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Emerging Roles of T Helper Subsets in the Pathogenesis of Asthma

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Abstract

The cardinal features of asthma include pulmonary inflammation and airway hyperresponsiveness (AHR). Classically, asthma, specifically allergic asthma, has been attributed to a hyperactive Th2 cell immune response. However, the Th2 cell-mediated inflammation model has failed to adequately explain many of the clinical and molecular aspects of asthma. In addition, the outcomes of Th2-targeted therapeutic trials have been disappointing. Thus, asthma is now believed to be a complex and heterogeneous disorder, with several molecular mechanisms underlying the airway inflammation and AHR that is associated with asthma. The original classification of Th1 and Th2 pathways has recently been expanded to include additional effector Th cell subsets. These include Th17, Th9 and Treg cells. Emerging data highlight the involvement of these new Th cell subsets in the initiation and augmentation of airway inflammation and asthmatic responses. We now review the roles of these recently classified effector Th cell subsets in asthmatic inflammation and the insights they may provide in addition to the traditional Th2 paradigm. The hope is that a clearer understanding of the inflammatory pathways involved and the mediators of inflammation will yield better targeted therapeutics.

Keywords

Asthma; Th17; Th2; Th1; Th9; Animal models

INTRODUCTION

Bronchial asthma is a respiratory disease that affects nearly one in ten individuals in the developed world (Fanta, 2009; Umetsu et al., 2002). Indeed, due to the persistent rise in the incidence and prevalence of this disease throughout the past 2–3 decades, it is now estimated that asthma has reached epidemic proportions. In fact, in industrialized nations, the most common chronic disease of childhood is asthma (van de Kant et al., 2009). Although current therapies are effective in suppressing disease symptoms, to date, there are no preventive treatments or cures for asthma. A better understanding of general pulmonary immunity and specifically, the initiators of inflammation associated with asthma, is therefore needed for development of new therapeutics.

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Inflammation in Asthma

The cardinal features of asthma include airway inflammation and airway hyperreactivity (AHR) which results in loss of pulmonary function. Symptoms of the disease comprise wheezing, breathlessness, chest tightness, and coughing due to reversible airway obstruction. Initially, studies indicated that asthma is an atopic disorder of the airways which involves activated T helper lymphocytes, specifically CD4⁺ Th2 cells. Analysis of bronchial biopsies and bronchoalveolar lavage (BAL) fluids from asthmatic patients showed an infiltration of Th2 lymphocytes, eosinophils and degranulated mast cells (Azzawi et al., 1990; Robinson et al., 1993; Robinson et al., 1992). These results framed research efforts for many years and suggested that, following exposure to allergen, activated Th2 cells and their cytokines, namely interleukin-4 (IL-4), IL-5, IL-9 and IL-13, orchestrate eosinophilic airway inflammation as well as stimulate B-cells to generate allergen-specific IgE antibody, and that these events ultimately lead to the release of preformed or newly synthesized inflammatory mediators from mast cells (Cohn et al., 2004; Wills-Karp, 1999).

However, recent studies have suggested that eosinophilic airway inflammation actually occurs in less than 50% of asthmatic cases (Douwes et al., 2002; Simpson et al., 2006). The most common form of asthma is allergic asthma (Barrios et al., 2006; Masoli et al., 2004), yet multiple and distinct forms of asthma exist. These other forms can be induced by exercise, air pollution, aspirin, or viral infection (Matangkasombut et al., 2009). Moreover, the type and intensity of inflammatory cellular infiltrates as well as the site of infiltration can vary depending upon the degree of disease severity and the form of asthma (Vignola et al., 1998). In addition, as many as 12 different types of inflammatory cells and more than 100 inflammatory mediators have been associated with the pulmonary inflammation that occurs in asthma (Anderson, 2008; Kiley et al., 2007). Thus, asthma is clearly a complex and heterogeneous disease. In order to fully understand the process of disease pathogenesis, it will be essential to identify the factors that initiate, intensify, and mediate the immune response that results in airway inflammation and hyperreactivity. Although the presence of Th2 cells and eosinophils can explain many features of asthma, it is becoming increasingly obvious that this paradigm may be too simplistic.

New T Helper Cell Subsets

In recent years, as the complexity of asthma has become better appreciated, newly emerging CD4⁺ Th cell subsets have been linked to general disease pathogenesis. It is evident that Th cell functions are considerably more complex and heterogeneous than originally thought. The original characterization of the Th1 and Th2 pathways has now been expanded to include additional Th cell subsets, each with their own cytokine repertoire and transcription factors.

