

The immunobiology of early asthma

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IT HAS BEEN RECOGNISED for decades that asthma is an inflammatory disease of the airways. In adult asthma, there is now good evidence that many of the disease-related traits (eg, eosinophilic airway inflammation, mast cell hyperdegranulation, atopy, raised IgE levels) may be largely attributable to inappropriate activation and cytokine production by CD4+ T cells. The few specific pathology studies that have been performed on tissues from infants and very young children with asthma have suggested that the same disease mechanisms that operate in adult asthma are likely to contribute to asthma in early childhood, at least in some form. As defects in lung function associated with asthma appear to occur within the first two years of life,

there is currently great hope that a detailed understanding of immune processes in early asthma may lead to specific preventive or curative interventions. However, at present, there is very little direct evidence of the actual immunological mechanisms that operate in very young children with asthma. Most of our current knowledge is based on animal models.

The so-called “T_H2 hypothesis” of asthma stems from studies in the late 1980s by Mosmann et al.^{1,2} Their observations of long-term, intensely stimulated mouse CD4+ lymphocytes *in vitro* revealed that they could be polarised into two distinct populations: type 1 helper T cells (“T_H1 cells”) and type 2 helper T cells (“T_H2 cells”). T_H1 cells produce interferon gamma (IFN-γ), tumour necrosis factor beta (TNF-β), and interleukins IL-2, IL-12 and IL-18. These cytokines help to clear intracellular pathogens and viruses and are linked to cell-mediated immunity. T_H2 cells, which produce IL-4, IL-5, IL-9 and IL-13, are important in fine-tuning B cell antibody production and defending against extracellular pathogens (especially helminths). Because T_H2 cytokines induce eosinophilia, IgE

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ABSTRACT

What do we know?

- CD4+ T cells are strongly implicated in asthma pathogenesis.
- The “T_H2 hypothesis” postulates two patterns of cytokine secretion by stimulated CD4+ T cells: a “T_H1” response and a “T_H2” response.
- T_H2-type cytokines (interleukins IL-4, IL-5, IL-9, IL-13) regulate eosinophilia, mast cell growth, IgE and mucus production and have been proposed as key regulatory factors in asthma.
- T_H1-type cytokines include interferon- γ , IL-2, IL-12, IL-18, and tumour necrosis factor beta. T_H2 responses are reciprocally inhibited by T_H1 responses in animal models, but this may not be so in asthma in humans.
- In humans, T_H1- and T_H2-type cytokines are often coexpressed in early asthma.

What do we need to know?

- Is cross-regulation between T_H1 and T_H2 immune biases truly lost in early asthma?
- Can induction of T_H1-type responses actually protect against asthma, as predicted by the “hygiene hypothesis”? If so, how might this induction be achieved safely in infants?
- Can the in-utero environment be subtly manipulated to minimise asthma risk?
- Does early childhood treatment with current anti-asthma drugs lead to long-term immune changes?

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production, mucus secretion and mast cell growth in animals *in vivo* and in human cells *in vitro*, they have been specifically linked to asthma.

The “T_H2 model” of asthma has been widely accepted, and in humans there is clear evidence for T_H2-like cytokine patterns in T cells obtained by bronchoalveolar lavage.^{3,4} Further evidence comes from recent studies of IL-12, which is a natural endogenous inhibitor of T_H2-type responses. Morahan et al have identified a close association between impairment of IL-12 expression (due to promoter polymorphism) and a markedly increased risk of developing severe asthma.⁵ However, detailed analysis of individual cytokine profiles in single T cells has proven that distinct T_H2 cells do not exist in humans — in both childhood asthma and adult asthma there is increasing evidence for a mixed T_H1/T_H2 cytokine pattern, in which IFN- γ and IL-5 are concurrently high.⁶

