T helper type-2 cytokine responses: potential therapeutic targets

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T helper (Th2) cell-mediated immune responses are associated with parasitic helminth infections and atopic disorders. The production of interleukin (IL)-4, IL-5, IL-9 and/or IL-13 by Th2 cells mediates a range of responses that can be protective or pathogenic. Progress has recently been made in elucidating the mechanism of Th2 immunity, which has therapeutic potential for the treatment of allergic diseases.

Introduction

Since Mossman, Cherwinski, Bond, Giedlin and Coffman [1] described selective cytokine production by distinct CD4$^+$ T helper (Th) cell sub-populations, the division of cells by their Th1 versus Th2 cytokine profile has become a prevailing paradigm in immunology. This functional dichotomy of T cells has led to the categorisation of many human diseases according to their association (protective or pathogenic) with preferential elevations in either Th1 or Th2 cytokines. Consequently, in order to ameliorate such Th1- or Th2-mediated diseases, various therapeutic strategies that selectively modify Th responses are being investigated. Several recent advances in the elucidation of regulatory mechanisms that induce Th cell responses, as well as characterisation of distinct molecules that are important in Th1 and Th2 regulation, have led to the identification of several novel therapeutic targets. In this review, we examine recent developments in the manipulation of Th2 responses that might have therapeutic potential, specifically in regard to the treatment of allergic diseases.

What is a Th2 cell response?

The development of an immune response involves an orchestrated interaction between various cell lineages. Exogenous antigens are processed by professional antigen-presenting cells, including dendritic cells (DCs), before presentation to T cells. When CD4$^+$ T cells are exposed to dual stimulation of major histocompatibility complex class II–peptide complexes on DCs and an appropriate co-stimulation, naive T helper (Th0) cells are activated and can then, depending on various intra- and extracellular stimuli, differentiate into either a Th1 or a Th2 cell (Figure 1). Although tightly regulated, the early differentiation of an uncommitted naïve cell into a distinct polarised Th1 or Th2 effector cell lineage has some plasticity [2*]. Stetson, Mohrs, Mallet-Designe, Teyton and Locksley [3*] have shown that, following experimental infection of mice with the pathogen Leishmania major, naïve T cells in the draining lymph nodes are able to initially produce interleukin (IL)-4; this is independent of the ultimate differentiation into either a Th1 or Th2 polarised cell response. Additionally, although Th2 responses are characterised by adaptive T and B cellular immune responses, a novel role for Th2 cells in innate eosinophil-mediated immunity was recently demonstrated [4**].

Th1 cells are characterised by the production of interferon (IFN)-γ, and are associated with cell-mediated responses, particularly resistance to intracellular pathogens. In contrast, Th2 cells secrete IL-4, IL-5, IL-9 and IL-13. Th2 responses are involved in humoral (B cell) responses and IgE production, as well as tissue fibrosis, mastocytosis and eosinophilia. Th2 responses are important in the resistance to infection with helminth parasites (i.e. worm infections). Although both Th responses are protective against certain infectious pathogens, they can themselves be pathogenic: Th1 cell responses can mediate autoimmune diseases, whereas dysregulation of Th2 responses is implicated in various atopic diseases. In recent years, the initial functional division of CD4$^+$ Th cells has been expanded to encompass a range of other cell lineages that selectively produce characteristic type 1 (IFN-γ and IL-12) or type 2 (IL-4, IL-5, IL-9 and IL-13) cytokines. Immune responses are also controlled by distinct and specialised populations of T cells, including regulatory T cells, Th3 cells and CD4$^+/CD25^+$ cells [5]. These cells regulate immune function through several mechanisms, such as the production of the cytokines IL-10 and transforming growth factor-β or via cell contact. Regulatory cells are increasingly being
implicated in the suppression of a range of diseases, including Th2-mediated pathologies.