These include regulatory T cells (Treg) (Robinson, 2009), Th17 cells (Louten et al., 2009) and most recently, Th cells that produce IL-9 (Th9 cells) (Soroosh and Doherty, 2009). The discovery of new Th cell subsets has motivated investigators to revisit and reinterpret existing models of autoimmunity and allergy, including asthma. Furthermore, T cell lineage commitment has recently been shown to be less rigid than previously appreciated (Zhou et al., 2009). Epigenetic modification of cytokine genes and key transcriptional regulator genes direct T cell differentiation and appear to allow T cells to adapt to specific effector profiles. In this review, we discuss the classic Th2 inflammation paradigm, its role in lung mucosal inflammation and the apparent limitations of this model, which underlie discrepancies observed in human asthma. We also discuss the role of Th1, Th9, Treg and Th17 cells in lung mucosal inflammation and asthma, and their potential role in resolving these discrepancies. Ultimately, insights into the role of Th cell subsets in asthma pathogenesis will allow design of new targets for therapy.

The Th2 Cell Paradigm

It is widely believed that Th2 cells initiate and perpetuate asthma. At the onset of detailed studies, CD4⁺ Th2 cells were found to be present in the lungs of asthmatic patients, particularly those with allergic asthma, as indicated by the fact that cells obtained from BAL fluids of asthmatic patients contain more IL-4 and IL-5 mRNA than do cells from BAL fluids of non-asthmatic patients or patients who have other lung diseases such as pneumonia or sarcoidosis (Robinson et al., 1992; Ying et al., 1997). Additionally, IL-4 and IL-5 expression in asthmatic patients is predominantly T cell-derived (Walker et al., 1994).

The specific contribution of Th2 lymphocytes has also been documented in classical allergen challenge studies. In these studies, sensitized asthmatics were exposed to aerosolized allergen and found to subsequently exhibit airflow obstruction, an influx of eosinophils and T lymphocytes, and an increase in Th2 cytokines in BAL and bronchial mucosa (Barrett and Austen, 2009). Furthermore, the level of T cell infiltrate observed in the BAL correlates with the severity of asthma (Kay, 1997). These results indicate an undoubtedly vital role for Th2 cells in the development for some forms of human asthma.

Mouse models of antigen-induced airway inflammation have also reinforced a central role for Th2 lymphocytes and their cytokines in asthma. Using an experimental ovalbumin (OVA) asthma model, mice receiving OVA-specific, DO11.10 TCR-transgenic Th2 cells, but not Th1 cells, developed hallmark traits of asthma, such as eosinophilic airway inflammation and AHR, following OVA challenge (Cohn et al., 1997; Cohn et al., 1998). Moreover, development of allergic lung inflammation could be prevented in OVA-sensitized wild-type (WT) mice by depletion of CD4⁺ T cells prior to allergen challenge (Gavett et al., 1994).

The specific roles of individual Th2 cytokines were elucidated through sensitization and airway challenge studies in mice using either transgenic cytokine overexpression, targeted gene deletion, or blocking anti-cytokine antibodies. The results from these studies demonstrated a central role for IL-4 in the generation of Th2 cells and B-cell IgE production; for IL-5 in the promotion of eosinophilic airway inflammation and damage; for IL-9 in the recruitment, proliferation, and activation of mast cells; and for IL-13 in the induction of AHR, goblet cell hyperplasia, mucus secretion, and fibrosis (Fig. 1) (Burrows et al., 1989; Holgate, 2008; Kay, 2006; Larche et al., 2003). As a result of activation of allergen-specific Th2 cells, allergen-specific IgE is produced and binds to IgE receptors on the surface of mast cells, which, after crosslinking by specific allergen, induces the activation of these cells. Mast cell activation then results in release of preformed mediators, such as histamine and leukotrienes, which directly affect airway smooth muscle and mucous glands, ultimately causing AHR (Galli, 1997; Lane and Lee, 1996; Rossi and Olivieri, 1997). Overall, these studies have led to the dominant concept that activated Th2 cells orchestrate pulmonary immune responses and mediate the lung inflammation and AHR that is seen in asthmatic patients.

LIMITATIONS OF THE TH2 PARADIGM

Despite clarifying many features of asthma, some clinical and molecular features that have been documented in human asthma are not adequately explained by the Th2 paradigm. Even as the Th2 inflammation model was being advanced, concerns were raised about whether it would actually lead to improved treatments for asthma (Anderson and Coyle, 1994; Coyle et al., 1995). First, the Th2 paradigm does not explain non-allergic asthma. Non-allergic asthma can be induced by exercise, air pollution, aspirin, or viral infection. In fact, viral infections are known to exacerbate asthma (Barrett and Austen, 2009). In addition, in the absence of an allergen, granulocytic cellular infiltration, including infiltration of

degranulated mast cells, can still occur and result in airway hyperreactivity, emphasizing the fact that a specific allergen is dispensable for non-allergic pulmonary inflammation.

