

Review Article

New concepts of cytokines in asthma: Is the Th2/Th1 paradigm out the window?

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Abstract: Although asthma is clearly associated with a systemic propensity for allergic T helper type 2 (Th2) cell cytokine responses, independent local immune events appear to be responsible for the development of allergic airways inflammation. There is growing interest in how local immune networks interact with resident airway cell populations such as epithelial cells, which are now also recognized as key producers of cytokines, chemokines and growth factors. As well as their recognized role in airway remodelling, epithelial cells are now thought to have a role in initiating events. This review examines the role of cytokines produced by these and other cells in the development of asthma. It also highlights emerging concepts that the excessive and inappropriate immune responses seen in allergic disease may be related to dysfunction of various interleukin-10 producing regulatory cell populations.

Key words: airway epithelium; asthma; cytokines; IL-10; IL-13; Th2 responses.

With the relatively recent recognition that asthma is an inflammatory disorder, there has been an enormous effort to identify and modify the contributing pro-inflammatory processes. The advent of molecular technology and more sophisticated detection systems has led the identification and functional characterization of a vast number of component cytokines, mediators, receptors and other molecules. However, despite this dramatic increase in 'component' knowledge, our understanding of the more dynamic interactions and relationships is still fairly rudimentary. Furthermore, technology has so far added dimensions of complexity without uncovering basic causal pathways. A key objective of future research must be to address these issues and provide a more dynamic understanding of disease pathogenesis and the contributing factors.

Despite limitations in current knowledge it is already evident that asthma and allergic disease are associated with both local and underlying systemic immune dysregulation. Cytokines appear to play a key role in the initiation and perpetuation of allergic inflammation. However, it is still not clear if this dysfunction is initiated locally in the mucosal surfaces (and related immune networks) where disease is manifest, or if it is due to a more generalized abnormality in host responses. There is growing evidence that both of these processes occur and interact to produce airways disease. This report provides an overview of recent literature in this area, including the current models of immune regulation, interactions between cytokines and local airway epithelial cells, and the role of cytokines in the prediction, assessment and treatment of disease.

THE ROLE OF TH2 CYTOKINES IN ASTHMA

An increase in activated CD4⁺ T helper (Th) cells in asthmatic airways after allergen challenge has been recognized for many

years,^{1,2} reinforcing an immunological basis for this disease. Strong associations with allergic disease have also provided a strong case for a primary role of T helper type 2 (Th2) cells in the development of asthma. Although 'deficient' Th1 function was initially proposed as an underlying factor, there is no conclusive evidence of impaired Th1 function in either asthma or atopy.^{3–5} However, there is a substantial body of evidence implicating most 'Th2' cytokines (interleukin (IL)-4, IL-5, IL-9, IL-13) in the expression and development of airways inflammation and hyperactivity (AHR). While all of these cytokines appear to have a role, the recent literature indicates that IL-13 is especially critical and may be 'sufficient and necessary' for the development of reactive airways disease, at least in animal models.⁶ Repeated exposure to IL-13 leads to murine airway inflammation, AHR and increased mucous production.^{7,8} While blocking the effects of other Th2 cytokines (IL-4, IL-5 and IL-9) results in an incomplete reduction in the asthmatic airways response in mice, blocking the effects of IL-13 completely abolishes asthmatic responses.⁹ Accordingly, IL-13 'knock out' mice also have reduced airways eosinophilia, inflammation, fibrosis and epithelial and mucous cell hyperplasia compared with wild-type mice.¹⁰ IL-13 appears to act directly on airways epithelial cells to produce the pathological features of asthma.⁶

In humans, most studies are more indirect, focusing on peripheral blood responses, in vitro airway models or bronchial alveolar lavage¹¹ studies, all of which have clear limitations. Although the events leading to airways inflammation may be independent of the processes leading to the atopic state, these are strongly associated. Compared to non-atopic individuals, atopics have higher Th2 IL-4,^{12–15} IL-5,^{16–19} IL-13^{15,18,19} and IL-9²⁰ peripheral blood mononuclear cell (PBMC) responses to allergens and other stimuli. Similar patterns are seen in asthmatics as a reflection of their atopic propensity. Few studies