Factors that affect the type and intensity of the Th1 or Th2 response induced are complex and multifactorial (Figure 1). Contributing factors include the type of infectious pathogen, dose of pathogen-derived antigen(s), duration and site of exposure, cell-type responsible for processing and presenting the antigen, and genetic factors. For example, antigens that induce a Th1 response when administered systemically are predisposed to stimulate Th2 responses when encountered in the lung [6]. A current area of intense investigation is the ability of pathogens to selectively prime DCs to induce naïve T cells to become Th1 or Th2 cells [7**]. Expression of Toll-like receptor (TLR)4 and TLR9 on DCs has been shown to be associated with selective stimulation of Th1 or Th2 responses, respectively [8,9,10*]. The area of pathogen recognition through TLR expression on cells is addressed elsewhere in this issue (see review by O’Neill, this issue).

Recently, two additional Th2 cytokines, IL-21 and IL-25, have been described. IL-25 is an example of the expanding role of bioinformatics in functional biology as, in contrast to the discovery of other Th2 cytokines by empirical cell studies, IL-25 was identified by BLAST searches for homologues of IL-17 family members [11]. Although IL-25 has significant sequence homology to IL-17, it is functionally very different and is potentially an important regulator of Th2 responses, as it can induce the production of IL-4, IL-5 and IL-13 [11,12]. IL-21 is also produced by Th2 cells, and may contribute to the expansion of a Th2 cell response by downregulating Th1 cell development [13*]. The completion of the mapping of the human genome raises further possibilities for discovering other Th2 cytokines or regulators.

**Th2 diseases**

Although Th2 cytokine responses are associated with helminth parasitic diseases, the most widespread Th2-mediated diseases in developed societies are the atopic disorders. Atopic disease symptoms range from the inconvenient to the fatal and include allergic rhinitis, atopic dermatitis, food allergy, asthma and anaphylaxis, all of which are characterised by an overproduction of Th2 cytokines that contribute to elevated levels of IgE and eosinophilia. In asthma, elevation of Th2 cytokines causes pulmonary pathology that includes airway inflammation, eosinophil infiltration, increased mucus production and airway hyperactivity, which induces the symptoms of the disease [14]. The causal association of atopy with urbanisation is intrinsic to the renewed interest in the hygiene hypothesis [7**]. The hygiene hypothesis proposes that exposure to infections, particularly bacterial, in early life provides protection against subsequent development of asthma and atopy, in part by programming cells to produce a more Th1-biased or regulatory phenotype upon stimulation. Although evidence for or against the hypothesis differ, a recent study of more than 20 000 subjects showed no inverse correlation between autoimmunity (Th1-mediated) and allergic (Th2-mediated) disease [15]. Evidently, the hypothesis needs further testing.

In addition to the causal relationship between elevations in Th2 cytokines levels and asthma, there is also complementary data supporting genetic associations. The Th2 cluster on human chromosome 5q23–35, containing loci for the Th2 cytokines IL-4, IL-5, IL-9, IL-13 and other potentially relevant genes, has been shown by various studies to have a significant linkage with total IgE levels (for one example, see [16]). A more recent study found an association between asthma severity and polymorphisms of another genetic marker in the 5q23–33 region, IL-12B, which encodes the functional p40 subunit of IL-12 [17]. IL-12p40 protein and mRNA were reduced in severe asthma [17]. As IL-12 can suppress Th2 responses (Figure 1), a lack of IL-12p40 may impair the capacity of asthmatics to reduce Th2 responses. As discussed above, TLR4 and TLR9 have been differentially associated with a regulatory role in inducing Th2 responses. Genetic analysis has shown no association between TLR4 and asthma susceptibility [18], whereas polymorphisms in TLR9 might be associated with asthma in certain populations [19].
Potential Th2 therapeutic targets

As outlined in Figure 2, there are several intra- and extracellular molecules associated with Th2 cells that are potential therapeutic targets. Although extracellular targets are often more accessible to intervention, the difficulties associated with developing successful intracellular therapies are balanced with the critical role of intracellular signalling in regulating Th2 disease onset and severity. Here, selected examples of extracellular and intracellular molecules important to Th2 responses are reviewed.

