Sandford AJ, Chagani T, Zhu S, *et al.* Polymorphisms in the IL4, IL4RA, and FCER1B genes and asthma severity. *J Allergy Clin Immunol* 2000; 106:135–140.
- 52 Beghe B, Barton S, Rorke S, *et al.* Polymorphisms in the interleukin-4 and interleukin-4 receptor alpha chain genes confer susceptibility to asthma and atopy in a Caucasian population. *Clin Exp Allergy* 2003; 33:1111–1117.
- 53 Burchard EG, Silverman EK, Rosenwasser LJ, *et al.* Association between a sequence variant in the IL-4 gene promoter and FEV(1) in asthma. *Am J Respir Crit Care Med* 1999; 160:919–922.
- 54 Novak N, Kruse S, Kraft S, *et al.* Dichotomic nature of atopic dermatitis reflected by combined analysis of monocyte immunophenotyping and single nucleotide polymorphisms of the interleukin-4/interleukin-13 receptor gene: the dichotomy of extrinsic and intrinsic atopic dermatitis. *J Invest Dermatol* 2002; 119:870–875.
- 55 Mitsuyasu H, Izuwara K, Mao XQ, *et al.* Ile50Val variant of IL4RA upregulates IgE synthesis and associates with atopic asthma. *Nat Genet* 1998; 19:119–120.
- 56 Izuwara K, Yanagihara Y, Hamasaki N, *et al.* Atopy and the human IL-4 receptor alpha chain. *J Allergy Clin Immunol* 2000; 106:S65–S71.
- 57 Takabayashi A, Ihara K, Sasaki Y, *et al.* Childhood atopic asthma: positive association with a polymorphism of IL-4 receptor alpha gene but not with that of IL-4 promoter or Fc epsilon receptor 1 beta gene. *Exp Clin Immunogenet* 2000; 17:63–70.
- 58 Risma KA, Wang N, Andrews RP, *et al.* V75R576 IL-4 receptor alpha is associated with allergic asthma and enhanced IL-4 receptor function. *J Immunol* 2002; 169:1604–1610.
- 59 Donfack J, Tsalenko A, Hoki DM, *et al.* HLA-DRB1*01 alleles are associated with sensitization to cockroach allergens. *J Allergy Clin Immunol* 2000; 105:960–966.
- 60 Howard TD, Koppelman GH, Zheng SL, *et al.* Gene-gene interaction in asthma: IL4RA and IL13 in a Dutch population with asthma. *Am J Hum Genet* 2002; 70:230–236.
- 61 Kruse S, Japha T, Tedner M, *et al.* The polymorphisms S503P and Q576R in the interleukin-4 receptor alpha gene are associated with atopy and influence the signal transduction. *Immunology* 1999; 96:365–371.
- 62 Hackstein H, Hecker M, Kruse S, *et al.* A novel polymorphism in the 5' promoter region of the human interleukin-4 receptor alpha-chain gene is associated with decreased soluble interleukin-4 receptor protein levels. *Immunogenetics* 2001; 53:264–269.
- 63 Kauppi P, Lindblad-Toh K, Sevón P, *et al.* A second-generation association study of the 5q31 cytokine gene cluster and the interleukin-4 receptor in asthma. *Genomics* 2001; 77:35–42.
- 64 Bottini N, Borgiani P, Otsu A, *et al.* IL-4 receptor alpha chain genetic polymorphism and total IgE levels in the English population: two-locus haplotypes are more informative than individual SNPs. *Clin Genet* 2002; 61:288–292.
- 65 Hershey GKK, Friedrich MF, Esswein LA, *et al.* The association of atopy with a gain-of-function mutation in the α subunit of the interleukin-4 receptor. *N Engl J Med* 1997; 337:1720–1725.
- 66 Rosa-Rosa L, Zimmermann N, Bernstein JA, *et al.* The R576 IL-4 receptor alpha allele correlates with asthma severity. *J Allergy Clin Immunol* 1999; 104:1008–1014.
- 67 van der Pouw Kraan TCTM, van Veen A, Boeije LCM. An IL-13 promoter polymorphism associated with increased risk of allergic asthma. *Genes Immun* 1999; 1:61.
- 68 Howard TD, Whittaker PA, Zaiman AL, *et al.* Identification and association of polymorphisms in the interleukin-13 gene with asthma and atopy in a Dutch population. *Am J Respir Cell Mol Biol* 2001; 25:377–384.
- 69 Liu X, Nickel R, Beyer K, *et al.* An IL13 coding region variant is associated with a high total serum IgE level and atopic dermatitis in the German multicenter atopy study (MAS-90). *J Allergy Clin Immunol* 2000; 106:167–170.
- 70 Leung TF, Tang NL, Chan IH, *et al.* A polymorphism in the coding region of interleukin-13 gene is associated with atopy but not asthma in Chinese children. *Clin Exp Allergy* 2001; 31:1515–1521.
- 71 Tsunemi Y, Saeki H, Nakamura K, *et al.* Interleukin-13 gene polymorphism G4257A is associated with atopic dermatitis in Japanese patients. *J Dermatol Sci* 2002; 30:100–107.
- 72 DeMeo DL, Lange C, Silverman EK, *et al.* Univariate and multivariate family-based association analysis of the IL-13 ARG130GLN polymorphism in the Childhood Asthma Management Program. *Genet Epidemiol* 2002; 23:335–348.
- 73 Randolph AG, Lange C, Silverman EK, *et al.* The IL12B gene is associated with asthma. *Am J Hum Genet* 2004; 75:709–715.
- 74 Lyon H, Lange C, Lake S, *et al.* IL10 gene polymorphisms are associated with asthma phenotypes in children. *Genet Epidemiol* 2004; 26:155–165.
- 75 Celedon JC, Lange C, Raby BA, *et al.* The transforming growth factor-beta1 (TGFB1) gene is associated with chronic obstructive pulmonary disease (COPD). *Hum Mol Genet* 2004; 13:1649–1656.
- 76 Hobbs K, Negri J, Klinnert M, *et al.* Interleukin-10 and transforming growth factor-beta promoter polymorphisms in allergies and asthma. *Am J Respir Crit Care Med* 1998; 158:1958–1962.
- 77 Pulleyn LJ, Newton R, Adcock IM, Barnes PJ. TGFbeta1 allele association with asthma severity. *Hum Genet* 2001; 109:623–627.
- 78 Arkwright PD, Chase JM, Babbage S, *et al.* Atopic dermatitis is associated with a low-producer transforming growth factor beta(1) cytokine genotype. *J Allergy Clin Immunol* 2001; 108:281–284.