



Targeting memory Th2 cells for the treatment of allergic asthma

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Abstract

Th2 memory cells play an important role in the pathogenesis of allergic asthma. Evidence from patients and experimental models indicates that memory Th2 cells reside in the lungs during disease remission and, upon allergen exposure, become activated effectors involved in disease exacerbation. The inhibition of memory Th2 cells or their effector functions in allergic asthma influence disease progression, suggesting their importance as therapeutic targets. They are allergen specific and can potentially be suppressed or eliminated using this specificity. They have distinct activation, differentiation, cell surface phenotype, migration capacity, and effector functions that can be targeted singularly or in combination. Furthermore, memory Th2 cells residing in the lungs can be treated locally. Capitalizing on these unique attributes is important for drug development for allergic asthma. The aim of this review is to present an overview of therapeutic strategies targeting Th2 memory cells in allergic asthma, emphasizing Th2 generation, differentiation, activation, migration, effector function, and survival.

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Keywords: Th2 cells; Immunological memory; Allergic asthma

Abbreviations: AHR, airway hyperresponsiveness; APC, antigen presenting cells; BCG, Bacille Calmette Guerin; Bcl, B-cell lymphoma; BLC, B-cell attracting chemokine; CKR, chemokine receptor; CpG, cytosine–phosphate–guanine CpG motifs; DCs, dendritic cells; IFN, interferon; Ig, immunoglobulin; IL, interleukin; ISS-ODNs, immunostimulatory oligodeoxynucleotides; JNK, c-Jun NH2-terminal kinase; LFA-1, lymphocyte function associated antigen-1; mAb, monoclonal antibody; MAPK, mitogen-activated protein kinases; MCP-1, monocyte chemoattractant protein 1; MDC, macrophage derived chemokine; MHC, major histocompatibility complex; NF- κ B, nuclear factor kappa B; ODNs, oligodeoxynucleotides; PDE, phosphodiesterase; PI3K, phosphoinositide 3-kinase; PG, prostaglandin; TARC, thymus activation regulated chemokine; TCR, T-cell receptors; Th, CD4⁺ T helper; (T reg) cells, T regulatory cells; TNF, tumour necrosis factor; TSLP, human thymic stromal lymphopoietin; VLA, very late antigen.

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1. Introduction

Allergic asthma is a chronic inflammatory disease of the conducting airways caused by an immune response directed against allergens. The syndrome affects both children and adults, resulting in loss of work and school days. It is responsible for numerous hospital visits, is a burden on the health system, and has important social and economic ramifications. Growing concern over the increase in incidence and prevalence in the last decades, in addition to increased morbidity and mortality associated with asthma, has intensified attempts to find new drug targets. Remarkably, the rise in incidence and prevalence of asthma in industrialized countries has not been adequately explained, and equally inexplicable is evidence showing that this trend is stabilizing (Lawson & Senthilselvan, 2005). Nevertheless, allergic asthma is a chronic, progressive illness, which is not influenced by diminished incidence rates. Moreover, certain patients are resistant to currently available drugs. The need for new and improved therapies is vital.

The best method of primary prevention for allergic disease is allergen avoidance. Environmental allergens, however, are impossible to elude; thus, the struggle to find improved treatments and cures persists. One strategy to cure disease is to prevent allergen sensitization, which currently is impossible because of our inability to determine susceptibility to allergic disease (atopy). Another approach is to suppress or eliminate memory CD4⁺ T helper (Th) 2 lymphocytes that mediate the allergic response and perpetuate allergic asthma. Th2 memory cells provide a valuable therapeutic focus in allergic asthma because of their specificity, which permits the treatment of disease without causing generalized immunosuppression.

Therapeutic strategies may be aimed at the prevention of disease or toward the inhibition of established disease. At present, it is difficult to construct novel treatments to inhibit disease initiation since the development of atopy cannot be predicted. New discoveries of genes associated with asthma, such as I-TIM1 (McIntire et al., 2004), arginase (Zimmermann et al., 2003), human thymic stromal lymphopoietin (TSLP; Soumelis et al., 2002), amongst other susceptibility

genes (Hakonarson et al., 2002; Hytonen et al., 2004; Jinnai et al., 2004; Nicolae et al., 2005; Noguchi et al., 2005), however, may make it possible to establish reliable biomarkers that precede the manifestations of allergic disease. Thus, therapeutic strategies inhibiting the initial generation of memory Th2 cells must also be considered. Suppressing or eliminating Th2 cells will be most likely to ameliorate disease when the specific targeting of the approach is directed against the allergen receptor, Th2 cell, or memory Th cell. The latter 2 approaches tend to be broader and less specific than when the precise allergen is known and targeted. Inhibiting Th2 cells using non-allergen-specific methods is beneficial because the major role for Th2 immunity is in parasite defense, the loss of which is likely inconsequential in industrialized countries with few pathogenic parasites. However, inhibition of memory Th cells would cause immunosuppression, unless it was specific for memory Th2 cells. An additional consequence of Th2 suppression is a potential alteration of the balance between Th1 and Th2 immunity, leading to increased Th1 cells and autoimmunity.

2. Allergic immunity

Allergens are defined as substances that induce allergy. Common inhaled allergens are proteins derived, for example, from tree, grass and weed pollens, dust mites, molds, and animals such as dogs and cats. Ingested allergens are proteins found, for example, in seafood, peanuts, milk, and eggs. Exposure to allergens in susceptible individuals leads initially to allergen sensitization and the generation of CD4⁺ Th2 cells. Subsequent allergen encounters activate previously sensitized Th2 cells resulting in an allergic immune response that is pathologically manifested in the eyes (conjunctivitis), nose (rhinitis), lungs (asthma), skin (atopic dermatitis, eczema), or circulatory system (anaphylaxis). In asthma, the activation of allergen-induced Th2 cells in the lungs leads to eosinophilic lung inflammation, edema, increased secretion of mucus, and recurring bronchospasm. Eventually, with repeated allergen exposures, the bronchial smooth muscle and basement membrane thicken, lung tissue is destroyed, and lung function diminishes. The major symptoms of allergen exposure are difficulty in breathing, shortness of breath, coughing, and wheezing, sometimes leading to respiratory distress and even death.

The current paradigm for an allergic response is represented in Fig. 1 and reviewed in Busse and Lemanske (2001). Allergen sensitization is initiated as allergen is taken up, internalized, and processed by antigen presenting cells (APC), such as dendritic cells (DCs) and macrophages. Intracellular allergens are transported to compartments, where they are proteolyzed into small peptide fragments (epitopes) that bind to major histocompatibility complex (MHC) class II molecules. The resulting MHC–peptide complexes migrate to the cell surface, where, together with

co-stimulatory molecules such as CD80 and CD86, stimulate T-cell receptors (TCR) on naive Th cells. The ligand–TCR interaction leads to signal transduction, activation, and differentiation. The transition between naive Th cells to activated Th2 cells is accompanied by an alteration in the expression of several cell surface receptors, production of Th2 cytokines interleukin (IL)–4, IL–5, IL–6, IL–9, IL–10, IL–13, and IL–25, and the ability to cooperate with B-cells to promote IgE synthesis.

Within the lungs, Th2 cells and their secreted products initiate a cascade of events that begins with the recruitment and activation of eosinophils, macrophages, and other inflammatory cells. The recruited eosinophils produce IL–3, IL–5, IL–6, IL–13, granulocyte-macrophage colony stimulating factor (GM-CSF), tumour necrosis factor (TNF), major basic protein, and eosinophil cationic protein. Recruited macrophages produce IL–1 β , IL–6, IL–8, and GM-CSF. The resulting allergen-specific IgE (produced by the cooperation between Th2 cells and B-cells) binds to both allergen and high-affinity Fc ϵ RI receptors on the surface of mast cells and transduces a signal that causes the release of IL–4, IL–5, TNF, leukotrienes, histamine, tryptase, prostaglandins (PG), and chymase. These mast cell mediators are responsible for inducing the immediate type hypersensitivity reaction or early phase response, which is followed by the more sustained, late phase response and is mediated and regulated by recruited leukocytes. Memory Th2 cells are required for the persistent production of allergen-specific IgE from B-cells, thus demonstrating that memory Th2 is important for early as well as late phase responses in allergic asthma. Allergen-specific IgE has a crucial role in clinical disease for the early phase response, but is less important in experimental animal models. The characteristic features of allergic asthma can be induced in B-cell- and antibody-deficient mice (Hamelmann et al., n.d.; Korsgren et al., 1997; MacLean et al., 1999), further supporting the central role for memory Th2 cells. The allergic immune response is thus a complex series of events orchestrated by allergen-specific Th2 cells that engage the participation of other leukocytes, respiratory epithelium, smooth muscle, and nervous tissue. It promotes eosinophilic inflammation, edema, smooth muscle contraction, and increased mucus production, resulting in airway obstruction and eventual lung damage.