Surface or extracellular targets

Cytokines

Arguably the most direct and simple approach to reduce Th2 responses is to remove or neutralise circulating Th2 cytokines. Here, we focus solely on IL-5 as an example of an individual cytokine that is being actively investigated as a potential therapeutic target in the treatment of Th2-mediated allergic diseases. IL-5 is a central mediator of the maturation of eosinophils in bone marrow, and also initiates activation, recruitment and survival of eosinophils. As asthmatics have elevated IL-5 protein levels in...
serum and marked eosinophil infiltration in bronchial biopsies, removing IL-5 is a proposed therapy for asthma. In support of a role for IL-5 in asthma, various experimental studies have shown a reduction in pulmonary inflammation, including eosinophilia, in IL-5-deficient mice or animals treated with neutralising anti-IL-5 monoclonal antibodies [20,21]. Furthermore, a recent paper has shown that transfer of eosinophils into allergen-primed mice induces airway damage, re-emphasising the importance of eosinophils in pulmonary inflammation [22]. However, despite cumulative evidence for a therapeutic role for IL-5 depletion in asthmatics, clinical administration of a humanised monoclonal antibody against IL-5 to treat asthma produced unexpected results. Leckie et al. [23] showed that, despite anti-IL-5 therapy depleting eosinophils in the blood and sputum of mild asthmatics, there was no effect on airway hyperresponsiveness. A more recent study confirmed that anti-IL-5 treatment did not significantly alter clinical parameters in mild asthmatics, which the authors attributed to the therapy merely reducing rather than eliminating eosinophils in the patients [24*]. Several reasons could explain the limited success achieved using anti-IL-5 therapy [25]. These include, firstly, differential expression of the IL-5 receptor on eosinophils in the periphery versus airways; secondly, direct effects of IL-5 on the airways [26]; thirdly, direct irreversible airway damage mediated by products from eosinophils; and finally, the role of compensatory processes. For example, the chemokine eotaxin is involved in eosinophil recruitment [27] and, in the absence of its receptor (CCR3), airway inflammation is altered [28,29].

The above example of IL-5 highlights the difficulties in preventing or altering a defined biological phenotype by depleting a single Th2 cytokine. In the past 12 months, various studies have further illustrated redundancy and overlap in function between the four major Th2 cytokines. IL-4, IL-5, IL-9 and IL-13 knockout mice (single, double, triple and quadruple knockouts) were generated and analysed for defined Th2 functional in vivo responses [30*]. Despite a surprising degree of plasticity in the capacity of deficient mice to generate Th2 responses in the absence of the various genes, it was shown that IL-4 is the most critical cytokine for Th2 responses [30*]. In the context of Th2-induced airway inflammation, similar redundancy in Th2 cytokines has been observed [31]. Thus, a therapy based on modifying a distinct pathological phenotype by neutralising an individual Th2 cytokine is possibly over-simplistic. Indeed, in support of this premise, blocking only IL-4 [32] or IL-5 (described above) in human clinical trials has had relatively limited therapeutic efficacy.

Cytokine receptors

Th2 cells, together with other cells, express receptors for IL-4, IL-5, IL-9 and IL-13 (Figure 2). As discussed above, the inability to reduce eosinophilia by neutralising IL-5 has been attributed to various factors, including differential expression of the IL-5 receptor. Currently, there is particular interest in antagonism of IL-4 and/or IL-13 receptors. Although IL-4 and IL-13 have distinct receptor chains (IL-4Rα, IL-13Rα1 and IL-13Rα2), they overlap in receptor usage (for a general commentary, see [33]). IL-4 can bind specifically to the type 1 receptor (comprising the IL-4Rα and common γ chain, IL-4Rγc), whereas both IL-4 and IL-13 can bind to the type II receptor (comprising IL-4Rα and IL-13Rα1 chains). IL-13Rα2 is specific for IL-13 and is found not only on the surface of cells but also extracellularly in a soluble form. Therapeutically, soluble IL-4R has been shown to alter IgE production in asthmatics [32]. In addition, recombinant soluble IL-13Rα2 has been used experimentally to ameliorate Th2 responses [34]. Significantly, the generation of mice deficient in IL-13Rα2 has shown that the receptor might act as a decoy in vivo to control the magnitude of Th2 responses [35,36]. Data showing that IL-13Rα2 regulates IL-13-induced fibrosis [35] extended findings from earlier studies [37,38], and raises the possibility of using IL-13Rα2 therapeutically as a decoy receptor to treat fibrotic diseases.