Animal models predict that, if primary T_H2 immune deviation (ie, bias towards the T_H2 response) could be prevented, early asthma might be preventable or curable. The molecular basis of primary immune sensitisation and immune deviation has therefore been intensively researched. It was rapidly appreciated that T_H1 and T_H2 immune deviations are mutually inhibitory. Cell biology studies have proven that the broad cytokine profile of CD4+ T cells

polarises under the influence of cytokines. This polarisation results in T cells producing a smaller number of cytokines in distinctive patterns that can be difficult to reverse if the driving stimulus is intense or prolonged. T_H2 immune deviation is driven by IL-4, produced by the CD4 cells themselves and by other cells, such as mast cells and perhaps some natural killer cell subpopulations. IL-4 is particularly effective when cytokines associated with the T_H1 response (IFN- γ , IL-12 and IL-18) are neutralised by gene manipulation or monoclonal antibodies.^{2,7} In the early stages of immune deviation, the cytokine profile remains plastic and can be easily redirected by simply changing the cytokine conditions. Thus, T_H2 responses can be suppressed by combinations of IFN- γ , IL-12 and IL-18, and intrinsic T_H1 cytokines reduce the intensity of the T_H2 response in animal models *in vivo*.^{8,9} However, as the intensity or duration of stimulation progresses, this plasticity is lost as cell-surface-receptor components and internal signalling molecules specific to T_H1 cytokine responses are selectively down-regulated. Similarly, T_H1 responses become refractory to redirection by IL-4. This effect is demonstrated *in vivo* by the observation that anti-IL-4 antibodies completely prevent asthma in animals if given before allergic sensitisation has occurred, but have no effect at all on established asthma.¹⁰

In infants and children, T_H2 immune deviation and allergic sensitisation develop and consolidate slowly over years, although, paradoxically, these responses are weaker than in children who do not develop asthma or allergy in later life.^{3,11} There has been great interest recently in the observation that the in-utero environment is strongly T_H2-biased (perhaps to prevent a cell-mediated immune response against the fetus?) and that the immune response in infants has a T_H2 bias that only slowly reverts to the adult default T_H1-biased pattern over the first three to five years of life.

Some researchers, using highly sensitive detection methods, have suggested that sensitisation to aeroallergens (eg, house-dust mite, cat allergen) occurs *in utero* and that antigen-specific T_H2-biased responses occur in T cells recovered from cord blood at birth. Even if this is true, it would seem likely that further postpartum development of the T_H2 compartment is necessary to develop the T_H2-biased armed effector T cells that coordinate tissue inflammation and damage.

As aeroallergens are ubiquitous, it remains unclear why some individuals develop asthma while others do not. This issue is further clouded by epidemiological studies showing an inconclusive (or, paradoxically, protective) relationship between domestic exposure to cat and house-dust mite allergens and the development of asthma.¹² It is clear from animal studies that the default immune response to aeroallergens is to develop “tolerance” (non-responsiveness), which may be mediated by lung macrophages. As aeroallergens are swallowed as well as breathed in, and as mucosal immune responses tend to be shared at anatomically distinct mucosal surfaces because of lymphocyte trafficking, there is currently great interest in whether normal tolerance occurs via the lungs or via the gut (in a manner analogous to food-

allergy tolerance) — or both. Respiratory infection also impairs tolerance.¹³ Subtle breakdown in tolerance, perhaps associated with differing patterns of gut flora or infection, might explain the rising global trend in asthma prevalence.

The “hygiene hypothesis” proposes that infections acquired early in life may protect children against asthma. Gram-negative bacteria (through lipopolysaccharide [LPS]-induced IFN induction), mycobacteria and most viruses strongly induce T_H1 responses. These pathogens, or their purified components, have been shown to prevent or lessen T_H2-type responses in animals.¹⁴ The hygiene hypothesis is strengthened by certain epidemiological evidence and by the recent discovery that polymorphisms in CD14, an LPS coreceptor, are associated with increased asthma risk in children.¹⁵ These genetic variants of CD14 may reduce the intensity of LPS responses, thereby reducing development of T_H1-type immunity.

It is clear that asthma is a complex disease involving multiple genetic determinants. The picture is complicated by the fact that, in established asthma, disease exacerbations may be associated with T_H1-type cytokine expression. Furthermore, T_H1-biased lymphocytes grown *in vitro* have been shown to worsen asthma, rather than suppress disease, when transferred to animals. Research on tolerance and its failure has also focused on the contribution of infections to the development of asthma, as inflammation induced by infection reduces tolerance to aeroallergens.¹⁶

The observation that more intense antigen stimulation of T cells *in vivo* tends to lead to tolerance rather than more severe disease raises the issue of whether the T_H2 hypothesis can account for varying degrees of asthma severity. Interleukin-13 (associated with the T_H2 response) is present in persistent disease, but its expression can occur independently of T cells once disease is established.¹⁷ Similarly, IL-4 (closely related to IL-13) can impair responses to steroids by inducing an inactive form of the steroid receptor.¹⁸ However, it seems more likely that separately inherited gene polymorphisms control disease severity independently of T_H2-biased cell populations, although T_H2 immune deviation may be necessary for primary disease induction. In adult asthma there is some doubt as to whether T_H2-biased T cell populations are needed to sustain long-established disease. Potent immunosuppressive agents, such as cyclosporin A, are poorly effective against established asthma, and even antibody-mediated depletion of CD4 lymphocytes seems to produce little clinical benefit.