Second, the Th2 paradigm predicts that eosinophilic infiltration and subsequent inflammation should provoke AHR, but no clear cause and effect exists (Alvarez et al., 2000a; Alvarez et al., 2000b). Studies have shown that atopy associated with eosinophilic activation, and AHR are not complementary (Woolcock and Peat, 2000). Th2 immunity is fundamental to atopy, and atopy is a risk factor for asthma, but atopy alone is a poor predictor of disease. In fact, most patients with allergic rhinitis and allergen sensitization do not exhibit AHR and therefore are not asthmatic (Corren, 1997). These results suggest that the presence of Th2 cells and allergen-specific IgE is not sufficient for development of asthma. Third, bronchial biopsies from asthmatic patients commonly have evidence of neutrophilic inflammation.

Neutrophils are frequently present in the lungs of asthmatics, particularly in patients with severe disease, or with corticosteroid resistant asthma (Green et al., 2002; Simpson et al., 2007; Wenzel et al., 1999). Fourth, therapeutics targeted to Th2 cells and their cytokines, which are often effective in Th2 animal disease models, have been found to have minimal effectiveness in the clinical setting. For instance, administration of antibodies that prevent binding of IgE to Fc RI has had limited therapeutic efficacy, as has inoculation of neutralizing anti-IL-5 antibodies. These treatments typically reduce eosinophilic inflammation but often do not affect AHR or the late asthmatic response.

Additionally, interventions such as anti-IL-4 antibodies or IL-4/IL-13 antagonists have weak effects and have also not reduced AHR in clinical asthma trials (Bryan et al., 2000; Holgate and Polosa, 2008; Leckie et al., 2000; Wenzel et al., 2007). These results suggest that immunological factors in addition to Th2 cells regulate asthma. Finally, asthmatic patients exhibit elevated levels of non-Th2 cytokines and factors, such as interferon (IFN)- γ (Cho et al., 2005; Nakao et al., 2001) and IL-17 (Bullens et al., 2006; Oboki et al., 2008). Taken together, these observations suggest that regulatory pathways in addition to Th2 cells and eosinophils may contribute to the development of the asthma phenotype. In spite of these contradictions, Th2 immunity has been shown to be clinically relevant specifically for childhood asthma with atopy as well as mild allergic adult asthma. Indeed, in these forms of asthma, Th2 directed therapies have shown to be effective (Wenzel et al., 2007). However, it is clear that asthma is complex and that Th2 immunity does not explain all forms of the disorder.

Activity of Th1 Cells

From its inception, the Th1/Th2 model has postulated that Th1 cells could have a beneficial effect on asthma by dampening the activity of Th2 cells. Indeed, Th1 cells have been shown to inhibit development and proliferation of Th2 cells (Abbas et al., 1996). IFN- γ , the hallmark cytokine produced by Th1 cells, has also been shown to abrogate IgE production and eosinophilia (Coffman et al., 1988; Iwamoto et al., 1996), and in animal models, exogenous administration of IFN- γ can result in suppression of allergic airway inflammation (Holgate and Polosa, 2008). Nevertheless, studies involving subcutaneous administration of recombinant human IFN- γ showed no improvement in asthmatic patients compared with controls (Boguniewicz et al., 1995). In fact, in human asthma, IFN- γ production is actually upregulated and appears to contribute to disease pathogenesis (Cho et al., 2005; Nakao et al., 2001).

For instance, in severe asthmatic patients, serum levels of IFN- γ increase during an asthmatic attack (Corrigan and Kay, 1990). Increases in IFN- γ have also been found in BAL cell cultures obtained from asthmatic patients, whether incubated alone or in the presence of

allergen (Cembrzynska-Nowak et al., 1993). In an attempt to counterbalance Th2 cell-induced AHR, allergen-specific Th1 cells were adoptively transferred into naïve mice and although these cells demonstrated an ability to migrate to the lungs, they were found to intensify severe airway inflammation and production of IFN- γ , which surprisingly, appeared to contribute to the activation of eosinophils (Hansen et al., 1999). It has recently been shown that the production of IFN- γ together with an established Th2-cell response, results in increased inflammation, possibly by damaging the epithelial cell barrier (Reisinger et al., 2005). Together these observations suggest that the role of Th1 cells in asthma is more complex than predicted. Indeed, Th1 and Th2 cells may not simply serve to counterbalance each other in a dichotomous manner, but rather may act together in a harmful manner in asthmatic individuals.