There are potential problems with the clinical use of recombinant monomeric soluble receptors to block cytokines. Such receptor constructs often have a short in vivo half-life and, as described above, cytokine receptors can consist of two heterogeneous chains. The use of ‘cytokine traps’ is a novel strategy that addresses these two shortcomings [39*]. Cytokine traps are soluble complexes consisting of extracellular domains of two different cytokine receptors that are fused to the constant region of human IgG1 (Fc). The use of two distinct receptor chains increases the cytokine binding affinity of the receptor complex, while the Fc portion extends the in vivo half-life, a strategy demonstrated in the previous example for IL-13Rα2 [34]. An IL-4 type I receptor cytokine trap (IL-4Rα and IL-4Rγc) was shown to bind IL-4 three times more potently in vitro than monomeric soluble IL-4Rα or IL-4Rα-Fc; the trap also blocked IL-4-mediated Th2 responses in mice in vivo [39*]. It will be of interest to see whether cytokine trap technology is ultimately used in the clinic.

There are several other Th2 cell surface markers that are of therapeutic interest (e.g. T1/ST2, inducible co-stimulator and cytotoxic T-lymphocyte-associated antigen-4) that are not discussed in this review. Chemokines and chemokine receptors can also affect Th1/Th2 polarisation by modifying cytokine production, as well as altering cell recruitment (see review by Lloyd and Rankin, this issue).

Intracellular targets

Transcription factor activation is involved in the initiation of both Th1 and Th2 responses. Several key intracellular targets are shown in Figure 2. Some transcription factors are Th2-specific, whereas others such as GATA3, although crucial for Th2 development and function, are also involved in different processes [2*]. Although there is a
hierarchical activation cascade during Th2 differentiation, knockout studies indicate that transcription factors are often functional despite the removal of upstream signals.

Signal transducer and activator of transcription-6 (STAT-6), a transcription factor induced by IL-4 and IL-13, plays a central role in Th2 cell differentiation. In the absence of STAT-6, there is a marked block in Th2 cell development [40]. In addition, epithelial cells that are devoid of STAT-6 are not affected by IL-13-induced airway hyperresponsiveness, and have reduced mucus production [41]. Following STAT-6 activation, levels of the transcription factor GATA-3 increase as the cell develops into a Th2 cell.

Another potential target is the family of intracellular proteins that regulates transcription factors, known as suppressors of cytokine signalling (SOCS). SOCS1 and SOCS3 strongly correlate with Th1 and Th2 expression, respectively [42]. Overexpression of SOCS3 in naïve T cells suggests that the default response is Th2, with SOCS3 being downregulated by the Th1-polarising cytokine IL-12. Additionally, overexpression of SOCS5, which is associated with Th1 cells, is able to affect Th2 development by inhibiting STAT-6 activation, through binding to the intracellular region of IL-4R [43]. Nuclear factors of activated T cells (NFATs) are important in Th2 cell development. Naïve T cells from mice deficient in both NFATc2 and NFATc3 are biased to differentiate into Th2 cells without IL-4 stimulation or the requirement for CD28 co-stimulation [44]. Also, interferon regulatory factor-4 synergises with NFATc2 and the IL-4-inducing transcription factor c-maf to augment IL-4 promoter activity, as well as to elicit significant levels of endogenous IL-4 production [45].

Altering transcription of gene enhancer sites and elements involved in chromatin remodelling could provide an alternative to targeting proteins. Dnase-1 hypersensitivity regions are important regulators of mRNA production that are found in conserved non-coding sequences (CNSs) between IL-4 and IL-13 within the Th2 gene cluster. Deletion of CNS-1 abrogates IL-4, IL-5 and IL-13 cytokine production, although mast cells can still produce IL-4 [46*]. Solymar, Agarwal, Bassing, Alt and Rao [47] have recently shown that deletion of CNS-2 also abrogates IL-4 induction. The transcription of cytokines in Th2-differentiated cells is partly controlled by CpG demethylation, which occurs around these Dnase-1 hypersensitivity sites and coincides with the appearance of consensus binding sites for GATA-3 [48]. In the absence of the methyl CpG binding domain protein-2, which is thought to be involved in gene silencing by bypassing transcription factors, Th cells have altered differentiation, making GATA-3 dispensable for IL-4 induction [49]. However, strategies targeting T cell-specific transcription factors might not be sufficient, as IL-4 can be produced through an indirect (non-T cell) pathway by mast cells [50].

