Th-2 cytokines in allergic disease

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The Th-1/Th-2 paradigm

The description of two subtypes of T helper cells based on cytokine profiles by Mosman and Coffman in 1986 was a major step forward in thinking on control of immune responses. Building on previous divisions of responses into predominantly humoral or predominantly cell-mediated, they described murine T cells clones that could be divided into either Th-1 producing IFN-γ and IL-2 but not IL-4 and IL-5 or Th-2 which produce IL-4 and IL-5 but not IFN-γ. The functional consequences of this division follow from the observation that IFN-γ was required for activation of macrophage function and cytotoxic T-cell responses in cell mediated immunity, whereas IL-4 unopposed by IFN-γ was essential in switching B-cells to IgE synthesis and IL-5 was involved in eosinophil development and survival. This was seen most elegantly in the response of different mouse strains to Leishmania infection which was shown to largely determined by the genetic tendency to mount either a healing Th-1 response or an inappropriate Th-2 response leading to disseminated disease. The potential relevance of Th-2 responses to atopic disease was rapidly apparent since IL-4 and IL-5 could explain both IgE and eosinophilic inflammation. These interactions are summarised in Figure 1.

Human Th-1 and Th-2 cells

Although a variety of cytokine profiles were described from human T helper clones, the work of the groups of Romagnani and Kapsenberg firmly established that polarised Th-1 and Th-2 clones could indeed be derived from humans and, in particular that Th-2 responses were prominent in allergen specific CD4+ T-cell clones. Although the principle of the Th-1/Th-2 concept applies in both mice and men, there are differences both in the cytokine profiles observed, the factors determining the response and the apparent stability of cytokine profile. Thus the work from study of murine T-cells and animal models needs to be confirmed in humans.
Fig. 1 Proposed interactions between allergen and Th-2 cells in asthma. Allergen is taken up by antigen presenting cells, cleaved into small peptides and complexed with MHC class II molecules. This complex is then recognised by Th-2 cells, which, with appropriate co-stimulation, proliferate and produce cytokines. IL-4 and IL-13 switch B-cells to IgE production, and IL-5 drives development, release and survival of eosinophils. IL-9 acts to increase IL-5 receptor expression. Mast cells, basophils and eosinophils themselves also produce type-2 cytokines and may thus amplify allergic inflammation. IgE cross linking on basophils and mast cells leads to histamine release and synthesis of lipid mediators and eosinophil degranulation and activation may contribute to airway hyper-responsiveness through lipid mediators and basic granule proteins.

Non-T-cell sources of type 2 cytokines

Fairly soon after the initial description of Th-1 and Th-2 cytokine patterns, it was shown that mast cells could produce type 2 cytokines\textsuperscript{11}. It is now established that eosinophils, basophils and some structural cells such as epithelial and endothelial cells may also produce cytokines and chemokines that amplify the inflammatory cascade\textsuperscript{12,13}. In addition, non-T-cell cytokines may influence the development of a Th-1 or Th-2 phenotype by responding T-cells: for example, IL-4 from basophils may
help drive a Th-2 response, whereas NK cell-derived IFN-γ might favour a Th-1 phenotype. Basophils and mast can support IgE switching by B-cells\textsuperscript{14}. How non-T-cell cytokine production is regulated and its role in the allergic response is at present uncertain.

The potential role of Th-2 cytokines in allergic disease

IgE regulation

The defining hallmark of atopic disease is production of specific IgE to allergens. The molecular regulation of IgE production from B-cells has been well defined\textsuperscript{15,16}. In particular, IL-4 or IL-13 are essential for the first step in isotype switching: generation of the \(I_{\text{imm}}\) immature mRNA transcript. A second signal, such as that from CD40/CD40L interaction, is required for IgE production. Other cytokines including IL-5\textsuperscript{17}, IL-6\textsuperscript{18}, and IL-9\textsuperscript{19} can enhance IgE production, whereas IFN-γ and IL-12 inhibit both isotype switch and IgE production. Thus, a Th-2 cytokine profile favours generation of specific IgE. It is of note that non-T-cells can also produce IL-4, and mast cells and basophils are capable of IgE switching\textsuperscript{14}. However, without cognate MHC/TCR interaction such IgE will not be allergen specific. IL-13 can be produced by human Th-1 as well as Th-2 T-cells, although IFN-γ from Th-1 cells would inhibit IgE switching.