Th9, a New Subset of T Helper Cells

IL-9 has generally been categorized as a Th2 cytokine (Kay, 2006; Larche et al., 2003). Recently, a new Th subset that preferentially produces IL-9 and that appears to be distinct from Th2 cells, has been reported to provide a unique contribution to immune responses (Dardalhon et al., 2008; Veldhoen et al., 2008). The IL-9-producing T cell lineage was discovered when it was found that, under certain circumstances, naturally arising CD4⁺CD25⁺ regulatory T cells (nTreg) and inducible regulatory T cells (iTreg) generated in the presence of TGF- β produced more IL-9 upon activation than Th2 cells (Liu et al., 2006; Lu et al., 2006). However, IL-9 production was found to be absent from Foxp3⁺ nTregs isolated from the thymus or Foxp3⁺ iTregs generated from naïve T cells (Veldhoen et al., 2008). However, committed Th2 cells cultured in the presence of TGF- β and IL-4, discontinue expressing the Th2 transcription factor, GATA3, as well as the Th2 cytokines, IL-4, IL-5 and IL-13, while initiating transcription of IL-9 (Veldhoen et al., 2008). These results highlight the differential role of TGF- β in T-cell differentiation such that, in the presence of IL-6, Th17 cell differentiation occurs (see below), but in the presence of IL-4, Th9 cells develop. These experiments, which are relevant to chronic diseases, identified a distinct population of IL-9-producing helper T cells which appear to have a role in allergic diseases as well as asthma.

Th9 Cells in Asthma

The role of IL-9 in asthma, as a Th2 cytokine, has long been recognized. Since the elucidation of Th9 cells and their potential inflammatory function, studies have begun revisiting the role of these cells in asthma. As was documented with other Th2 cytokines, IL-9 mRNA-positive cells have been shown to be elevated in bronchial biopsies from asthmatic patients compared to normal patients or patients with chronic bronchitis or sarcoidosis (Shimbara et al., 2000; Ying et al., 2002). IL-9 levels also increase following allergen challenge of asthmatics compared to normal controls (Erpenbeck et al., 2003a). In the airways, the CD3⁺ lymphocyte population was found to be the primary source of IL-9 (Erpenbeck et al., 2003b; Tsicopoulos et al., 2004).

The specific role for IL-9 in asthma pathogenesis has primarily been investigated in murine models. Upon systemic overexpression in IL-9 transgenic mice, there was increased AHR, heightened eosinophilic infiltration and increased levels of IgE production following antigen challenge (McLane et al., 1998). However, overexpression of IL-9 was able to enhance asthmatic inflammation only in the presence of specific antigen since the transgenic mice had no evidence of eosinophilic inflammation in the absence of antigen challenge. In contrast, when IL-9 was selectively overexpressed in the lung, using a clara cell (CC10) promoter, eosinophilic airway inflammation, mast cell accumulation as well as AHR occurred without antigen challenge (Temann et al., 1998). In addition, these transgenic mice demonstrated increased mucus production and fibrosis, features of airway remodeling

associated with chronic asthma. The role of IL-9 in asthma has also been evaluated using IL-9 deficient mice. Using the OVA-induced asthma model, one group found no changes in AHR, cellular infiltration, mucus production or peribronchial inflammation in IL-9 gene deficient mice compared to WT mice (McMillan et al., 2002).

However, another group reported a significant decrease in AHR and eosinophilic infiltration after administration of an IL-9 blocking antibody at the antigen challenge phase (Cheng et al., 2002). Thus, IL-9 may not be required for the initiation of asthma, but it might play a role in sustaining and modulating subsequent airway responses to antigen. Indeed, the presence of IL-9 is strongly associated in both mice and humans with mast cell accumulation in the airways, as well as AHR and goblet cell metaplasia (Longphre et al., 1999). Also, IL-9 can significantly contribute to the recruitment of mast cells, as immature cells, from the bone marrow and their subsequent differentiation *in vivo* (Lu et al., 2006).

Undoubtedly, IL-9 maintains the ability to significantly contribute to asthma, specifically chronic asthma. Analysis of specimens from allergic patients has revealed that T cells can be a major source of IL-9 and mouse models have indicated that IL-9 has multiple effects in the development and maintenance of allergic inflammation and airway remodeling. However, it is unknown whether IL-9-secreting T cells are distinct from Th2 cells or whether Th2 cells can be reprogrammed to become Th9 cells. Overall, it appears that T cells are a significant source of IL-9, which can have multiple effects in the development and, likely more importantly, in the maintenance of airway inflammation.