The central role for allergen-specific Th2 effector and memory cells has been shown in allergic asthma in mice and humans (Robinson et al., 1992; Garlisi et al., 1995; Larche et al., 2003; Leigh et al., 2004a). Several studies demonstrate that Th2 cells are in airways and lungs of asthmatics and experimental animals (Larche et al., 2003). However, the importance of Th2 cells in disease pathogenesis is emphasized by the following 2 observations in mice: (1) the elimination of Th2 cells abolishes allergic immunity (Gavett et al., 1994), and (2) adoptive transfer of allergen-specific Th2 cells induces passive allergic asthma in naive recipients (Cohn et al., 1997; Li et al., 1998; Wise et al., 1999). A

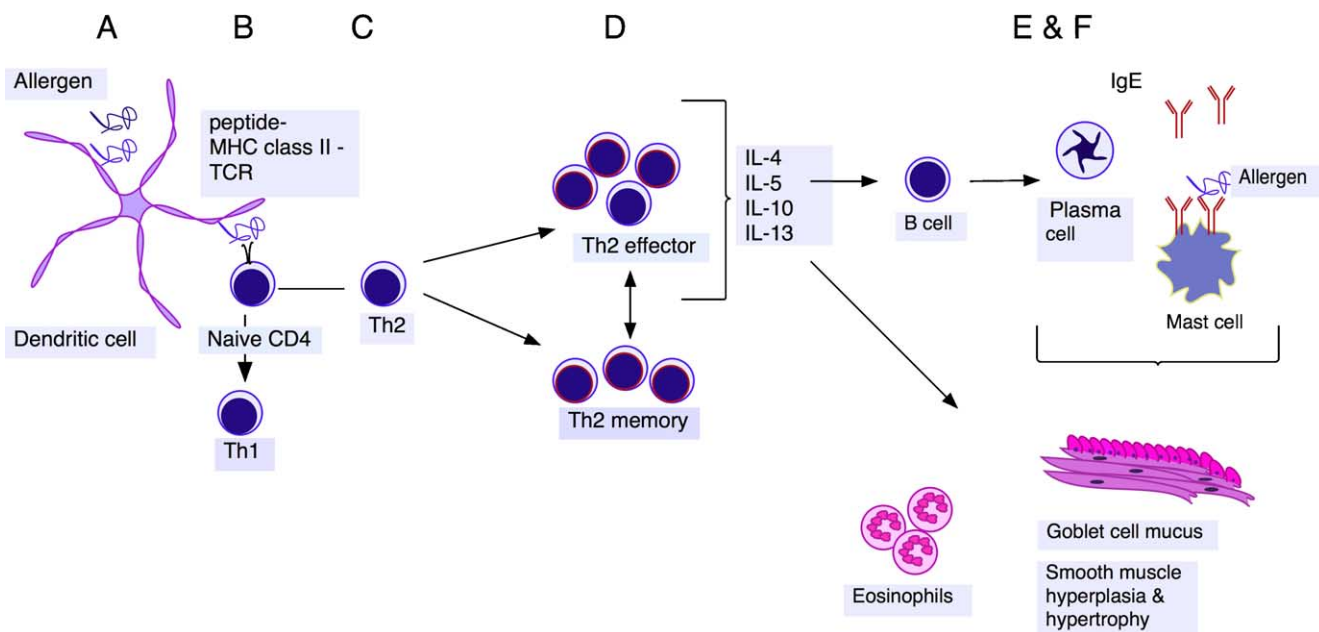


Fig. 1. Allergic immunity underlies the induction of allergic asthma. (A) Allergen uptake, processing, and presentation. Allergen is taken up by APC (macrophages or DCs; DC is depicted) in the lungs. It is then processed into peptides that are complexed within the cell with MHC class II and are transported to the cell surface. Complexes then interact with TCR on naive CD4⁺ Th cells. (B) Stimulation of naive CD4 T-cell; T-cell activation and up- and down-regulation of cell surface molecules. (C) Differentiation into Th2 (and maybe Th1 cells). (D) Clonal expansion of Th2 effector and memory cells. (E) Production of Th2 cytokines. Effector cytokines induce the differentiation of eosinophils and recruit them and other leucocytes to the lungs and stimulate B-cell differentiation, production of IgE (B-cell help). (F) IgE specifically binds allergen and cross-links Fcε receptors on mast cells, causing the release of vasoactive mediators leading to smooth muscle contraction, mucus hypersecretion, edema, and eventually, airway remodeling and lung destruction.

myriad of additional studies further support a crucial role for Th2 cells and their cytokines in allergic asthma (Corrigan & Kay, 1992; Renz et al., 1992; Brusselle et al., 1994; Kopf et al., 1995; Foster et al., 1996; Gavett et al., 1997; Hamelmann et al., 1997; Hogan et al., 1997; Corry et al., 1998; Wills-Karp et al., 1998; Zhu et al., 1999; Webb et al., 2000; Foster et al., 2002; Grunig et al., 2002; Kuperman et al., 2002).

3. Th2 cell generation

Why generate a Th2 immune response to allergens? In any battle, the main objective is to provide a first-rate defense while limiting collateral damage. Unfortunately, in the battle against pathogens, the immune system occasionally is either unable to adequately defend itself or causes unintentional harm by responding inappropriately. The immune system has evolved a defense mechanism for protecting against pathogens, which includes a strategy to avoid responding against innocuous, nonpathogenic foreign and self-antigens. Failure to distinguish between harmful and harmless foreign antigens leads to useless Th2 immune responses to environmental inhaled and ingested protein allergens.

Initial sensitization and repeated encounters with antigen require antigen presentation by APC to Th cells and induce T-cell activation (leading to proliferation, effector func-

tions), Th1 or Th2 differentiation, or T-cell tolerance. The mechanisms determining which response is generated are not completely known. However, there are some general rules: (1) Th1 cells arise during viral and bacterial infections and in response to transplanted organs and self-antigens, (2) Th2 cells are provoked by allergens (in susceptible individuals) and parasites, and (3) T-cell tolerance is generated towards noninfectious ingested and inhaled foreign antigens and self-antigens. The exact mechanism underlying how allergens provoke Th2 cell differentiation is unknown. A large number of factors may be involved, including the nature, quality, and quantity of the allergen, the route of first encounter (inhalation, ingestion, injection, or skin exposure), and antigen processing and presentation. On one hand, it is possible that susceptible or atopic individuals have genetic influences that lead to allergic immunity, regardless of the factors listed above. A hypothetical example is that the difference between an atopic and nonatopic individual might be the level of human thymic stromal lymphopoietin produced by lung epithelial cells (Soumelis et al., 2002). Increased production of human thymic stromal lymphopoietin would alter the lung micro-environment and bias towards Th2 cell immunity irrespective of the initial conditions and allergen exposure. Another example is that atopic individuals may have impaired regulatory mechanisms, such as T regulatory (T reg) cells, which might also determine whether Th2 immunity is initiated (Piccirillo & Shevach, 2004). On the other hand,

atopy may be a genetically determined variation of the usual sequence of events. Its frequency indicates positive selective factors and a potential biological advantage, for instance, in the handling of parasitic infections. Nevertheless, a better understanding of genetic influences during sensitization would enable us to predict atopy and implicates prophylactic allergen vaccination in childhood for inhibiting Th2-mediated allergic immunity.

4. Immunological memory

Immunological memory is an important protective mechanism that enables host organisms to respond more rapidly and vigorously to pathogens that have been previously encountered. It is the reason why repeated infection with the same pathogen may not cause recurrent illness; it is also the basis of vaccine development. Immunological memory for bacterial and viral infections may be life saving, but, when directed against allergens, may cause morbidity and mortality. Memory immune responses are long lived, antigen specific, and are characterized by a rapid and robust response to MHC class II–antigenic peptide complexes on APC. The mechanisms underlying the generation of memory immune responses are not known.