Interleukin-5 and eosinophils

Just before the description of Th-1 and Th-2 cells, IL-5 had been defined as a cytokine with specific action in the development, priming and survival of eosinophils\textsuperscript{5}. Some activity is also seen on basophils and human B-cells\textsuperscript{21,22}. Although IL-5 shares a common \(\beta\) receptor subunit with IL-3 and GM-CSF\textsuperscript{23,24}, and initial studies suggested that all three cytokines could act to cause eosinophil development from bone marrow progenitors\textsuperscript{25}, our recent studies suggest that IL-5 itself, but not IL-3 or GM-CSF up-regulates IL-5Rα expression and that human eosinophil development is largely IL-5 dependent\textsuperscript{26}.

Evidence for Th-2 cell involvement in atopic allergic disease

Baseline disease

Assessment of mRNA expression in broncho-alveolar lavage cells from atopic asthmatic subjects showed a predominant Th-2 pattern\textsuperscript{27}, and
numbers of cells expressing both IL-4 and IL-5 mRNA were correlated with measures of disease severity, such as bronchial responsiveness or forced expiratory volume in 1 s. In addition, IL-4 and IL-5, but not IFN-γ, protein levels were increased in BAL fluid from atopic asthmatics when compared to control subject, and allergen specific Th-2-type clones could be isolated from the respiratory mucosa of atopic subjects. Similarly, allergen-specific Th-2 cells were isolated from lesional skin in atopic dermatitis, and a Th-2 cytokine mRNA profile was demonstrated in skin biopsies. More recently, allergen specific T-cell lines from BAL from atopic asthmatics were shown to produce IL-5: this was from both CD4 and CD8 cells. In addition, by combining immunohistochemical staining to identify cell phenotype with in situ hybridization for cytokine mRNA, IL-4 and IL-5 mRNA were predominantly localised to CD4+ cells in the airway mucosa from asthmatic subjects, with lesser contributions from CD8+ cells, mast cells, and eosinophils. Other Th-1 and Th-2 cytokines have been assessed in asthma: some reports find increased expression of IL-10, and increased IL-13 expression is also reported.

**Non-atopic asthma**

Non-atopic asthma is characterised by asthma, generally occurring later in life than atopic asthma, without clinical or laboratory evidence of IgE sensitization to aero-allergens. Bronchial biopsies from such asthmatics show eosinophil infiltration and activated T-cells in the bronchial mucosa, as in atopic asthma. Although one study did not detect IL-4 in concentrated BAL fluid from non-atopic subjects, more recent biopsy studies have reported a Th-2 cytokine profile at both the mRNA and protein level. In addition, increased numbers of cells bearing high affinity IgE receptors were detected in bronchial biopsies from non-atopic asthmatics when compared to control subjects. What the role of IgE in this non-atopic variant of asthma is, and whether it is directed against specific antigens, remain to be established. It is of note that Valenta and colleagues have recently described a human IgE binding ‘auto-allergen’.

**Allergen challenge**

The late phase response to allergen challenge has been used to model chronic allergic inflammation, and is characterised by T-cell activation and eosinophil and neutrophil infiltration. Studies of cytokine mRNA expression also support activation of a Th-2-type response in skin, nose and lung biopsies obtained 24 h after allergen challenge.
Response to treatment

Numbers of cells expressing mRNA for IL-4 or IL-5 both fell after corticosteroid treatment of asthma in a double blind, placebo controlled study, and there was a small increase in IFN-γ mRNA expressing cells. That such changes might be relevant to clinical improvement was supported by the work of Leung et al. who showed a similar fall in IL-5 mRNA expressing cells in subjects responding to oral prednisone, but not in a group of subjects whose asthma did not improve with steroid therapy. The role of T-cells in asthma was also supported by the demonstration of clinical efficacy and steroid-sparing activity of cyclosporin A in severe asthma, and by the inhibition of the late, but not early, asthmatic response to allergen challenge by this inhibitor of T-cell activation. In addition, a non-depleting anti-CD4 monoclonal antibody was also shown to improve lung function in severe steroid-dependent asthmatics.