**Is inducing a Th1 response the answer?**

One postulate to ameliorate Th2 disease is to exploit the reciprocal regulation of Th1 and Th2 responses (Figure 1) by selectively increasing Th1 cytokines, thereby reducing Th2 cytokine production. One such approach involves exploiting the propensity of pathogens to selectively induce polarised Th responses. A recent example of this strategy involved the vaccination of mice with heat-killed *Mycobacterium vaccae*, a potent stimulator of Th1 responses, which profoundly affected aspects of allergic disease [51**,52**,53**]. The therapeutic effects of *M. vaccae* on airway hypersensitivity, as well as eosinophilia, were not only due to Th1 biasing but also to the induction of a regulatory cell population. The ability to generate regulatory cells that can specifically impair Th2 cell responses might have important therapeutic potential. It is highly pertinent that a lysophosphatidylserine from *Schistosoma mansoni*, a potent Th2-stimulating worm infection, has been shown to modulate DCs, through TLR2, to induce IL-10-producing regulatory T cells [54**].

**Th2 gene silencing**

The recent use of gene silencing by RNA interference (RNAi) might offer a new approach to treat Th2 diseases via selective ablation of Th2 genes. Previous antisense strategies have indicated the therapeutic possibilities of Th2 gene therapy. Treatment with GATA-3 antisense oligonucleotides reduces IL-4 production by Th2 cells, as well as impairing airway reactivity in mice [55]. Although there is mounting evidence of the successful use of RNAi in various systems, it is only recently that the therapeutic potential of RNAi has been demonstrated in a murine model [56*]. Following injection of small interfering RNA targeting the Fas gene, mice had lower hepatocyte Fas protein and mRNA for 10 days, and were protected from two different forms of autoimmune liver damage [56*]. RNAi will be an interesting approach to impair individual or multiple genes involved in Th2 responses described in this review. However, in regard to development of RNAi-based therapies, questions remain about the stability and specificity of gene silencing in *vivo*.

**Conclusions**

New therapeutic strategies to control Th2-mediated responses are required to address the increasing incidence of atopic disease in modern societies. Studies on functional redundancy and compensation in Th2 responses indicate that it is unlikely that a single strategy will be effective as a broad-spectrum therapy for Th2-mediated disease. The recent progress in our understanding of the mechanism of initiation and control of Th2 cell responses will eventually lead to new therapeutic strategies. However, the reactivation of latent tuberculosis in patients with Crohn’s disease or rheumatoid arthritis that were treated with anti-tumour necrosis factor-α raises a note of caution regarding potential unexpected side effects of any anti-cytokine therapy [57]. Therefore, with respect to the
future use of therapies to ameliorate Th2 responses, it is interesting to ponder whether curing the modern Th2 foe, asthma, could leave us more susceptible to the old Th2 enemy, worms?

Acknowledgements

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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


An informative and comprehensive review of Th1 (IFN-γ) and Th2 (IL-4) cell commitment.


Using tetramers to an immunodominant Leishmania major antigen (LACK) and bicistronic IL-4 reporter mice, the authors tracked the early kinetics of increases in LACK-specific Th2 cells during an in vivo immune response. LACK-/-/IL-4-/- cell frequency rose 124-fold in four days from a basal level of 15 cells in a naive lymph node.


This paper shows a unique role for Th2 cells in regulation of innate immunity in the control of eosinophil function.


The authors use molecules from various pathogens to selectively stimulate DCs to induce Th1 or Th2 cell responses.


The authors show that IL-21 is produced by Th2 cells. IL-21 regulated the development of IFN-γ-producing Th1 cells, and thereby may amplify a Th2 response.


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