The therapeutic time window in which to manipulate the immunobiology of early asthma may be small indeed. Nevertheless, there is very strong evidence from animal models that, should they be proven safe, immune modulators may be very useful in treating early disease (*see page S66*¹⁹).

References

1. Mosmann TR, Cherwinski H, Bond MW, et al. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol* 1986; 136: 2348-2357.

2. 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.
3. Holt PG, Macaubas S, Prescott S, et al. Primary sensitization to inhalant allergen. *Am J Respir Crit Care Med* 2000; 162(3 Pt 2): S91-S94.
4. 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.
5. Morahan G, Huang D, Holt BJ, et al. Association of IL-12B promoter polymorphism with severity of atopic and non-atopic asthma in children. *Lancet* 2002; 360: 455-459.
6. Salvi SS, Babu KS, Holgate ST. Is asthma really due to a polarized T cell response toward a helper T cell type 2 phenotype? *Am J Respir Crit Care Med* 2001; 164: 1343-1346.
7. Erard F, Wild MT, Garcia-Sanz JA, et al. Switch of CD8 T cells to noncytolytic CD8-CD4-cells that make Th2 cytokines and help B cells. *Science* 1993; 260: 1802-1805.
8. Coyle AJ, Tsuyuki S, Bertrand C, et al. Mice lacking the IFN-gamma receptor have impaired ability to resolve a lung eosinophilic inflammatory response associated with a prolonged capacity of T cells to exhibit a Th2 cytokine profile. *J Immunol* 1996; 156: 2680-2685.
9. Gavett SH, O'Hearn DJ, Li X, et al. Interleukin 12 inhibits antigen-induced airway hyperresponsiveness, inflammation, and Th2 cytokine expression in mice. *J Exp Med* 1995; 182: 1527-1536.
10. Coyle AJ, Le Gros G, Bertrand C, et al. Interleukin-4 is required for the induction of lung Th2 mucosal immunity. *Am J Respir Cell Mol Biol* 1995; 13: 54-59.
11. Holt PG, Macaubas C, Stumbles PA, Sly PD. The role of allergy in the development of asthma. *Nature* 1999; 402(6760 Suppl): B12-B17.
12. Lau S, Illi S, Sommerfeld C, et al. Early exposure to house-dust mite and cat allergens and development of childhood asthma: a cohort study. Multicentre Allergy Study Group. *Lancet* 2000; 356: 1392-1397.
13. Tsitoura DC, Kim S, Dabbagh K, et al. Respiratory infection with influenza A virus interferes with the induction of tolerance to aeroallergens. *J Immunol* 2000; 165: 3484-3491.
14. Tulic MK, Wale JL, Holt PG, Sly PD. Modification of the inflammatory response to allergen challenge after exposure to bacterial lipopolysaccharide. *Am J Respir Cell Mol Biol* 2000; 22: 604-612.
15. Koppelman GH, Reijmerink NE, Colin Stine O, et al. Association of a promoter polymorphism of the CD14 gene and atopy. *Am J Respir Crit Care Med* 2000; 163: 965-969.
16. Tsitoura DC, Kim S, Dabbagh K, et al. Respiratory infection with influenza A virus interferes with the induction of tolerance to aeroallergens. *J Immunol* 2000; 165: 3484-3491.
17. Wills-Karp M, Luyimbazi J, Xu X, et al. Interleukin-13: central mediator of allergic asthma. *Science* 1998; 282: 2258-2261.
18. Kam JC, Szeffler SJ, Surs W, et al. Combination IL-2 and IL-4 reduces glucocorticoid receptor-binding affinity and T cell response to glucocorticoids. *J Immunol* 1998; 151: 3460-3466.
19. Anderson GP. Therapeutic prospects for early asthma. *Med J Aust* 2002; 177 Suppl Sep 16: S66-S69. □