Allergen immunotherapy has been used to control atopic allergic disease for many years, and evidence also suggests that this too may target Th-2 T-cell activation, since reduced allergen induced IL-4 and IL-5 production with increased IFN-γ responses were seen after clinically successful treatment.

Animal models of allergic disease

Mouse models of allergic asthma yield varying data in different mouse strains and different sensitization and challenge regimens. Most models involve initial sensitization by intraperitoneal route with adjuvant and subsequent inhaled challenge. They are thus models of allergen challenge and some way from human asthma. Nonetheless, such models can elicit airway eosinophilia and hyper-responsiveness, and allow careful molecular dissection of the immunology of such responses. Although some caution must be applied in extrapolating to human disease, data from genetic studies and from gene manipulation in such models have elegantly identified themes for future research in human disease.

Gene knock-out studies and use of blocking antibodies established the importance of IL-4 and IL-5 in eosinophilic and IgE responses and AHR in animal models of allergen challenge, although the relative role of these cytokines varies according to the strain studied. Targeting T-cells with anti-CD4 antibodies and experiments with adoptive transfer of antigen-specific Th-2 cells have shown that Th-2 cells can certainly induce airway eosinophilia and BHR. Recent experiments suggest that IL-4 and IL-5 are not the only route to T-cell-dependent AHR. Hogan et al. showed that, in IL-4 gene targeted BALB/c mice, anti-IL-5 antibody treatment reduced airway eosinophilia but did not block AHR,
whereas depleting CD4+ T-cells in mice lacking both IL-4 and IL-5 did abolish residual AHR. More recently, an important role for IL-13 was suggested. Wills-Karp et al. showed that a soluble IL-13Rα-human IgG-Fc fusion protein, which blocks mouse IL-13 but not IL-4, could inhibit AHR in a mouse allergen challenge model, without reducing airway eosinophilia or serum antigen-specific IgE. Grunig et al. showed similar data, although their experiments did suggest some reduction in eosinophilia, and went on to show that IL-4R deficient mice did not acquire the AHR upon Th-2 transfer that was seen in wild type controls. These data suggest that IL-13 and IL-4 may act to produce AHR by mechanisms that do not involve IgE or eosinophils. Whether this is also so in humans remains to be established.

Transgenic expression of IL-5, IL-9, IL-11 or IL-13 under the control of a promoter directing lung specific over expression of these cytokines can induce AHR in mice without allergen challenge. The airway pathology in such models varies, but variable mast cell and eosinophil expansion is seen. It is likely that such experiments induce a cascade of cytokines and chemokines in the lung, and definition of the patterns seen may pinpoint important contributors to the AHR seen in these models. Some such transgenic animals, in particular those overexpressing IL-11 and IL-13 also show some evidence of changes reminiscent of the airway remodelling that characterises chronic asthma, such as sub-epithelial collagen deposition, and may give information on these processes.

IL-9 may of particular interest in allergic disease. As mentioned above, transgenic overexpression of IL-9 alone is sufficient for eosinophilic airway inflammation and AHR in mice. Murine studies also show that IL-9 is genetically linked to BHR in different strains. Human linkage studies have also implicated IL-9 in asthma. Receptor variants of IL-9 have been described which may explain differential sensitivity to the cytokine. We have recently shown that IL-9 can up-regulate IL-5Rα on developing eosinophils and may act as an eosinophil survival factor.

Animal models have also defined the interaction of IL-5 with eotaxin and other chemokines in eosinophil mobilisation from the bone marrow. Collins et al. showed synergy between systemically delivered IL-5 with local eotaxin in recruitment of eosinophils, and both were shown to act in release of a bone marrow pool of eosinophils.

**Factors regulating Th-2 development, recruitment and phenotype expression**

**Development of Th-1 and Th-2 cells**

A variety of factors has been shown to act in driving developing naive T-cell responses in either the Th-1 or Th-2 direction. The best defined is
the cytokine environment, but the antigen dose, antigen presenting cell, local hormone and prostaglandin milieu can also influence the outcome. As the molecular control of T-cell differentiation is defined, these data can be better understood. An important factor in Th-1/Th-2 development is the loss or retention of IL-12 responsiveness, determined at the level of expression of IL-12Rβ2. IL-4, unopposed by IFN-γ (or IFN-α in human cells) directs loss of IL-12Rβ2 expression and thus drives to Th-2 phenotype. In contrast IFN-γ (IFN-α in humans) directs retention of IL-12Rβ2, and thus in the presence of IL-12, Th-1 development. More recently, the role of IL-1 family members induced in innate immune responses has been shown. In particular, IL-18 synergises with IL-12 in Th-1 phenotype expression, whereas Th-2 cells are responsive to IL-1α. Indeed, IL-18R has been suggested as a phenotypic marker of Th-1 cells, whereas another IL-1R family member ST2/T1 appears restricted to Th-2 cells. The ligand for ST2/T1 remains to be defined.