Treg Cells

Another pivotal subset of CD4⁺ T cells, namely regulatory T cells (Treg cells), has recently been identified as having a distinct role in asthma pathogenesis. Regulatory T cells are characterized by the expression of the transcription factor Foxp3 (Forkhead box p3) and the IL-2 receptor (CD25), and are known to produce the inhibitory cytokines IL-10 and TGF- β (Wing et al., 2006). Treg cells have been strongly associated with suppression of allergic responses in murine models of asthma. For example, adoptive transfer of Treg cells following the onset of allergic airway inflammation, has been shown to downregulate established inflammation and prevent airway remodeling (Kearley et al., 2008).

Further studies demonstrated that the suppressive ability of the adoptively transferred, antigen-specific Treg cells was dependent on IL-10. Not only did the adoptively transferred Treg cells produce IL-10, but these cells also induced IL-10 production from bystander recipient CD4⁺ T cells (Kearley et al., 2005). In agreement with these findings, depletion of Treg cells before allergen sensitization was found to enhance the severity of airway inflammation and AHR (Lewkowich et al., 2005). There is also strong evidence in humans that Treg cells inhibit Th2 cell responses, which suggests that atopy can result from an imbalance between Th2 cells and Treg cells (Fig. 1) (Larche, 2007). Interestingly, there are substantial decreases in the frequencies of allergen-specific IL-10-producing Treg cells, and increases in IL-4-producing T cells, in allergic individuals compared to healthy, nonatopic individuals (Akdis et al., 2004), which further highlights the close interplay between Treg and T effector cells in asthma.

The Role of IL-10

One significant feature of Treg cell function is the role of IL-10. IL-10 has been shown to be an effective immunosuppressive mediator of allergic responses in the lung, as well as other mucosal sites. Targeted deletion of IL-10, specifically in Treg cells, results in development of spontaneous colitis as well as increased AHR and inflammation in mice exposed to inhaled allergen (Grunig et al., 1997; Rubtsov et al., 2008). IL-10 dampens Th2 effector cell

activation and inhibits both early- and late-phase asthmatic responses, including both mast cell activation and eosinophilic inflammation (O'Garra et al., 2008). Furthermore, the administration of exogenous IL-10 can inhibit AHR and alleviate asthma pathology.

It can also inhibit IgE production while promoting IgG4 production, an immunoglobulin isotype generally believed to be protective against allergic responses (Till et al., 2004). To further test the ability of IL-10 to inhibit inflammation, the gene encoding IL-10 was instilled intratracheally into IL-10 deficient mice following allergen challenge and was found to significantly suppress airway inflammation and AHR (Fu et al., 2006). The role of IL-10 in asthma has also been investigated in human allergic and asthmatic patients. There are substantial reductions in IL-10 transcript and protein levels, and increased amounts of proinflammatory cytokines, in the BAL fluids and in alveolar macrophages of asthmatic patients compared to healthy control subjects (John et al., 1998).

Additionally, a polymorphism in the human IL-10 gene promoter, which results in reduced IL-10 expression, has been correlated with severe asthma (Lim et al., 1998). Interestingly, recent evidence suggests that depending on the cytokine milieu, Th2 cells may convert into Th cells that express IL-10 (Dardalhon et al., 2008). Indeed, IL-10-producing Treg cells are key players in the complex immunological mechanisms designed to maintain homeostasis in the lung. Overall, this evidence suggests that IL-10-producing Treg cells are able to suppress inflammatory immune responses while lack of these cells heightens the inflammation seen in asthma.

Th17 cells

The Th1/Th2 paradigm has recently been expanded to include Th17 cells (Steinman, 2007). IL-17-producing T cells were first isolated from human rheumatoid synovial tissue (Aarvak et al., 1999) and found to be induced by microbial lipopeptides (Infante-Duarte et al., 2000). These results ultimately led to the hypothesis that IL-17A production actually designates a distinct subset of CD4⁺ T helper cells that principally functions in inflammatory reactions. Soon afterwards, use of IL-17-deficient mice (Nakae et al., 2002; Nakae et al., 2003) and antibody-mediated IL-17A neutralization (Bush et al., 2002) demonstrated that IL-17-producing T cells mediate pathology in autoimmune models. IL-17 production was found to be promoted *in vitro* by IL-23, which stimulated CD4⁺ Th cells distinct from either the Th1 nor Th2 pathway (Aggarwal et al., 2003).