have attempted to define differences in cytokine responses in atopics with and without airways disease, although higher IL-9 responses,²⁰ attenuated pro-Th1 IL-12 responses²¹ and variously altered IL-10 responses²¹ have been noted (discussed further below). In a long-term follow-up study,²² persistent asthmatics had reduced Th1 (IFN- γ) responses to house dust mite compared to adults with resolved asthma, but both groups had higher IL-5 and IL-13 responses compared to non-atopic controls. In this context, Th2 responses reflected the atopic state rather than airways disease. Acute asthma has also been associated with higher levels of circulating IL-4, IL-5 and IL-13 compared to asymptomatic asthmatics,²³ but it is not clear if this is related to systemic or local airways factors. Genetic variants of IL-13 (Arg 110 Gln) have been associated with asthma, and homozygotes have been noted to have increased serum IL-13 levels²⁴ suggesting functional differences. However, while IL-13 plays a central role in animal models, the contribution in humans is less clear.

INTERACTIONS BETWEEN TH2 CYTOKINES AND AIRWAY EPITHELIUM

Despite the clear association with atopy, there is accumulating evidence that airways disease occurs through independent processes in 'Th2 predisposed' individuals. Local airway cells, and epithelial–mesenchymal interactions, are now believed to play a critical role in asthma pathogenesis.²⁵ Epithelial cells, which produce many chemokines, cytokines and growth factors, have been shown to be essential for Th2-induced disease expression in animal asthma models.^{6,26} Th2 cytokines have also been shown to have direct effects on human epithelial cells.²⁷ Together, these observations suggest that asthma is the culmination of both local epithelial dysfunction and generalized Th2 propensity (atopic predisposition).

IL-4 and IL-13 have direct effects on human lung epithelial cells, impairing epithelial barrier function and wound healing.²⁸ IL-13 and transforming growth factor beta (TGF β) also act synergistically on human airway fibroblasts to increase production of eotaxin-1, a powerful eosinophil chemoattractant.²⁷

Recent work suggests that these effects may be mediated by direct effects on airways epithelial cells^{6,26} without the need for an associated inflammatory infiltrate. Kupermann *et al.*⁶ demonstrated that mice lacking the vital Th2 signal transducer and activator of transcription 6 (STAT6) were protected from all of the IL-13 induced features of airways disease. Reconstitution of STAT6 only in airway epithelial cells was sufficient to lead to all of the features of IL-13-induced AHR and mucous production. Together, these findings suggest that IL-13 may account for many of the airway abnormalities in asthma, and that direct actions on epithelial cells may be central to the expression of disease. The mechanisms are still unclear. Other cell targets for IL-13 are still not well defined, and many IL-13 effects appear to be independent of known pathways (via IL-4 receptors).¹⁰

ROLE OF THE TH2 PARADIGM IN ASTHMA PATHOGENESIS – RECOGNIZED LIMITATIONS

The clear association between asthma and other allergic disease has led to the working model that similar patterns of immune dysregulation may be common to these conditions. Indeed, many studies^{12–20} confirm that patients with this group of atopic conditions have an increased propensity for Th2 responses to allergens. Although there are clear human parallels

with the Th1/Th2 paradigm originally described in rodents, the role of this in disease development is unclear. While many asthmatics are atopic, a number of other factors appear to contribute to the local inflammation and eventual airway remodelling. Furthermore, the presence of Th2 responses in atopic asthma may not represent a simple skewing or polarization of responses along a Th1–Th2 continuum as previously supposed. In fact, many recent studies suggest that affected individuals are more generally responsive to allergens with increases in both Th1 and Th2 responses,^{3,4} suggesting a breakdown of more fundamental regulatory processes, likely to be common to many aspects of the immune response. Finally, while there are clear associations between allergy and asthma, it appears that separate processes are involved in the atopic diathesis and airways inflammation. Asthma is a heterogeneous condition, which probably arises from different predisposing genetic and environmental factors in different individuals, and this may account for the lack of clear genetic or environmental causal pathways. Thus, while the Th1/Th2 paradigm has provided a very good model for describing allergic airways disease, it does not encapsulate the complexity of *in vivo* immune interactions in diseases such as asthma.