Studies of transcriptional regulation of cytokine expression suggest a number of factors important in determining murine IL-4 production, including GATA3, c-maf, NIP-45 and NFAT. Indeed overexpression of GATA3 was suggested to favour Th-2 development. However, other investigators suggest that GATA3, together with NFκB, may be more important in control of IL-5 expression, and retroviral gene transfer to Th-1 cells down regulated IFN-γ production, suggesting that effects on IL-4 may be indirect. It will be important to define whether similar mechanisms apply in regulating Th-2 phenotype expression in humans, although increased GATA3 expressing cells were seen in bronchial biopsies from asthmatic subjects. Recent data suggest that IL-4, and IL-2, expression is regulated in a mono-allelic fashion. Thus, only one of the two IL-4 alleles is expressed in developing Th-2 cells. In addition, further experiments are elucidating the epigenetic modification including histone de-acetylation and DNA methylation involved in directing gene expression, together with the role of cell cycling and passage of such epigenetic imprinting to determine, for example, IL-4 cells in progeny. Understanding the molecular regulation of human Th-2 cytokine expression may help in understanding the complex genetics of asthma and atopy, and provide opportunities for regulating cytokine production in disease.

Although much work has been done on murine Th-1 and Th-2 development, and some of this has been extended to human T cells, there is still a need for information on the factors driving Th-2 responses during initiation of allergic disease and in maintaining the Th-2 phenotype in human allergen-specific memory T cells.

Recruitment of Th-1 and Th-2 cells

With the explosion of information on chemokines it has become clear
that Th-1 and Th-2 cells can express different chemokine receptors and respond to different chemokines. It is also clear that chemokine receptor expression by T cells varies with activation status and cytokine environment, so that the picture in vivo may be different from that seen in isolated T cell clones. Current evidence suggests that human polarised Th-1 cells express CXCR3 and are more responsive to its ligand IP-10, whereas Th-2 lines express CCR3, CCR4 and CCR8 and respond to eotaxin, TARC and I-309. Th-2 clones were reported to have reduced expression of CCR5. It will be interest to determine the chemokine receptor profile and responsiveness of allergen-specific T-cells in vivo, and to determine whether different chemokines are involved in recruitment, retention and activation of Th-2 cells at sites of allergic inflammation.

Differential responses of Th-1 and Th-2 cells to p- and e-selectin have been reported, and whether different adhesion pathways act in selection of Th-1 or Th-2 responses in humans remains to be established.

Conclusions and potential for regulating Th-2 responses

There is now considerable evidence that Th-2 type T-cell responses play a role in human atopic allergic disease. The factors that drive such a response in initiating the allergic diathesis will be important, and may act even in utero. The relative role of continued 'new' Th-2 T-cells and memory responses in perpetuating human allergic disease and control of these processes will be important. The role of individual cytokines of the Th-2 ‘family’ will become clearer with the use of blocking antibodies in human studies. Although initial understanding of the reciprocal regulation of Th-1 and Th-2 cells and data from immunotherapy studies were interpreted to suggest that inducing a Th-1 response might be beneficial in control of allergic inflammation, recent mouse studies suggest otherwise, and an allergen-specific Th-1 response might also induce pathology. Of more interest is the possibility of inducing T-cell unresponsiveness, as had been described for both conventional allergen immunotherapy and peptide therapy. In particular, the description of regulatory T-cells, such as the murine Th-3, or murine and human Tr-1, that inhibit through cytokines such as IL-10 and TGF-β, is of interest. Further understanding of normal immunological regulation of Th-1 and Th-2 responses may hold the key to targeted manipulation of pathological Th-2 responses in atopic allergic disease.

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