It appears, however, that a subpopulation of antigen-specific memory Th cells is generated during an immune response to antigen and/or allergen. Two proposed theories explain how memory Th cells are generated. The first suggests that naive Th cells differentiate into 2 distinct subpopulations, 1 effector and the other memory during antigen sensitization (Farber & Ahmadzadeh, 2002; Farber, 2003; Chandok & Farber, 2004). The second states that naive T-cells differentiate into effector cells, which then undergo further differentiation into memory cells. In the latter model, interruption during sensitization would simultaneously prevent the generation of effector and memory cells, whereas in the former model, it might be possible to discriminate the effects on effector and memory Th cells. In support of a nonlinear model of differentiation, investigators have shown that antigen sensitization generates 2 populations of cells, non-cytokine-producing cells and interferon (IFN) γ –cytokine-producing cells (Wu et al., 2002). The IFN γ –producing cells were short lived, whereas the non-cytokine-producing cells persisted in vivo and developed into long-term memory cells that produced cytokines upon secondary antigen challenge. These results suggest that initial antigen sensitization leads to the production of both effector and memory Th cell populations and implies that preventing memory cells at initial sensitization may 1 day be possible. It is possible, however, that these models are not mutually exclusive, but this has yet to be determined. Furthermore, recent evidence suggests that early T-cell differentiation is associated with highly heterogeneous populations, supporting a more complicated process than

the proposed linear or nonlinear differentiation models (Song et al., 2005).

Patients with allergic asthma maintain Th cells in their lungs (Yurovsky et al., 1998) and during remission have elevated nitric oxide breath levels indicative of persistent lung inflammation (Bates & Silkoff, 2003). These data are consistent with observations that memory T-cells home to nonlymphoid tissue and contribute to systemic immunity (Mackay & von Andrian, 2001; Masopust et al., 2001; Reinhardt et al., 2001; Cauley et al., 2002; Mojtavavi et al., 2002; Roberts & Woodland, 2004). They act as tissue-based sentinels providing protection against pathogens, supported by data showing that antiviral CD8+ T-cells in lungs of virally infected mice persist within the lungs for long periods of time and provide protection against reinfection with virus (Roberts & Woodland, 2004). Similarly, allergen-specific memory Th2 cells have been shown to reside within the lungs of mice for their lifetime after recovering from a single episode of allergic asthma (Mojtabavi et al., 2002). Mice recuperate from acute allergic asthma rapidly, but evidence of immunological memory is illustrated by the maintenance of elevated serum allergen-specific IgG1 and persistent inflammatory lung infiltrates containing lymphocytes and macrophages (without eosinophils). Moreover, secondary aerosol allergen challenge induces rapid lung Th2 cell activation and cytokine secretion and plasma cell differentiation, and increased airway antibody titres in the lungs and the development of eosinophilic lung inflammation, mucus hypersecretion, airway hyperresponsiveness (AHR), and allergen-specific IgE. Repeated allergen exposures stimulate memory Th2 cells, induce asthma exacerbations, and eventual lung remodeling and damage (Wills-Karp et al., 1998; Zhu et al., 2001; Blackburn et al., 2003). While the data suggest that Th2 cells have an important role in the early and acute/intermediate stages of allergic asthma, a recent study determined that AHR and remodeling were T-cell independent in mice treated with chronic allergen exposures, thus challenging the role of Th2 cells in disease perpetuation (Leigh et al., 2004b). This finding suggests that treating memory Th2 cells during mild and moderate asthma may be more effective than during severe disease.

Allergic asthma is characterized by remissions and exacerbations, and thus, the challenge is to either prevent disease relapse or inhibit ongoing disease. Targeting Th2 cells during distinct phases of disease may be challenging because there are at least 2 populations of memory cells, resting memory and effector memory Th cells, and are known to differ with respect to activation state, cell surface phenotype (CD45, CD44, CD62L, and CCR7), cytokine production, migration (or lack thereof), and longevity (Farber & Ahmadzadeh, 2002; Farber, 2003). We have found that during remission from allergic asthma, persistent memory Th2 cells in the lungs are quiescent (Mojtabavi et al., 2002). They have a resting cell phenotype and cannot easily be distinguished in wild-type mice from other Th cells until they are reactivated with allergen. Thus, they are

distinguished most reliably based on their antigen/allergen-specific effector functions. During an exacerbation, resting memory cells become activated effectors that secrete cytokines and circulate based on changes in cell surface homing receptors. Therefore, resting and memory effector cells are distinct populations that need to be targeted differently. For resting memory cells, it is more important to target the factors involved in their maintenance within the lungs and to attempt to prevent activation. Conversely, during an exacerbation, suppressing activation, effector functions, and migration of effector memory cells would ameliorate disease. However, it is not that simple. There is evidence demonstrating that the memory CD4⁺ T-cell population is heterogeneous, consisting of functionally distinct (Harari et al., 2005), resting, and effector memory cells (Ahmadzadeh et al., 2001). Collectively, these data suggest that when targeting memory Th2 cells in allergic asthma, one must address a diverse memory cell population with differential activation states and cells that inhabit distinct locations.

5. Targeting memory Th2 cells

The following sections will focus on the current paradigm of Th2 cell generation, activation, effector functions, and maintenance of immunological memory depicted in Fig. 2 and how it is possible to interfere with them to treat allergic asthma. It will begin with the initial encounter with allergen through to the processes that perpetuate Th2 cells and disease. The first step is allergen capture by APC and its subsequent handling and presentation, which converts a naive Th cell into an allergen-specific Th2 cell (differentiation). Th1 and T_{reg} cells might also be generated and downmodulate the Th2 immune responses. Allergen-specific Th2 activation results in clonal expansion (increase in the number) and subsequent effects. These include an up-regulation of cell surface receptors that enable effector cells to migrate, secrete effector cytokines, and help B-cells produce IgE, which together lead to allergic immunity. Remission is established by the elimination of allergen, which is correlated with the resolution of symptoms and active inflammation. Recovery is characterized by the elimination of effector Th2 cells by homeostatic mechanisms and the survival of predominantly quiescent, resting allergen-specific memory cells. These cells reside in lymphoid and lung tissue and reactivate to become allergen-specific effector cells upon subsequent allergen exposure. Each step in this pathway can be interrupted and has the potential to attenuate disease.

5.1. Allergen handling and presentation targets

The immune system decides whether to ignore or respond to allergen. When allergen is recognized and not ignored, the decision then becomes whether to generate a

Th1 or Th2 response or to actively suppress the response (immune tolerance). This decision occurs during allergen recognition and handling by APC, but the factors involved in the decision are not completely known. The uptake and processing of allergen together induce APC maturation, which is influenced, in turn, both by the character and quantity of the allergen, as well as the location and microenvironment during processing and presentation (Fig. 2). The maturation of APC is associated with changes in cell surface molecule expression (i.e., costimulatory molecules, chemokine and cytokine receptors), migration capacity, cytokine production, and the expression of MHC class II–peptide complexes. This permits APC to migrate and set up a local cytokine milieu that subsequently influences the Th response during antigen presentation. Although the effects of allergen recognition, processing, and presentation are important during initial contact with allergen, they continue to influence Th2 cell responsiveness upon repeated encounters with allergen and potentially are involved in the maintenance of long-lived memory Th2 cells. There are numerous examples in which interference with allergen handling and subsequent Th2 reactivity is a strategy used for the treatment of allergic asthma.

5.1.1. Dendritic cell targets

DCs are the most potent APC. They have diverse functions attributable, in part, to distinct subsets. Airway DCs are scavenger cells that take up inhaled particulate and soluble antigens (Hammad et al., 2002) and appear to maintain immunological homeostasis (Gajewska et al., 2003; Stumbles et al., 2003). Immature lung DCs capture antigen and allergen, mature, and migrate to draining lymph nodes, where they present MHC class II–peptide and stimulate naive and memory Th2 cells. Thus, DCs are important in the initiation and perpetuation of Th2 cell-mediated allergic asthma. The role of DCs in the induction and maintenance of Th2-mediated allergic asthma has been illustrated recently in 2 transgenic mouse models. In 1 model, the Runx3 transcription factor was genetically deleted, resulting in mice with DCs that are insensitive to TGFβ and are more mature (Fainaru et al., 2004). This causes increased T-cell activation and the development of spontaneous eosinophilic lung inflammation, airway remodeling, and mucus hypersecretion. In a second transgenic model, mice were generated by inserting a CD11c⁺ promoter-driven diphtheria toxin receptor (van Rijt et al., 2005). Upon treatment with diphtheria toxin, CD11c⁺ cells were eliminated. Using these mice, investigators demonstrated that CD11c⁺ DCs were critical for the induction of allergic asthma. Taken together, these results demonstrate that the inhibition of DC antigen presentation may inhibit Th2 cell activation, differentiation, and potential survival.