For instance, mice that lack the IL-12 p40 subunit, which is shared by IL-23, or the p19 subunit that is specific to IL-23, were found to develop resistance to autoimmune diseases due to the absence of IL-17-producing T cells (Cua et al., 2003; Harrington et al., 2005; Murphy et al., 2003; Park et al., 2005). Although IL-23 can enhance IL-17 expression, actual Th17 cell differentiation from uncommitted cell precursors in mice requires IL-6 and TGF- β , both *in vitro* (Veldhoen et al., 2006) and *in vivo* (Bettelli et al., 2006; Mangan et al., 2006). On the other hand, IL-6 plus IL-1 or IL-21 have been found to be critical for Th17 development in humans (Manel et al., 2008). These cytokines, acting via STAT3, induce increased expression of the key transcription factors for Th17 cell differentiation namely, retinoic acid-related orphan receptors (ROR) γ and ROR α (Ivanov et al., 2006). The identification of these specific transcription factors further supports the concept that IL-17-producing cells represent a distinct T helper cell lineage.

The production of IL-17A, IL-17F, IL-22, and, to a lesser extent, tumor necrosis factor (TNF) and IL-6, currently defines the Th17 pathway (Bettelli et al., 2008). Th17 cells are now accepted to represent a third CD4⁺ Th subset, which has led to the resolution of some inconsistencies in the Th1/Th2 paradigm. Additionally, IL-17 has been implicated in asthma development (Hellings et al., 2003). Thus, a full understanding of the effector functions of

Th17 cells during pulmonary inflammation may be key to the ultimate control of asthma pathogenesis.

Th17 Cells and Asthma Pathogenesis

Increased expression of IL-17A has been detected in the lungs, sputum, BAL fluids, and sera of asthmatic patients (Barczyk et al., 2003; Bullens et al., 2006; Chakir et al., 2003; Laan et al., 2002; Wong et al., 2001), and IL-17A expression is correlated with the extent of AHR following administration of the lung irritant, methacholine (Barczyk et al., 2003; Kolls et al., 2003).

Additionally, IL-17 production was found to have a significant role in collagen deposition and airway remodeling, traits of chronic asthma (Molet et al., 2003). IL-17A and/or IL-17F can orchestrate local inflammation by inducing human bronchial fibroblasts and airway smooth muscle cells to release proinflammatory cytokines such as TNF- α , IL-1 β , granulocyte colony-stimulating factor (G-CSF), and IL-6, as well as the neutrophil chemotactic proteins, IL-8 and CXCL1/Gro- α (Fig. 1) (Molet et al., 2001). IL-17A can also act in concert with IL-6 to induce human bronchial epithelial cells to produce the mucus proteins MUC5B and MUC5AC (Chen et al., 2003; Kawaguchi et al., 2001a; Laan et al., 1999). Previously, goblet cell hyperplasia (a hallmark asthmatic trait) was linked, albeit weakly, to Th2 cytokine production. The more recent results, in addition to other studies (Oda et al., 2005; Wakashin et al., 2008), now convincingly show that IL-17 maintains increased mucin gene expression in the airways and may underscore a significant role of IL-17 in airway inflammation.

Animal studies have further implicated Th17 cells and their cytokines in provocation of lung inflammation. Over-expression of IL-17A in the lung has been found to result in neutrophilic infiltration and elevated levels of granulopoietic factors such as CXCL1/Gro- α , IL-8, and G-CSF (Laan et al., 1999; Schwarzenberger et al., 1998). Conversely, mice deficient for IL-17A or IL-17A receptor (IL-17RA) expression exhibited diminished pulmonary neutrophil recruitment in response to allergen challenge (Nakae et al., 2002). Taken together, these results suggest that IL-17A plays a pivotal role in lung inflammation, especially in those cases of severe asthma in humans that are characterized by a neutrophil-dominant cellular infiltrate. However, the role of IL-17 in murine models of allergic asthma, which are typically Th2-cell-mediated, has been more obscure. On the one hand, following sensitization and allergen challenge in an OVA asthma model, IL-17A- or IL-17RA-deficient mice showed impaired induction of OVA-specific T cells, reduced eosinophilic inflammation, and lower levels of serum IgE production (Durrant et al., 2009; Nakae et al., 2002; Schnyder-Candrian et al., 2006), suggesting that IL-17 is required for the initiation of asthma.