The associated increase in Th2 allergic disease with progressively 'cleaner' environments has been recently explained in terms of reduced bacterial burden and a consequent reduction in the strong pro-Th1 stimuli required for immune maturation. Thus, the 'hygiene hypothesis' was initially explained in terms of a shift in favour of Th2 responses because of reduced pro-Th1-inducing microbial exposure. However, this does not explain the paradoxical observation that 'Th1 diseases' (such as type 1 diabetes) have also increased in the same period. This complexity also suggests that simple strategies to alter Th1/Th2 responses may not be successful in complex immunological disorders such as asthma.

THE ROLE OF IL-10 AND OTHER REGULATORY CYTOKINES: NEW CONCEPTS OF ASTHMA PATHOGENESIS

More recently, a new theory has emerged. This proposes that environmental changes have in some way modified regulatory systems such that they inadequately prevent over-expression of inappropriate Th1 and Th2 responses in predisposed individuals. This has led to investigation of pathways with known homeostatic and immunomodulatory effects, particularly of IL-10 and TGF β regulatory pathways.

A key study recently demonstrated that administration of mycobacteria can reduce airway inflammation in animals, and that this effect was mediated by regulatory T cells producing both TGF β and IL-10.²⁹ This strongly supports the notion that in addition to their Th1 effects, microbial elements also induce important regulatory pathways.³⁰ Thus, while the 'hygiene hypothesis' may yet hold true, the proposed mechanisms are probably more complex than originally proposed.

The gut is believed to be central in the development of counter-regulatory immune function. The gastrointestinal tract and associated mucosal immune system is an IL-10 and TGF β dominant environment, promoting mucosal immune responses and systemic immune tolerance.³⁰ It is of note that germ-free animals that do not achieve normal gut colonization have profound disorders of immune tolerance including both autoimmunity and allergy.³¹

Although initially classified as a Th2 cytokine, IL-10 is produced by a much wider range of cell types (dendritic cells, monocytes, macrophages, CD4+ and CD8+ T cells, natural

killer cells, neutrophils and epithelial cells), and is now viewed more as a potent immunoregulatory cytokine. It plays an important role modulating and dampening excessive immune responses across the whole immune system. IL-10 is also produced by a recently described T-cell subset; the CD4+ CD25+ regulatory T cell.³² Interleukin-10 inhibits T-cell activation, immunoglobulin (Ig)E production, eosinophil recruitment and many other aspects of allergic inflammation.^{33,34}

There is some evidence that IL-10 responses are attenuated in atopy, but this is not conclusive in either animal models or humans. Allergic patients demonstrate reduced IL-10 responses after viral infections, with reduced IL-10 detected in nasal lavage fluid³⁵ and following *in vitro* PBMC stimulation.³⁶ This suggests impaired ability to increase production of this regulatory cytokine after an inflammatory stimulus. Another recent study described reduced IL-10 production by allergen-specific CD8+ T cells from atopic individuals.³⁷ However, IL-10 responses by viral specific CD8+ T cells were unimpaired compared to non-atopic controls, suggesting that effects could be stimulus specific. Findings in animals appear to depend on the models used. A number of IL-10 'knock out' murine models demonstrate increased AHR, eosinophilia and exaggerated Th2 responses compared with wild-type mice,³⁸ although other reports are conflicting.³⁹ Not surprisingly, IL-10 deficient animals also develop spontaneous inflammatory bowel disease.⁴⁰

The role of IL-10 expression in human asthmatic airways is less clear. Lower IL-10 (protein) levels have been measured in bronchial alveolar lavage samples from human asthmatics,⁴¹ who also show reduced IL-10 responses by their PBMC (in response to endotoxin) compared with control subjects.⁴¹ However, other studies have demonstrated higher expression of IL-10 messenger ribonucleic acid in asthmatic airways⁴² and gut mucosa.⁴³ It has been proposed that this may reflect dysregulation of IL-10 expression in the presence of chronic inflammation³⁰ with inadequate effector mechanisms.³³ Of note, inhaled corticosteroids increase IL-10 responses by alveolar macrophages,⁴⁴ suggesting that this may contribute to the anti-inflammatory action of these medications. Less is known about the role of TGF β in the development of airways pathology. Both IL-10 and TGF β gene polymorphisms have been noted in association with asthma and atopy.⁴⁵

Because IL-10 is also produced by epithelial cells, it may be an important mediating factor in many of the interactions between systemic immune responses and local airways disease. This is an area of intensive ongoing research.