Distinct DC subpopulations play a role in Th differentiation and the class of immune response. Myeloid DCs (Lambrecht et al., 2000) and CD16⁺ DCs (Rivas-Carvalho et al., 2004) induce allergic asthma, plasmacytoid DCs (de

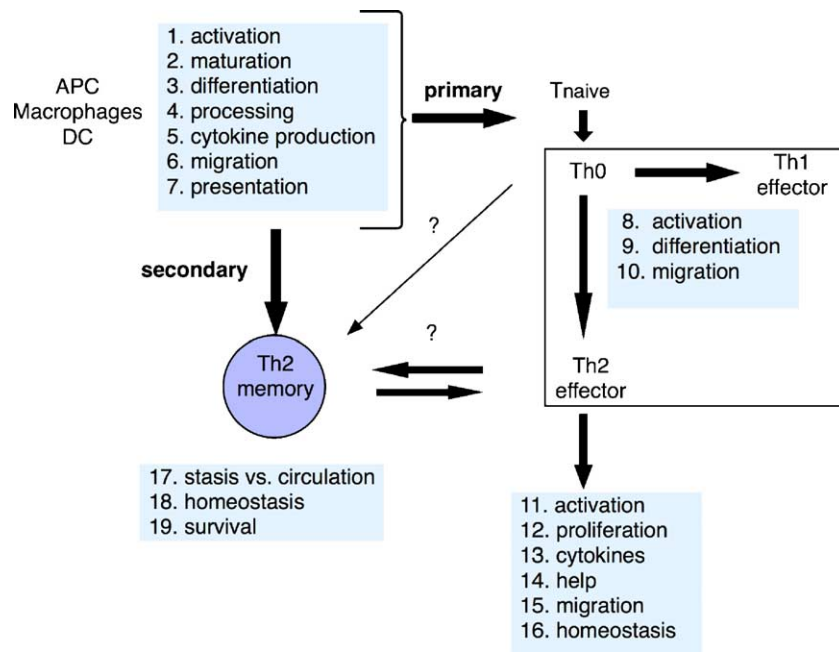


Fig. 2. Th2 memory cell activation, differentiation, generation, and effector function. (A) The processes leading to allergic asthma start with the primary generation and secondary stimulation of Th2 cells, which both require antigen/allergen presentation. Allergen is captured by macrophages and/or DCs, which become activated, mature, and differentiate. APC then produce cytokines, such as IL-12, migrate, and present allergen peptides to naive Th or memory Th2 cells. (B) The naive Th cell becomes an undifferentiated Th0 cell that undergoes activation and differentiation into Th1 or Th2 effector cells and are able to migrate. (It is not clear whether the naive or Th0 cells that differentiate directly to Th2 memory cells are generated during early sensitization.) Furthermore, it is also not obvious when or if Th1 and T reg cells that inhibit Th2 effectors are generated. (C) Effector Th2 cells (derived from Th0 or from Th2 memory cells) are activated, proliferate (clonally expand), produce Th2 cytokines, help B-cells produce Ig, migrate, and are maintained by homeostatic mechanisms. (D) Memory Th2 cells remain in the lymphoid and nonlymphoid tissue or in the circulation and are long lived. Interrupting any of these 20 processes can reduce the symptoms and pathological consequences of allergic asthma.

Heer et al., 2004) are protective, ICOS–ICOSligand regulatory DCs suppress Th2 cells (Akbari & Umetsu, 2005), and IL-10 secreting DCs induce tolerance (Akbari et al., 2001). Recent studies indicate that the administration of the Flt3 ligand, a growth factor for DCs, prevents and inhibits established allergic asthma in mice (Edwan et al., 2004; Edwan et al., 2005), thereby demonstrating that influencing maturation and differentiation of DCs alters Th2 responsiveness and disease. These data show a pivotal role for DCs in Th cell immune responsiveness to inhaled allergens and provide a myriad of potential targets for the treatment of allergic asthma, for example, inhibition of differentiation of distinct Th2-inducing DC subsets.

The role of DCs in allergic asthma is further underscored by the observation that there are intrinsic differences between DCs in asthmatic and healthy individuals. Asthmatics have higher numbers of airway DCs and they are functionally different from nonasthmatic DCs (Bertorelli et al., 2000; Upham et al., 2002; Lambrecht & Hammad, 2003; Upham & Stumbles, 2003). In vitro experiments show that allergen-pulsed DCs from asthmatic patients preferentially stimulate T-cells to produce IL-4, a cytokine particularly important for promoting Th2 differentiation, compared with healthy controls that induce IFN γ production (Hammad et al., 2002). In other studies, DCs treated with PGE2 have reduced IL-12 production, which leads to increased Th2 cell differentiation (Kalinski et al., 1998). This finding was

substantiated by a more recent study showing that monocyte-derived DCs from asthmatics have increased PGE2 production, leading to augmented Th2 differentiation (Long et al., 2004). Collectively, these findings suggest that DCs in asthmatic patients promote Th2 cells; they are supported by a recent study showing that DCs from the allergic patients induce Th2 cell proliferation and differentiation associated with an up-regulation of activation markers and transcriptional evidence of differential gene regulation on both DCs and T-cells (Lindstedt et al., 2005). It will be necessary to determine whether the variation between normal and asthmatic DCs is related to a primary difference or secondary one, caused by conditioning, or selection of DCs in the asthmatic milieu.

The mechanism underlying the role of DCs in determining the outcome of an immune response might be related to the nature of the antigen. DCs have been shown to discriminate between helminth and microbial antigens by inducing a response that is biased towards the type of antigen. For example, adoptive transfer of DCs incubated with both Schistosoma egg antigen (Th2-inducing antigen) and *Propionibacterium acnes* (Th1-inducing antigen) into naive recipients induced mutually exclusive, helminth-specific Th2 responses and bacterial-specific Th1 responses (Cervi et al., 2004). That is, all anti-helminth responses were Th2 and all antibacterial responses were Th1. Furthermore, helminth and bacterial antigens were taken up differently

and tracked to distinct intracellular compartments, implying that antigen drives differential antigen processing and presentation, which leads to Th1 versus Th2 immunity. In other studies, *Schistosoma mansoni* and *Vibrio cholerae* toxin caused DC-dependent Th2 cell differentiation, whereas *Bordetella pertussis* induced Th1 cell development (de Jong et al., 2002).

DC-mediated Th differentiation might function through cytokines secreted by DCs during antigen processing and/or presentation. DCs that overexpress IL-12 polarize towards Th1 immunity in vitro and in vivo (Kuipers et al., 2004). The importance of IL-12 is further demonstrated in studies showing that the epithelial derived human thymic stromal lymphopoietin activates CD11c⁺ DCs by increasing IL-12 production by DCs (Watanabe et al., 2005), leading to increased IL-4, IL-5, IL-13, and TNF, but reduced IL-10 and IFN γ production from Th cells (Soumelis et al., 2002). Additionally, allergoids (chemically modified allergens with reduced IgE binding affinity compared with the native allergen, but similar immunogenicity) reduce DC and macrophage IL-12 but increase IL-10 production (Kahlert et al., 2000). It is likely that the type of the antigen and APC processing and presentation influences Th cell immune responses and is probably the basis for the effectiveness of allergen immunotherapy (see Section 5.4.1).

DC migration, chemokine production, and chemokine receptor (CKR) expression contribute to allergic asthma. Chemokines up-regulated in allergic asthma are CCL2 (monocyte chemoattractant protein 1 [MCP-1]), CCL17 (thymus activation regulated chemokine [TARC]), and CCL22 (macrophage derived chemokine [MDC]; Conti & DiGiacchino, 2001; Soumelis et al., 2002; Bochner et al., 2003). MCP-1 regulates the homing of DCs and appears to modulate DC function (Ohshima et al., 2004). Alterations of chemokine production during maturation, for example, selective down-regulation of CCL18 (DC-CK1) during maturation (Vulcano et al., 2003) and differential expression of CXCL13 (B-cell attracting chemokine [BLC]) in DC subsets (Visser et al., 2001), affect Th cells and suggest that DC migratory patterns contribute to Th immunity. The migration of DC during allergic asthma has been demonstrated by the observation that peroxisome proliferator-activated receptor (PPAR) γ activation with agonists such as rosiglitazone inhibit DC migration from the airway to draining lymph nodes, thereby reducing T-cell priming (Angeli et al., 2003).