Similarly, blocking the effects of IL-17 following OVA challenge in mice that were sensitized epicutaneously, resulted in reduced neutrophil influx into the lung and reversal of bronchial hyperreactivity (He et al., 2007). However, IL-17 neutralization with anti-IL-17A mAb in an OVA-challenge asthma model also caused elevated eosinophilic infiltration and IL-5 production in BAL (Schnyder-Candrian et al., 2006), suggesting an inhibitory role of IL-17A on established Th2-driven allergic immune responses. Furthermore, when IL-6-deficient mice, which are defective in Th17 cell development, were epicutaneously sensitized to allergen, Th2 cells were activated and the animals developed exacerbated pulmonary eosinophilic inflammation (Wang et al., 2000). These results suggested that treatment with exogenous IL-17 would alleviate established Th2-mediated asthma. Indeed, recombinant IL-17 administered i.n. during the challenge phase of allergen appeared to inhibit asthma responses such as AHR and eosinophilic inflammation (Schnyder-Candrian et al., 2006). These findings underscore a crucial role of this cytokine in pulmonary

inflammation but also emphasize a need for further clarification of this pathway in the disease process.

In addition to Th2 cell-driven murine models of allergic asthma, the role of IL-17A has been studied in Th17-dominant models of asthma. Mice deficient in T-bet, the transcription factor necessary for Th1 cell development, have been shown to develop an allergic immune response that is dominated by neutrophilic influx that is associated with increased expression of IL-17A, but not IL-4 or IL-5 (Durrant et al., 2009; Fujiwara et al., 2007). Furthermore, OVA challenge of DO11.10 OVA-specific TCR transgenic mice has been reported to result in neutrophilic inflammation without an increase in serum IgE (Wilder et al., 2001).

In this model, Th1 and Th17 cells/cytokines, but not Th2 cells/cytokines, have been found to be increased in BAL from DO11.10 and OTII mice exposed to OVA inhalation (Nakae et al., 2007). Following neutralization of IL-17A during the OVA challenge phase in T-bet deficient mice, neutrophil-mediated lung inflammation was alleviated as well as AHR (Durrant et al., 2009). Similarly, IL-17-deficient DO11.10 mice (Nakae et al., 2002) and IL-17-deficient OTII mice (Nakae et al., 2007) demonstrated decreased neutrophilic airway inflammation and AHR following OVA challenge. Taken together, these observations indicate that IL-17/Th17 cells are responsible for airway neutrophilia in asthma models that use T-bet deficient or OVA-specific TCR transgenic mice.

Several studies have also described the potential role of IL-17 in human lung disease and it has been shown that IL-17 can induce pulmonary inflammation (Sharkhuu et al., 2006). Specifically, accumulating evidence implicates IL-17 in the development and progression of human asthma. IL-17 expression is upregulated after allergen challenge, and *in vitro* allergen stimulation of T cells from atopic asthmatic patients leads to enhanced IL-17 production compared to the levels seen in nonallergic control subjects (Hashimoto et al., 2005; Kawaguchi et al., 2001b). As has been seen with other cytokines, the level of IL-17A expression correlates with the severity of AHR in patients. Interestingly, IL-17A has also been shown to induce the release of the eosinophil-recruiting chemokine eotaxin from airway smooth muscle cells, thus likely aiding in eosinophilic inflammation, and both IL-17A and IL-17F have been shown to be able to induce the release of inflammatory mediators, such as CXCL1/Gro- and IL-8, from human eosinophils *in vitro* (Cheung et al., 2008; Rahman et al., 2006).

Although eosinophilic airway inflammation is recognized as a hallmark feature of disease in some asthmatic patients, recent studies indicate a significant role for neutrophils in asthma (Monteseirin, 2009). Although approximately 50% of asthma cases demonstrate eosinophilic inflammation, most other cases of asthma exhibit an increase in airway neutrophils and interleukin 8 (IL-8) (Douwes et al., 2002). Indeed, the severity of asthma may be determined by the balance between IL-4 and IL-17 levels as well as the amount of eosinophilic versus neutrophilic infiltration in the lung. It is possible that IL-17 has a subdominant role in allergic asthma, but a central role in nonallergic asthma in which neutrophils play a critical role. Overall, Th17 cells have an undeniable role in asthma disease pathogenesis and as their role is clarified, this cell pathway will become an attractive target for asthma.

Targeting Th17 cells with a Th1 Cytokine

Many therapeutic approaches for regulating asthmatic responses have relied upon the Th1/Th2 paradigm and numerous studies have compellingly demonstrated that Th1 cells can inhibit the development of Th2-mediated airway inflammation (Coyle et al., 1996). However, therapeutic strategies based on the Th1/Th2 model have thus far led to disappointing outcomes in some asthmatic patients (see above). For instance, IL-12, a key

cytokine in modulating the balance between Th1 and Th2 cells, inhibits AHR and airway eosinophilia after antigen challenge in animal models (Kips et al., 1996; Schwarze et al., 1998).