THE USE OF CYTOKINE RESPONSES TO PREDICT DEVELOPMENT OF DISEASE

The detection of Th2 responses in the neonatal period may not represent a tendency for atopic disease, as both immaturity of Th1 (and antigen presenting cell function) and the pro-Th2 effects of maternal hormones in pregnancy promote Th2 responses in the perinatal period.⁴⁶ However, a number of studies suggest that persistence of this profile beyond the first months of life is associated with subsequent allergic disease.⁴⁷ It is therefore possible that future technologies may offer the potential to assess the propensity of an individual to a particular pattern of response.

There are a number of associations between early patterns of immune responses and either genetic risk of allergic disease (family or parental allergy) or subsequent allergic disease, although these associations are not specifically with asthma. Although Th1 function is generally less mature at birth, more

significant immaturity in Th1 capacity has been associated with genetic risk or subsequent atopic disease.⁴⁸ Similarly, a number of studies have reported that stronger neonatal Th2 responses (particularly IL-13) are associated with a subsequent increased risk of atopy.^{49–51} At this stage, these measures do not appear to have any predictive value, but support the concept that the processes which lead to immune dysregulation are initiated very early in life. With the development of allergy prevention strategies, there is a growing need to more accurately predict children at the highest risk for both atopy and related airways disease.

THE USE OF CYTOKINE RESPONSES TO ASSESS THE COURSE OR THE SEVERITY OF DISEASE

Although there have been some reports linking cytokine profiles to severity of disease, including higher serum IL-5 and lower IFN- γ levels,²³ none of these is of value in the clinical context. Although cytokine responses may also vary with disease activity and as a result of immunotherapy, this currently has no practical application.

METHODS OF MODIFYING CYTOKINE RESPONSES AS THERAPEUTIC STRATEGIES

Although the mechanisms were not understood until recently, strategies to modify cytokine responses have been employed to effectively reduce atopic responses for almost a century. Traditional allergen-immunotherapy is now known to result in long-term decreases in the production of allergen specific Th2 cytokines (IL-4 and IL-5) and an increase in Th1 (IFN- γ) responses.^{52–54} The initial phases of immunotherapy are associated with increased CD25 expression (characteristic of regulatory populations), with increases in both IL-10 and IL-12 signalling from tissue macrophages and other antigen presenting cells.⁵⁵ IL-10 is believed to play an important role in inducing and maintaining T-cell anergy.⁵⁶ While the atopic response is modulated, it is not clear what effects immunotherapy has on independent processes involved in airways inflammation. While traditional allergen immunotherapy is of value in some patients, it can have significant side effects and may not be feasible in individuals with multiple allergies.

Growing knowledge of immune pathways has provided a new range of potential targets for immune modulation. However many, including monoclonal antibodies to human IgE,^{57,58} target downstream events and may not alter or reverse the underlying pathological processes. Over the last 5–10 years, Th2 and Th1 cytokines became more logical targets. Early attempts were of only limited success, possibly because of limited awareness of the importance of regulatory mechanisms at that time. Although subcutaneous administration of recombinant human (pro-Th1) IL-12 resulted in significantly reduced eosinophils in blood and sputum of mild allergic asthmatic patients,⁵⁹ there were no differences in histamine-induced airways-hyper-responsiveness or the late asthmatic reaction compared with placebo administration. Similarly, a single intravenous infusion of monoclonal anti-IL-5 prevented allergen-induced eosinophilia (sputum and blood) but did not affect airways hyper-responsiveness or the late asthmatic reaction.⁶⁰ These studies provide a cautionary tale, that is, effectively targeting one component of the allergic response may not address the complexity of airway inflammation. More recent cytokine targets, including IL-4, IL-9, IL-13, their receptors, transcription factors (such as STAT6) and chemokine receptors

(such as CCR3) may hold some promise but may ultimately face the same issues. Potential side effects and unknown long-term consequences of these strategies remain of concern.