5.1.2. Alveolar macrophage targets

Macrophages are abundant APC in the lungs; however, their role in Th cell differentiation is controversial. Alveolar macrophages are able to both inhibit and stimulate Th2 cells. Alveolar macrophages co-incubated with CD4⁺ T-cells from atopic asthmatic patients exhibited increased IL-5 production and aggravated allergic asthma (Tang et al., 1998). Moreover, allergic asthma in mice could be induced by the administration of antigen-pulsed intraperitoneal

macrophages (Lambert et al., 1996). By contrast, Th1-stimulating alveolar macrophages inhibited Th2 responses and allergic asthma in mice (Tang et al., 2001). In addition, intratracheal instillation of immortalized antigen-pulsed spleen macrophages induced a Th1 immune response (Pynaert et al., 2003). In other studies, alveolar macrophages induced neither a Th1 nor Th2 response, but inhibited T-cell activation (Upham et al., 1995), a result that could be modulated by GM-CSF, IL-4, TGF β , and TNF (Bilyk & Holt, 1993). The lack of T-cell activation may be related to the ability of alveolar macrophages to induce Th cell anergy (Strickland et al., 1996), which correlates with their ability to inhibit pulmonary DC antigen presentation (Holt et al., 1993). Intravenous instillation of macrophages pulsed with protein antigens, but not peptide antigens, inhibited allergic asthma by inducing IL-10 (Janssen et al., 2002), suggesting a role in the generation of T reg cells by alveolar macrophages.

Macrophages play an additional role in the inflammatory response in allergic asthma that is associated with adenosine (Tilley & Boucher, 2005). Adenosine has been shown to play a protective role in disease, suggesting the possibility of using adenosine modulators in the treatment of allergic asthma (Sun et al., 2005; Trifileff et al., 2005). Although the macrophage has been considered the forgotten cell of asthma (Peters-Golden, 2004), it remains an important component of the immune response to foreign antigens/allergens and may eventually lead to novel targets for therapeutic intervention.

5.1.3. Costimulation targets

The interaction between CD80/CD86 and CD28/CTLA4 during TCR stimulation is important for Th cell activation (Greenfield et al., 1998). CD28 signaling is critical for Th2 differentiation from both naive and memory Th cells (Skapenko et al., 2001). Interfering with APC function by inhibiting costimulation has been shown to prevent the generation and activation of Th2 cells (Larche et al., 1998; Harris & Ronchese, 1999; Jaffar et al., 1999a, 1999b; Broeren et al., 2000; Lordan et al., 2001).

CD28-deficient mice lack Th2 differentiation and are unable to mount Th2 immune responses (Mathur et al., 1999b; Burr et al., 2001), and interfering with B7-CTLA4 in the CD28-deficient mice leads to Th1 but not Th2 activation, suggesting a selective role for costimulation in Th2 differentiation (Mathur et al., 1999b; Burr et al., 2001). Antibody blocking studies reveal that anti-CD80 and anti-CD86 antibodies have an effect on allergic asthma. In 1 study, they partially inhibited allergic asthma in mice with only anti-CD86 blocking IgE production and in combination abrogated disease (Mathur et al., 1999b). In another study, anti-CD86, but not anti-CD80, reduced allergic asthma in mice (Haczku et al., 1999) and anti-B7-2 monoclonal antibody (mAb) treatment inhibited all features of allergic asthma, but anti-B7-1 mAbs only suppressed lung inflammation (Keane-Myers et al., 1998). Further

evidence for the important role of costimulation in asthma is shown by CTLA4-IgG prevention and inhibition of established allergic asthma in mice (Van Oosterhout et al., 1997; Deurloo et al., 2001) and its ability to block the release of IL-5 and IL-13 from allergen-stimulated bronchial explant cultures from allergic patients (Jaffar et al., 1999a, 1999b). Additionally, there is evidence that CTLA-4 exon 1 polymorphism is associated with AHR, indicating that this difference could account for disease susceptibility or severity (Lee et al., 2002). Mechanistically, Th2 cell differentiation is associated with chromatin remodeling and hyperacetylation of histones H3 and H4 in the nucleosomes of IL-4, IL-13, and IL-5 genes. CD28 costimulation selectively enhances histone hyperacetylation of the IL-5 gene locus mediated through nuclear factor kappa B (NF- κ B) activation and GATA-3, demonstrating a selective role of CD28 costimulation during Th2 cell differentiation (Inami et al., 2004). B7-DC is an additional costimulatory molecule belonging to the B7 family expressed on DCs and activated macrophages (Latchman et al., 2001; Tseng et al., 2001) and binds to PD-1 on T-cells and acts as a negative regulator (Nishimura & Honjo, 2001). Treatment with B7-DC cross-linking antibody prevented allergic asthma in mice (Radhakrishnan et al., 2004). Taken together, these results indicate that costimulation plays a significant role in allergic asthma and can be manipulated to specifically downmodulate Th2-mediated allergic disease.

5.2. Th2 cell differentiation targets

5.2.1. Transcription factor targets

Following activation by peptide–MHC class II, Th cells differentiate into Th1 or Th2 cells. Intracellular factors involved in the transition have been identified. GATA-3 and c-Maf are selective transcription factors involved in Th2 differentiation and cytokine production. Conversely, T-bet is a transcription factor that determines Th1 cell differentiation and appears important for maintaining a Th1/Th2 balance. The selectivity of these transcription factors can be potentially utilized to specifically inhibit Th2 differentiation.

5.2.2. GATA-3

GATA-3 is a transcription factor that selectively regulates Th2 cell differentiation and is important in allergic asthma (Zhang et al., 1997a, 1999; Ray & Cohn, 1999). It is expressed at high levels in asthmatic T-cells (Caramori et al., 2001). GATA-3 expression must be sustained to maintain the Th2 phenotype (Pai et al., 2004), apparently by continuous chromatin remodeling of specific Th2 cytokine gene loci during cell division (Yamashita et al., 2004b). Ablation of GATA-3 in cells leads to increased DNA methylation at the IL-4 gene locus and decreased histone hyperacetylation at the IL-5 gene locus with resulting decreased cytokine expression (Yamashita et al., 2004b). Taken together, these data imply that inhibiting GATA-3 expression causes inhibition of Th2 cells.

Furthermore, there are important regulators of GATA-3 that provide additional targets for suppressing GATA-3 activity and potentially treating disease. For example, Schnurri-2 is a zinc finger protein that appears to regulate NF- κ B and GATA-3 and plays a regulatory role in Th2 cell differentiation (Kimura et al., 2005). Schnurri-2-deficient mice have increased Th2 differentiation owing to constitutive activation of NF- κ B and enhanced GATA-3 expression. Another example of a GATA-3 regulator is B-cell lymphoma (Bcl)-6, which is a transcriptional repressor protein that represses GATA-3. Evidence of its crucial role is shown in Bcl-6-deficient mice that develop more severe allergic asthma (Kusam et al., 2003). Inhibitors capable of downmodulating GATA-3 include GATA-3 antisense oligonucleotides (Finotto et al., 2001) and the Chinese herbal formula (MSSM-002; Srivastava et al., 2004); both inhibit established allergic asthma.

5.2.3. c-Maf

c-Maf is another Th2 cell-specific transcription factor (Ho et al., 1996; Hwang et al., 2002) that is selective for IL-4 gene transcription (Kim et al., 1999). Asthmatic patients have more c-Maf + T-cells in the sputum and bronchial biopsies compared with healthy controls (Taha et al., 2003). The importance of this transcription factor in asthma is illustrated in c-Maf-transgenic mice that demonstrate spontaneous Th2 cell differentiation (Ho et al., 1998) and in c-Maf-deficient mice that are unable to generate Th2 cells, presumably owing to reduced IL-4, but surprisingly maintain normal IL-13 and IgE responses (Kim et al., 1999). Furthermore, So-Cheong-Ryong-Tang, a Korean traditional medicine, suppresses c-Maf and specifically inhibits Th2 cells (Ko et al., 2004b). Selective c-Maf inhibitors provide a potential specific Th2 inhibitor for allergic asthma.

5.2.4. T-bet

T-bet (a member of the T-box family) and the proto-oncogene E26 transformation-specific-1 (Ets-1; an essential functional co-factor of T-bet; Grenningloh et al., 2005) play a key role in IFN γ -producing Th1 cells (Szabo et al., 2000; Mullen et al., 2001; Afkarian et al., 2002; Avni et al., 2002; Finotto et al., 2002; Gorelik et al., 2002; Mullen et al., 2002; Peng et al., 2002; Szabo et al., 2002). The overexpression of T-bet by retroviral gene transduction in primary T-cells induces IFN γ production by inducing chromatin remodeling at the IFN γ gene locus and via direct transactivation of the IFN γ gene promoter. Significantly, T-bet-deficient mice develop spontaneous Th2 disease (Finotto et al., 2002), demonstrating important regulation of the Th1/Th2 balance.