However, when asthmatic patients were treated with recombinant IL-12, there was no significant effect on AHR or on the asthmatic reaction (Bryan et al., 2000). These results suggest that either IL-12 is ineffective in its ability to regulate Th1/Th2 imbalances in humans, which from animal studies seems to be unlikely, or there is an incomplete understanding of the mechanisms mediating the asthmatic response. In any case, the experience in clinical trials has reiterated the need for a better understanding of the molecular mechanisms that drive immune responses in the lung.

Of particular interest are recent studies showing that T-bet is significantly decreased in the airways of asthmatic patients (Finotto et al., 2002) and that polymorphisms of this gene in humans are correlated with airway inflammation and AHR (Munthe-Kaas et al., 2008; Raby et al., 2006). T-bet is a T-box transcription factor and is crucial for Th1 cell differentiation and IFN- γ production. In the absence of T-bet, Th cell responses following allergen challenge have been shown to be predominantly Th17-mediated (Durrant et al., 2009; Fujiwara et al., 2007). Recently, IL-12 was used to target Th17 cell-mediated lung inflammation in a T-bet deficient OVA asthma model. In the absence of T-bet, there was little to no IFN- γ expression; however, antigen-induced airway inflammation as well as AHR was almost completely suppressed following treatment with IL-12 during the OVA challenge phase.

Surprisingly, this suppression was found to be dependant on IL-10, which acted through inhibiting IL-6 and TGF- β (Fig. 2) (Durrant and Metzger, submitted for publication). These results suggest that in a Th17 cell-mediated model, IL-12 has a strong immunosuppressive, IFN- γ -independent effect and could represent a novel mechanistic advance in which the Th1-inducing cytokine, IL-12, inhibits inflammatory Th17 cytokine production through induction of IL-10.

CONCLUSION

Asthma is now regarded as a complex and heterogeneous disorder. The historical view of this disease as solely a Th2-mediated condition has been challenged by inconsistencies observed in clinical presentation, by responses to targeted treatments (or lack thereof), and by variations in pathogenic phenotypes as well as inflammatory cellular infiltrates. Indeed, the degree of disease variability suggests that other Th immune responses, beyond the canonical Th2 pathway, have important and possibly complementary roles in pulmonary inflammation and AHR.

Despite significant attempts into understanding the basic immune mechanisms involved in asthma, the prevalence of this disorder has continued to rise and the lack of efficacy of anti-inflammatory treatments has been disappointing. The emerging body of evidence from human and mouse models has now demonstrated that in addition to Th2 cells, Th1, Th17, Treg and Th9 cells, each have unique abilities to influence airway inflammation and hyperresponsiveness. We predict that continued identification of different lymphoid cell subsets and delineation of their precise roles in asthma initiation and exacerbation will lead to new insights that ultimately will allow development of novel and better targeted therapeutic approaches for human asthma.

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Figure 1.

T helper cell subsets in asthma. As allergens enter the airway, professional antigen-presenting cells take up the allergen and present it to naïve CD4⁺ T helper cells. This leads to the activation of allergen-specific Th2 cells. Th2 cells produce IL-4, which induces B-cell activation and IgE antibody production; IL-5, which induces eosinophil recruitment; and IL-13, which promotes mucus production and AHR. In addition to Th2 cells, Th17 cells can also be activated. Th17 cells produce IL-17 and IL-22. These cytokines induce epithelial cells to produce the proteins MUC5B and MUC5AC and granulopoietic factors such as IL-8 and G-CSF, which cause neutrophil recruitment and expansion. There is evidence that Th1-cell responses might also be responsible for some of the pathogenic features observed in patients suffering from chronic forms of allergy, e.g., IFN- has been shown to exacerbate an established asthmatic response. IL-9 produced by Th9 cells leads to the recruitment, development, and activation of mast cells. Upon degranulation, IgE-sensitized mast cells release both preformed and newly synthesized mediators in sensitized individuals. These include histamine and leukotrienes, which promote airway inflammation and mucus production. IL-10-producing Treg cells have an immunosuppressive effect on Th2 cell activation that occurs during asthmatic reactions, especially in decreasing production of IgE antibody.

Figure 2.

A novel cytokine-based therapy in asthma. Allergic inflammation associated with asthma has been characterized as primarily an eosinophilic/Th2-mediated disease. However, in the absence of T-bet, the master transcription factor necessary for Th1 cell development and IFN- γ production, allergic inflammation is largely mediated by a neutrophilic/Th17 cell response. The Th1 cell promoting cytokine, IL-12, can inhibit this Th17-mediated inflammation and AHR in the absence of IFN- γ production, through induction of pulmonary IL-10 expression.