Methods of enhancing 'regulatory' pathways are growing in popularity, and range from simple oral administration of probiotic bacteria to the administration of highly developed plasmid DNA vaccines which encode allergic epitopes in the presence of immunostimulatory bacterial CpG motifs.⁶¹ Rather than have specific targets in the immune cascade (which are still poorly understood), these strategies appear to act through enhanced regulatory TGF β and IL-10 responses²⁹ and may directly reduce airway inflammation, in addition to atopic immune responses. Accordingly, in sensitised animals, mycobacteria can improve pulmonary function⁶² and reduce airway inflammation,⁶³ eosinophilia⁶⁴ and expression of adhesion molecules.⁶⁵ In humans, there is also preliminary evidence that bacterial preparations (intestinal flora and mycobacterial products) can improve atopy⁶⁶⁻⁶⁹ and lung function in asthmatics.⁷⁰ It is not clear if previous concerns about increasing the risk of autoimmune disease (which were based on assumptions that suppressing Th2 responses could lead to Th1 overexpression) are no longer justified if regulatory pathways are the main avenue of influence. These issues and basic mechanisms need to be addressed by ongoing research. Ideally these methods will eventually be replaced by strategies targeting the prevention of allergic responses (immunoprophylaxis).

Asthma and allergic disease remain the most common diseases in the modern world, making it essential to determine the casual pathways and underlying mechanisms, in the hope that this may lead to more definitive treatment and prevention strategies. A more complete understanding of the processes involved in local and systemic immune development is a crucial part of this process.