5.3. Inhibition of Th2 cells by Th1 cells

5.3.1. Th1 cell targets

The concept that there is a Th1/Th2 balance in the immune system is based on the dominant immune class responses to pathogens. Bacteria and viruses generate

predominantly Th1 responses while parasites generate Th2 responses and the resulting Th1 and Th2 cells produce cytokines that appear to mutually inhibit Th2 and Th1 cells, respectively. For example, Th1 cells reduce Th2-mediated allergic asthma in mice (Huang et al., 2001; Irifune et al., 2005). However, cotransfer of Th1 and Th2 cells is necessary for allergic asthma induction and, in some cases, augments preexisting allergic disease (Cohn et al., 1997; Hansen et al., 1999; Lee et al., 1999a; Randolph et al., 1999a, 1999b; Aronica et al., 2004a, 2004b). Conflicting results suggest that Th1 cells and their cytokines are capable of either inhibiting or enhancing allergic asthma. Utilizing Th1 immunity and cytokines in order to treat allergic asthma potentially has both positive and negative aspects. Especially important is the possibility of enhancing Th1 immunity and unintentionally producing autoimmunity. Despite disparate results, Th1 cells and their secreted products appear to predominantly downmodulate Th2 responses.

5.3.2. Interferon γ

The Th1 cytokine, IFN γ plays an especially important downregulatory role in allergic asthma. Th1 and CD8⁺ T-cell-derived IFN γ suppresses Th2 cells (Suzuki et al., 2002). Administered IFN γ inhibits Th2 lymphocyte differentiation and effector function and allergic asthma in mouse models (Hofstra et al., 1998b; Hayashi et al., 2002). The genetic overexpression of IFN γ (Gao et al., 2002) and mucosal IFN γ gene transfer leading to the expression of IFN γ in the lung epithelium (Li et al., 1996) inhibit allergic asthma in mice. There are, however, conflicting results which show that IFN γ -deficient mice have similar Th2 cell-mediated allergic inflammation compared with wild-type mice but have reduced immunoglobulin (Ig) and persistent disease, indicating that IFN γ is important for the resolution of inflammation (Coyle et al., 1996; Yoshida et al., 2002b). Additionally, treatment with anti-IFN γ suppresses AHR and chronic lung inflammation without altering lung eosinophilia or influencing airway remodeling (Hessel et al., 1997; Kumar et al., 2004). Finally, in severe steroid-dependent asthmatics, the administration of IFN γ was ineffective (Boguniewicz et al., 1993). These results are somewhat difficult to explain. However, in a recent study, a linear model of maturation of IFN γ cells has been proposed that implies a fundamental shift in the way we interpret how the balance of cytokines is regulated and may eventually provide a more satisfying understanding of the role of Th1-producing IFN γ cells in allergic disease (Loza et al., 2005).

5.3.3. Interleukin-12

IL-12 is essential for Th1 differentiation, cytokine production and proliferation; it is also a key suppressor of Th2 cells (Barnes, 2002; Caramori & Adcock, 2003). Patients with allergic asthma have reduced IL-12 production (Barnes, 2002; Caramori & Adcock, 2003) and have been successfully treated with IL-12 (Bryan et al., 2000; Matsuse

et al., 2003; Kuipers et al., 2004). However, clinical efficacy in 1 study with IL-12 was limited despite a reduction in blood and sputum eosinophilia; efficacy was also hampered by adverse side effects (Bryan et al., 2000). Successful treatment with IL-12 has also been shown in mouse allergic asthma models and in numerous experimental systems (Gavett et al., 1995; Kips et al., 1995, 1996; Lack et al., 1996; Bruselle et al., 1997; Kim et al., 1997; Hofstra et al., 1998a; Hogan et al., 1998; Schwarze et al., 1998; Lee et al., 1999b; Sur et al., 2000; Kuribayashi et al., 2002; Rais et al., 2002; Ogawa et al., 2003). IL-12 is an important mediator involved in Th cell differentiation that is especially relevant to antigen presentation (see DC target section) and in downmodulating Th2 immunity with Th1-mediated strategies. Although IL-12 is a potential target for the downmodulation of Th2 cells, its essential role in Th1 immunity cannot be compromised, and therefore, inhibiting IL-12 may not be a useful approach in allergic disease.

5.3.4. Interleukin-18

IL-18 is a cytokine that induces IFN γ and has a controversial role in allergic asthma because it also induces the development of Th2 cells, increases IL-4 and IL-13 production by NK cells, mast cells, and basophils (Nakanishi et al., 2001), induces allergic asthma in mouse models (Tsutsui et al., 2004), and generates memory Th1 cells via IL-4 and STAT6 pathways that exacerbate airway inflammation (Sugimoto et al., 2004). IL-18 levels in BAL are lower in patients with asthma (Ho et al., 2002), although they are higher in serum (Wong et al., 2001), and appear to correlate with disease activity (Tanaka et al., 2001). IL-18-deficient animals have diminished airway inflammation to inhaled antigen challenge but increased AHR and eosinophilia (Kodama et al., 2000). The administration of IL-18 increases IgE, Th2 cytokines, and eosinophilia (Kumano et al., 1999; Wild et al., 2000) but, in contrast, has also been shown to inhibit and induce IgE (Yoshimoto et al., 1997, 2000). The inhibition of IgE was related to an IL-12- and IL-18-mediated increase in IFN γ and subsequent reduction of IgE, while the induction of IgE observed in the later study was related to IL-18 induction of naive Th cells into IL-4-producing cells in vitro. Experiments using an IL-18 allergen-fusion DNA treatment have shown a reduction in AHR (Maecker et al., 2001), and IL-18 cDNA in a replication-deficient adenovirus system administered into the respiratory tract reduced allergic asthma (Walter et al., 2001). Collectively, these data suggest that IL-18 may be a target for disease, but its dual role in immunity needs to be clarified.

5.3.5. Interleukin-15, tumour necrosis factor, and TGF β

Cytokines such as, IL-15, TNF, and TGF β downmodulate Th2 cells. The expression of IL-15 is increased in the lungs of asthmatics compared with normal subjects (Muro et al., 2001) and plays a role in allergic asthma in mouse models (Ishimitsu et al., 2001; Yajima et al., 2002;

Ruckert et al., 2005). TNF is a cytokine that is used as a target for treating autoimmune disease. Inhaled recombinant human TNF induces airway inflammation and increases AHR, indicating a potential role for TNF in asthma (Thomas & Heywood, 2002). However, neutralizing anti-TNF antibodies only partially inhibit asthma and have no effect on eosinophilia (Hessel et al., 1997). Furthermore, TNF receptor-deficient mice have normal Th2 responses, suggesting that TNF does not play a critical role in allergic asthma (Rudmann et al., 2000). In contrast, immunization of mice with AutoVac TNF106, a recombinant murine TNF molecule containing a foreign immunogenic Th epitope, induces neutralizing anti-TNF autoantibodies, which reduce TNF levels and eosinophil infiltration in the BAL, thus illustrating a role for TNF in allergic inflammation and providing a novel strategy for treatment (Zuany-Amorim et al., 2004). TGF β is produced by many cells, including regulatory T-cells, which play a downregulatory role in allergic asthma (Zuany-Amorim et al., 2002) and may be involved in the mechanism underlying allergen immunotherapy (Till et al., 2004). TGF β effects were shown recently in mice heterozygous for deletion of the TGF β 1 gene, which express ~30% of wild-type TGF β 1 protein levels and develop increased lung eosinophilia, mucus secretion, high levels of Th2-cytokines, decreased IFN γ , and increased IgE (Scherf et al., 2005). Targeting these cytokines for the treatment of allergic asthma may be useful but may result in undesired immunosuppression.

5.3.6. Th1 pathogens

Pre- or coexisting Th1 polarizing infections are capable of downmodulating Th2 immunity. In some circumstances, T-cells primed with antigen in complete Freund's adjuvant (contains *Mycobacterium* proteins) inhibit a model of antigen-induced tracheal eosinophilia through IFN γ secretion (Sano et al., 1999). *Mycobacterium tuberculosis* has received attention because it induces predominantly Th1-mediated immunity and has a long-term history of safe utilization in the form of Bacille Calmette Guerin (BCG; Smit et al., 2004). BCG immunization establishes Th1 immune responses that protect against allergic sensitization (Herz et al., 1998; Major et al., 2002; Tsai et al., 2002; Sayers et al., 2004) and suppresses Th2-mediated immune responses in animals with established disease (Koh et al., 2001; Hopfenspirger & Agrawal, 2002; Choi & Koh, 2003). Specifically, mycobacterial cell wall lipoglycans, lipoarabinomannan, phosphatidylinositol mannan (Sayers et al., 2004), and *M. tuberculosis* chaperonins (Riffo-Vasquez et al., 2004) are components of *M. tuberculosis* with suppressive activity. Whole killed *Mycobacteria* or *Listeria* monocytogenes have been used to successfully inhibit AHR and eosinophilia in mouse models (Hansen et al., 2000; Zuany-Amorim et al., 2002). The mechanism of inhibition with *M. tuberculosis* and BCG in the mouse model appears due to the skewing of Th2 cells toward a Th1 phenotype (Sano et al., 1999). However, the administration

of whole bacterium appears to induce T reg cells rather than skewing of Th2 to Th1 immunity (Zuany-Amorim et al., 2002).

The role of other microbials augmenting Th1 and simultaneously diminishing Th2 immunity has been shown. In conflicting studies, prior infection with Influenza A virus led to more severe allergic asthma (Dahl et al., 2004) or to suppressed lung eosinophilia and augmented AHR and goblet cell metaplasia (Wohlleben et al., 2003). Clinical studies have indicated that the incidence of allergy in children relates to enteric lactobacteria populations and can be reduced by supplementation with Th1-promoting probiotic lactobacilli and bifidobacteria (Cross & Gill, 2001). Cholera toxin (Wiedermann et al., 1998), *Mycoplasma pneumoniae* (Chu et al., 2003), or administration of lipoprotein I from *Pseudomonas aeruginosa* (Revets et al., 2005) decreases Th2 responses and reduces the ability to induce allergic asthma. Potential treatments that bias immune responses away from Th2 toward Th1 immunity may be effective in allergic asthma, although there are some conflicting results. BCG immunization has been used as an adjuvant with immunotherapy and showed no clinical improvement (Arikan et al., 2004), but there has been an abundance of reports showing that augmenting Th1 immunity with microbial CpG improves clinical effectiveness (see Section 5.3.7). Pathogens that induce an adaptive Th1 immune response also engage the innate immune system, particularly in the lung (Basu & Fenton, 2004). Microorganisms utilize the Toll-like receptors on the surface of cells, such as macrophages to induce activation and the production of cytokines. These effects subsequently influence the microenvironment leading to a Th1 cytokine milieu and downmodulating allergic asthma. Toll-like receptor agonists have been used successfully to treat allergic asthma in animal models (Velasco et al., 2005).

5.3.7. Immunostimulatory oligodeoxynucleotides

An important mechanism underlying microbially induced Th1 immunity is based on immunostimulatory sequences of bacterial DNA. Oligodeoxynucleotides (ODNs) containing unmethylated cytosine–phosphate–guanine CpG motifs (CpG-ODNs) combined with antigen DNA result in DNA vaccines with immunostimulatory oligodeoxynucleotides (ISS-ODNs) that are supposed to resemble bacterial DNA (Kitagaki et al., 2002; Hussain & Kline, 2003; Hussain et al., 2004; Jarman & Lamb, 2004). An example of bacterial DNA downmodulating allergic disease has been shown in an experiment in which whole-cell *B. pertussis* was introduced in mice and found to inhibit IL-4 and IL-5, allergic lung inflammation, and AHR. The mechanism appeared to be mediated by *B. pertussis* DNA containing unmethylated CpG (Kim et al., 2004). ISS-ODNs powerfully skew immune responses towards Th1 and suppress Th2 responses, with concomitant increases in IL-6, IL-10, IL-12, IL-18, and IFNs, up-regulation of costimulatory molecules, and induction of Th1 class switching of allergen-

specific human T-cells in vitro (Hussain et al., 2002a, 2002b, 2004; Kitagaki et al., 2002; Chiang et al., 2003; Hussain & Kline, 2003). ISS-ODNs have also been used to successfully inhibit allergic rhinitis (Caramori & Adcock, 2003) and allergic asthma (Jahn-Schmid et al., 1999; Kline et al., 1999; Sur et al., 1999; Serebrisky et al., 2000; Broide et al., 2001; Hussain & Kline, 2001; Jain et al., 2002; Chiang et al., 2003; Jain et al., 2003; Vissers et al., 2004b).

5.4. Th2 cell activation targets

Suppression of the effects of Th2 cells can be achieved by inducing anergy (Th2 cell unresponsiveness) or by elimination of the cells themselves. Several commonly used drugs, as well as some others that are in development, attempt to inhibit Th2 cell activation, proliferation, or survival. The most common T-cell suppressive strategies in use for allergic asthma are immunotherapy and steroids. Other inhibitors of T-cell activation for allergic asthma include calcineurin inhibitors, methotrexate, and nondepleting mAbs directed against T-cells. Additionally, there are several compounds that antagonize molecules involved in cell signaling and metabolism, including phosphodiesterase 4 (PDE4), mitogen-activated protein kinases (MAPK), p38 MAPK, c-Jun NH2-terminal kinase (JNK), and phosphoinositide 3-kinase (PI3K), in addition to a variety of others. Suppressive approaches for established Th2 cells in allergic disease are especially useful if they prevent the activation of memory Th2 cells and inhibit activated effector cells. Interfering with signal transduction is a useful strategy for activated cells that have up-regulated activation and effector pathways. However, blocking signaling molecules may not effectively function in resting memory Th2 cells that do not have active signal transduction. Moreover, approaches for inhibiting Th2 immunity are promising especially if they are allergen specific or Th2 cell specific to avoid general immunosuppression.

5.4.1. Allergen immunotherapy

Allergen immunotherapy is well-tolerated and effective in allergic disease in humans and mouse models (Liu et al., 2003; Kay & Larche, 2004; Norman, 2004; Larche & Wraith, 2005). Allergens in many forms suppress allergic responses. They include allergenic extracts, purified proteins, purified peptides, and peptide epitopes. Their effectiveness has been clearly documented using intradermal, subcutaneous, inhalation, intranasal, oral, and sublingual routes of administration (Haselden et al., 1999, 2001; Ali et al., 2002, 2004; Canonica & Passalacqua, 2003; Canonica et al., 2004; Gardner et al., 2004b; Norman, 2004; Bahceciler et al., 2005; Verhoef et al., 2005). The evolution of classical immunotherapy has been toward noninjectable forms of therapy (Cannonica & Passalacqua, 2003) and toward modified allergens, including peptides designed to react with T-cells and not to cross-link IgE (Haselden et al., 2001; Larche & Kay, 2004; Larche et al., 2004; Verhoef et al.,

2005), allergens conjugated with polyethylene glycol (Lavoie et al., 2003), or provided as genetically modified plant vaccines (Smart et al., 2003), chimeric human–cat fusion proteins combining a truncated human IgG-Fcγ1 and cat allergen (Zhu et al., 2005), DNA vaccines encoding allergen (Li et al., 2005), or in an oral microencapsulated forms (TePas et al., 2004). Increasing allergen dosing has also demonstrated improved effectiveness (Reefer et al., 2004).

The mechanism of action of allergen immunotherapy is unknown (Bousquet, 2005). Two main theories propose to explain how allergen inhibits memory Th2 cells: (1) skewing from Th2 to Th1 immunity and (2) generation of inhibitory T reg cells. There is evidence for both mechanisms. Allergen immunotherapy causes elevation of Th1 antibody isotypes and allergen-specific IgG (mainly IgG4), which blocks IgE-dependent histamine release from basophils; it augments IgE-mediated antigen presentation to T-cells, increases Th1 cytokines, and induces differential apoptosis (Benjaponpitak et al., 1999; Majori et al., 2000; Wachholz et al., 2002; Fu et al., 2003; Wachholz et al., 2003; Gardner et al., 2004a). However, some studies show that there is no alteration in the Th1/Th2 ratios in patients receiving allergen immunotherapy (Huang et al., 2003). Allergen immunotherapy also increases IL-10 and TGFβ production and correlates with the generation of regulatory CD4+CD25+ T-cells (Till et al., 2004) that are capable of suppressing allergen-specific Th2 memory cells (Akdis et al., 1998; Francis et al., 2003; Jutel et al., 2003; Zemmann et al., 2003; Gardner et al., 2004b; Nouri-Aria et al., 2004; Reefer et al., 2004; Robinson et al., 2004; Smith et al., 2004; Stock et al., 2004; Vissers et al., 2004a; Alexander et al., 2005; Verhoef et al., 2005). Conversely, in a recent study with sublingual immunotherapy in children, there were no changes in T-cell parameters (Rolink-Werninghaus et al., 2005), suggesting an entirely different mechanism. Irrespective of whether inhibitory Th1 or T reg cells are generated, the precise mechanisms leading to allergen-induced suppression remain unclear.