REFERENCES

- Azzawi M, Bradley B, Jeffery PK *et al.* Identification of activated T lymphocytes and eosinophils in bronchial biopsies in stable atopic asthma. *Am. Rev. Respir. Dis.* 1990; **142**: 1407-13.
- Robinson D, Hamid Q, Bentley A, Ying S, Kay AB, Durham SR. 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**: 313-24.
- Ng TW, Holt PG, Prescott SL. Cellular immune responses to ovalbumin and house dust mite in egg-allergic children. *Allergy* 2002; **57**: 207-14.
- Smart JM, Kemp AS. Increased Th1 and Th2 allergen-induced cytokine responses in children with atopic disease. *Clin. Exp. Allergy* 2002; **32**: 796-802.
- Kimura M, Yamaide A, Tsuruta S, Okafuji I, Yoshida T. Development of the capacity of peripheral blood mononuclear cells to produce IL-4, IL-5 and IFN-gamma upon stimulation with house dust mite in children with atopic dermatitis. *Int. Arch. Allergy Immunol.* 2002; **127**: 191-7.
- Kuperman DA, Huang X, Koth LL *et al.* Direct effects of interleukin-13 on epithelial cells cause airway hyperreactivity and mucus overproduction in asthma. *Nat. Med.* 2002; **8**: 885-9.
- Wills-Karp M, Luyimbazi J, Xu X *et al.* Interleukin-13: central mediator of allergic asthma. *Science* 1998; **282**: 2258-61.
- Grunig G, Warnock M, Wakil AE *et al.* Requirement for IL-13 independently of IL-4 in experimental asthma. *Science* 1998; **282**: 2261-3.
- Temann UA, Ray P, Flavell RA. Pulmonary overexpression of IL-9 induces Th2 cytokine expression, leading to immune pathology. *J. Clin. Invest.* 2002; **109**: 29-39.
- Kumar RK, Herbert C, Yang M, Koskinen AM, McKenzie AN, Foster PS. Role of interleukin-13 in eosinophil accumulation and airway remodelling in a mouse model of chronic asthma. *Clin. Exp. Allergy* 2002; **32**: 1104-11.
- O'Hehir R, Bal VQ, Uint D *et al.* An *in vitro* model of allergen dependent IgE synthesis by human B cells: comparison of the response of an atopic and non atopic individuals to *Dermatophagoides* spp. *Immunology* 1989; **64**: 627-31.
- Byron K, O'Brien R, Vaigos G, Wootton A. *Dermatophagoides pteronyssinus* ii-induced interleukin-4 and interferon- γ expression by freshly isolated lymphocytes of atopic individuals. *Clin. Exp. Allergy* 1994; **24**: 878-83.
- Imada M, Simons FE, Jay FT, Hayglass KT. Allergen-stimulated interleukin-4 and interferon-gamma production in primary culture: responses of subjects with allergic rhinitis and normal controls. *Immunology* 1995; **85**: 373-80.
- Gabrielsson S, Paulie S, Roquet A *et al.* Increased allergen-specific Th2 responses *in vitro* in atopic subjects receiving subclinical allergen challenge. *Allergy* 1997; **52**: 860-5.
- Kimura M, Tsuruta S, Yoshida T. IL-4 production by PBMC 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.
- 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.
- Till S, Dickason R, Huston D *et al.* IL-5 secretion by allergen-stimulated CD4+ T cells in primary culture: relationship to expression of allergic disease. *J. Allergy Clin. Immunol.* 1997; **99**: 563-9.
- 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.
- Macaubas C, Prescott S, Smallacombe T *et al.* Perinatal and early childhood cytokine responses to environmental allergens. In: Sanderson C, ed. *IL-5: from Molecule to Drug Target*. Marcel Dekker Inc., New York, 1999, 119-26.
- Jenmalm MC, Van Snick J, Cormont F, Salman B. Allergen-induced Th1 and Th2 cytokine secretion in relation to specific allergen sensitization and atopic symptoms in children. *Clin. Exp. Allergy* 2001; **31**: 1528-35.
- Tomita K, Lim S, Hanazawa T *et al.* Attenuated production of intracellular IL-10 and IL-12 in monocytes from patients with severe asthma. *Clin. Immunol.* 2002; **102**: 258-66.
- Smart JM, Horak E, Kemp AS, Robertson CF, Tang ML. Polyclonal and allergen-induced cytokine responses in adults with asthma. resolution of asthma is associated with normalization of IFN-gamma responses. *J. Allergy Clin. Immunol.* 2002; **110**: 450-6.
- Lee YC, Lee KH, Lee HB, Rhee YK. Serum levels of interleukins (IL) -4, IL-5, IL-13, and interferon-gamma in acute asthma. *J. Asthma* 2001; **38**: 665-71.
- Arima K, Umeshita-Suyama R, Sakata Y *et al.* Upregulation of IL-13 concentration *in vivo* by the IL13 variant associated with bronchial asthma. *J. Allergy Clin. Immunol.* 2002; **109**: 980-7.
- Holgate ST, Davies DE, Lackie PM, Wilson SJ, Puddicombe SM, Lordan JL. Epithelial-mesenchymal interactions in the pathogenesis of asthma. *J. Allergy Clin. Immunol.* 2000; **105**: 193-204.
- Venkayya R, Lam M, Willkom M, Grunig G, Corry DB, Erle DJ. The Th2 lymphocyte products IL-4 and IL-13 rapidly induce airway hyperresponsiveness through direct effects on resident airway cells. *Am. J. Respir. Cell Mol. Biol.* 2002; **26**: 202-8.
- Wenzel SE, Trudeau JB, Barnes S *et al.* TGF-beta and IL-13 synergistically increase eotaxin-1 production in human airway fibroblasts. *J. Immunol.* 2002; **169**: 4613-9.
- Ahdieh M, Vandenbos T, Youakim A. Lung epithelial barrier function and wound healing are decreased by IL-4 and IL-13 and enhanced by IFN-gamma. *Am. J. Physiol. Cell Physiol.* 2001; **281**: C2029-38.
- Zuany-Amorim C, Sawicka E, Manlius C *et al.* Suppression of airway eosinophilia by killed Mycobacterium vaccae-induced allergen-specific regulatory T-cells. *Nat. Med.* 2002; **8**: 625-9.
- Wills-Karp M, Santeliz J, Karp CL. The germless theory of allergic disease: revisiting the hygiene hypothesis. *Nat. Rev. Immunol.* 2001; **1**: 69-75.

- 31 Sudo N, Sawamura S, Tanaka K, Aiba Y, Kubo C, Koga Y. The requirement of intestinal bacterial flora for the development of an IgE production system fully susceptible to oral tolerance induction. *J. Immunol.* 1997; **159**: 1739–45.
- 32 Shevach EM. Certified professionals: CD4 (+) CD25 (+) suppressor T cells. *J. Exp. Med.* 2001; **193**: F41–6.
- 33 Koulis A, Robinson DS. The anti-inflammatory effects of interleukin-10 in allergic disease. *Clin. Exp. Allergy* 2000; **30**: 747–50.
- 34 Umetsu DT, DeKruyff RH. Interleukin-10: The missing link in asthma regulation? *Am. J. Respir. Cell Mol. Biol.* 1999; **21**: 562–3.
- 35 Corne JM, Lau L, Scott SJ, Davies R, Johnston SL, Howarth PH. The relationship between atopic status and IL-10 nasal lavage levels in the acute and persistent inflammatory response to upper respiratory tract infection. *Am. J. Respir. Crit. Care Med.* 2001; **163**: 1101–7.
- 36 Gentile DA, Patel A, Ollila C *et al.* Diminished IL-10 production in subjects with allergy after infection with influenza A virus. *J. Allergy Clin. Immunol.* 1999; **103**: 1045–8.
- 37 Seneviratne SL, Jones L, King AS *et al.* Allergen-specific CD8 (+) T cells and atopic disease. *J. Clin. Invest.* 2002; **110**: 1283–91.
- 38 Grunig G, Corry DB, Leach MW, Seymour BW, Kurup VP, Rennick DM. Interleukin-10 is a natural suppressor of cytokine production and inflammation in a murine model of allergic bronchopulmonary aspergillosis. *J. Exp. Med.* 1997; **185**: 1089–99.
- 39 Makela MJ, Kanehiro A, Borish L *et al.* IL-10 is necessary for the expression of airway hyperresponsiveness but not pulmonary inflammation after allergic sensitization. *Proc. Natl Acad. Sci. USA* 2000; **97**: 6007–12.
- 40 Kuhn R, Lohler J, Rennick D, Rajewsky K, Muller W. Interleukin-10-deficient mice develop chronic enterocolitis. *Cell* 1993; **75**: 263–74.
- 41 Borish L. IL-10: evolving concepts. *J. Allergy Clin. Immunol.* 1998; **101**: 293–7.
- 42 Robinson DS, Tsicopoulos A, Meng Q, Durham S, Kay AB, Hamid Q. Increased interleukin-10 messenger RNA in atopic allergy and asthma. *Am. J. Respir. Cell Mol. Biol.* 1996; **14**: 113–7.
- 43 Lamblin C, Desreumaux P, Colombel JF, Tonnel AB, Wallaert B. Overexpression of IL-10 mRNA in gut mucosa of patients with allergic asthma. *J. Allergy Clin. Immunol.* 2001; **107**: 739–41.
- 44 John M, Lim S, Seybold J *et al.* Inhaled corticosteroids increase interleukin-10 but reduce macrophage inflammatory protein-1 α , granulocyte-macrophage colony-stimulating factor, and interferon- γ release from alveolar macrophages in asthma. *Am. J. Respir. Crit. Care Med.* 1998; **157**: 256–62.
- 45 Hobbs K, Negri J, Klinnert M, Rosenwasser LJ, Borish L. Interleukin-10 and transforming growth factor- β promoter polymorphisms in allergies and asthma. *Am. J. Respir. Crit. Care Med.* 1998; **158**: 1958–62.
- 46 Prescott S, Macaubas C, Holt B *et al.* Transplacental priming of the human immune system to environmental allergens: universal skewing of initial T-cell responses towards Th-2 cytokine profile. *J. Immunol.* 1998; **160**: 4730–7.
- 47 Prescott S, Macaubas C, Smallacombe T *et al.* Development of allergen-specific T-cell memory in atopic and normal children. *Lancet* 1999; **353**: 196–200.
- 48 Tang MLK, Kemp AS, Thorburn J, Hill D. Reduced interferon gamma secretion in neonates and subsequent atopy. *Lancet* 1994; **344**: 983–5.
- 49 Spinazzi F, Agea E, Russano A *et al.* CD4+ IL13+ T lymphocytes at birth and the development of wheezing and/or asthma during the 1st year of life. *Int. Arch. Allergy Immunol.* 2001; **124**: 497–501.
- 50 Kopp MV, Zehle C, Pichler J *et al.* Allergen-specific T cell reactivity in cord blood: the influence of maternal cytokine production. *Clin. Exp. Allergy* 2001; **31**: 1536–43.
- 51 Ohshima Y, Yasutomi M, Omata N *et al.* Dysregulation of IL-13 production by cord blood CD4+ T cells is associated with the subsequent development of atopic disease in infants. *Pediatr. Res.* 2002; **51**: 195–200.
- 52 Secrist H, Chelen CJ, Wen Y, Marshall JD, Umetsu DT. Allergen immunotherapy decreases interleukin 4 production in CD4+ T cells from allergic individuals. *J. Exp. Med.* 1993; **178**: 2123–30.
- 53 Jutel M, Pichler WJ, Skrbic D, Urwyler A, Dahinden C, Muller UR. Bee venom immunotherapy results in decrease of IL-4 and IL-5 and increase of IFN- γ secretion in specific allergen-stimulated T cell cultures. *J. Immunol.* 1995; **154**: 4187–94.
- 54 McHugh SM, Deighton J, Stewart AG, Lachmann PJ, Ewan PW. Bee venom immunotherapy induces a shift in cytokine responses from a TH-2 to a TH-1 dominant pattern: comparison of rush and conventional immunotherapy. *Clin. Exp. Allergy* 1995; **25**: 828–38.
- 55 Hamid QA, Schotman E, Jacobson MR, Walker SM, Durham SR. Increases in IL-12 messenger RNA+ cells accompany inhibition of allergen-induced late skin responses after successful grass pollen immunotherapy. *J. Allergy Clin. Immunol.* 1997; **99**: 254–60.
- 56 Akdis CA, Blaser K. Mechanisms of allergen-specific immunotherapy. *Allergy* 2000; **55**: 522–30.
- 57 Fahy JV. Reducing IgE levels as a strategy for the treatment of asthma. *Clin. Exp. Allergy* 2000; **30** (Suppl. 1): 16–21.
- 58 Fick RB, Fox JA, Jardieu PM. Immunotherapy approach to allergic disease. *Immunopharmacology* 2000; **48**: 307–10.
- 59 Bryan S, O'Connor B, Matti S *et al.* Effects of recombinant interleukin-12 on eosinophils, airways hyper-responsiveness, and the late asthmatic reaction. *Lancet* 2000; **356**: 2149–53.
- 60 Leckie M, ten Brinke A, Khan J *et al.* Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airways hyper-responsiveness, and the late asthmatic reaction. *Lancet* 2000; **356**: 2144–8.
- 61 Spiegelberg HL, Tighe H, Roman M, Broide D, Raz E. Inhibition of IgE formation and allergic inflammation by allergen gene immunization and by CpG motif immunostimulatory oligodeoxynucleotides. *Allergy* 1998; **53**: 93–7.
- 62 Hopfenspirger MT, Agrawal DK. Airway hyperresponsiveness, late allergic response, and eosinophilia are reversed with mycobacterial antigens in ovalbumin-prensensitized mice. *J. Immunol.* 2002; **168**: 2516–22.
- 63 Tsai JJ, Liu YH, Shen HD, Huang SH, Han SH. Prevention of Der p2-induced allergic airway inflammation by mycobacterium-bacillus Calmette-Guerin. *J. Microbiol. Immunol. Infect.* 2002; **35**: 152–8.
- 64 Major T, Wohlleben G, Reibetanz B, Erb KJ. Application of heat killed Mycobacterium bovis BCG into the lung inhibits the development of allergen-induced Th2 responses. *Vaccine* 2002; **20**: 1532–40.
- 65 Yang X, Fan Y, Wang S *et al.* Mycobacterial infection inhibits established allergic inflammatory responses via alteration of cytokine production and vascular cell adhesion molecule-1 expression. *Immunology* 2002; **105**: 336–43.
- 66 Cavallo GP, Elia M, Giordano D, Baldi C, Cammarota R. Decrease of specific and total IgE levels in allergic patients after BCG vaccination: preliminary report. *Arch. Otolaryngol. Head Neck Surg.* 2002; **128**: 1058–60.
- 67 Ota MO, Vekemans J, Schlegel-Haueter SE *et al.* Influence of mycobacterium bovis bacillus Calmette-Guerin on antibody and cytokine responses to human neonatal vaccination. *J. Immunol.* 2002; **168**: 919–25.
- 68 Barlan IB, Tukenmez F, Bahceciler NN, Basaran MM. The impact of *in vivo* Calmette-Guerin bacillus administration on *in vitro* IgE secretion in atopic children. *J. Asthma* 2002; **39**: 239–46.
- 69 Arkwright PD, David TJ. Intradermal administration of a killed mycobacterium vaccae suspension (SRL 172) is associated with improvement in atopic dermatitis in children with moderate-to-severe disease. *J. Allergy Clin. Immunol.* 2001; **107**: 531–4.
- 70 Choi IS, Koh YI. Therapeutic effects of BCG vaccination in adult asthmatic patients: a randomized, controlled trial. *Ann. Allergy Asthma Immunol.* 2002; **88**: 584–91.