5.4.2. Glucocorticoids

Steroids are the most effective drugs for allergic asthma. They have direct effects on T-cell activation and cytokine expression in the airways of asthmatics (Robinson et al., 1993; Leung et al., 1995; Bentley et al., 1996; Barnes, 1998), although their mechanism of action is only partially owing to T-cell inhibition. Inhaled corticosteroids inhibit T-cell recruitment and diminish T-cell numbers in the airway (Masuyama et al., 1994; Gemou-Engesaeth et al., 1997; Umland et al., 1997; Hamilos et al., 1999; O'Sullivan et al., 2003). This may be a result of apoptosis of resident T-cells, but the mechanism is unclear because corticosteroids can protect against TCR-driven apoptosis (Ashwell et al., 2000) and T-cells from asthmatics tend to be resistant to Fas-induced apoptosis (Jayaraman et al., 1999). Steroid withdrawal is often associated with disease relapse, with

increased eosinophils in bronchial biopsies and airway T-cells showing an independent role for airway T-cells in mediating disease exacerbations (Castro et al., 2004). Remarkably, exposure to long-term treatment with steroids may lead to paradoxical increase in Th2 cytokines (Wiley et al., 2004). Steroids remain the most effective treatment of disease. A recent study found that inhalation with once-daily budesonide therapy in patients with mild persistent asthma was safe and well tolerated in both children and adults (Sheffer et al., 2005).

5.4.3. Calcineurin inhibitors

Suppressing Th2 memory cells by interfering with activation and signal transduction may inhibit disease, but, without selectivity, these drugs and compounds will induce immunosuppression. Cyclosporin A inhibits calcineurin NF-AT transcription factor, resulting in the suppression of allergen-specific T-cell proliferation, IL-2, IL-4, IL-5, and GM-CSF, induction of T-cell apoptosis, and decreased Bcl-2 gene product levels (Umland et al., 1999; Ying et al., 2003). Cyclosporin A has been shown to inhibit allergen-induced IL-5 and GM-CSF in BAL, eosinophils in blood and bronchial mucosa, and to have a steroid sparing effect, but its efficacy was limited (Nizankowska et al., 1995; Lock et al., 1996; Eynott et al., 2003). SAR943 (32-deoxorapamycin) inhibits eosinophilic and lymphocytic lung infiltration and IL-4 and IL-5 but has no effect on chronic eosinophilia (Eynott et al., 2003). Sirolimus (rapamycin) inhibits antigen-specific T-cell proliferation and IL-5 production in PBMCs from patients with allergic asthma (Powell et al., 2001). Concerns about adverse side effects and generalized immunosuppression with systemic administration of calcineurin inhibitors have limited their therapeutic usage to topical formulations of tacrolimus and pimecrolimus that are very effective in atopic dermatitis.

5.4.4. Anti-CD4 antibodies

Antibodies directed at Th cells have been attempted with some success and some caution (Li et al., 1999; Kon et al., 2001). Nondepleting anti-CD4 mAbs have been shown to provoke a Th1 to Th2 shift, induce tolerance to allergens during initial priming, inhibit T-cell proliferation, and stimulate a distinct pattern of tyrosine phosphorylation in T-cells (Li et al., 1999). Keliximab is a chimeric antibody against CD4 that reduces circulating CD4⁺ cells and is effective in asthma (Kon et al., 2001). However, it is not clear whether this approach can also inhibit Th1 cells leading to Th1 immunosuppression.

5.4.5. Phosphodiesterase 4 inhibitors

PDE4 is a dominant PDE isoenzyme in inflammatory cells that is involved in the regulation of cAMP. Atopic patients have high expression of the isoform, PDE4 (Chan & Hanifin, 1993). There are PDE4 selective inhibitors that reduce hydrolysis of intracellular cAMP, resulting in decreased proliferation as well as IL-4 and IL-5 production

(Torphy, 1998). Roflumilast, piclamilast, rolipram, AWD 12-281, and cilomilast are selective PDE4 inhibitors that are effective in allergic asthma (Harbinson et al., 1997; Bundschuh et al., 2001; Giembycz, 2001; Kumar et al., 2003; Kuss et al., 2003; Draheim et al., 2004; Lipworth, 2005), and combinations of inhibitors, such as, cilostazol (PDE 3), RO 20-1724 (PDE 4), and sildenafil (PDE 5), also inhibit allergic asthma in mice (Clayton et al., 2004).

5.4.6. Signal transduction inhibitors

Inhibiting proteins and enzymes involved with T-cell signal transduction are possible targets. Imatinib mesylate, 4-[(4-methyl-1-piperazinyl)methyl]-N-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-phenyl]benzamide methanesulfonate, inhibits receptor tyrosine kinase-mediated activation and suppresses allergic asthma (Wong & Leong, 2004; Berlin & Lukacs, 2005). MAPK are involved in signal transduction pathways and inflammation. Specifically, inhibitors of p38 MAPK may preferentially inhibit Th2 cytokines. For example, U0126, a specific MAPK/ERK kinase inhibitor (Duan et al., 2004; Chialda et al., 2005) and an inhaled p38 MAPK antisense oligonucleotide (Duan et al., 2005) inhibit allergic immunity in mice. In addition, SP600125 (anthra [1, 9-cd] pyrazole-6 (2H)-one) inhibits JNK (Chialda et al., 2005; Nath et al., 2005), and combination compound, CGH2466, which inhibits adenosine A1, A2b, and A3 receptors, p38 MAPK, and PDE4D inhibit allergic asthma in mice (Trifileff et al., 2005). PI3K has a regulatory role in Th2-cell cytokine secretion, airway inflammation, and airway hyperresponsiveness in mice and can inhibit the activated phosphorylation of protein kinase B with a dominant negative form of the class IA PI3K subunit (Myou et al., 2003). The effectiveness and specificity of targeting signal transduction for allergic asthma might be improved by analyzing data with mathematical models. In a recent study of the MAPK pathway, it was shown that the analysis of multiple variables dependent upon the input conditions could shed light on the overall biology (Barbano et al., 2005). Modeling of experimental and in silico data offers new opportunities for drug discovery for signaling and metabolic pathways.

5.4.7. Miscellaneous inhibitors

A series of additional, potential T-cell targets is being investigated. TIM-1, a member of the T-cell Ig domain, mucin-like domain gene family, encodes a transmembrane protein expressed by Th cells and is genetically linked to asthma (polymorphic TIM-1 variants associated with past infection with hepatitis A virus and protection against atopy; McIntire et al., 2004). Recently, TIM-1 was found to bind to TIM-4, a receptor expressed on APC. Interruption of TIM-1–TIM-4 binding with either a soluble TIM-1–Ig fusion protein or TIM-4–Ig fusion protein reduced T-cell proliferation (Meyers et al., 2005). This may be a new target for the treatment of allergic asthma. Mycophenolate mofetil, an

inhibitor of inosine monophosphate dehydrogenase that inhibits lymphocyte proliferation, and sirolimus (rapamycin) both inhibit allergen-specific T-cell proliferation and IL-5 production in PBMCs from atopic asthmatic patients (Powell et al., 2001). The antifolate agent methotrexate has been used as an adjunct to high dose inhaled corticosteroids (Bardin et al., 1993; Corrigan, 2002) and so may a newly synthesized methotrexate derivative, MX-68, which inhibits AHR, airway inflammation, IL-4 and IL-5, and serum IgE and IgG levels in guinea pigs (Nagao et al., 2004). Resiquimod (R-848), an imidazol derivative, reduced AHR and BAL eosinophils and lymphocytes and inhibited mucus gland hyperplasia and decreased Th2-cytokine production in the lung while raising IL-12 and IFN γ (Quarcoo et al., 2004). MLD987 is a macrolide compound that acts locally upon inhaled or intratracheal instillation to inhibit T-cell activation, proliferation, and cytokine release has diminished the intensity of allergic asthma in rats (Hersperger et al., 2004).

5.5. Th2 cell migration targets

Recruitment of Th2 cells into the lungs is important for the pathogenesis of disease. However, the ability of Th2 cells to circulate in and out of the lungs is important for disease exacerbation and immunological memory. The driving force mediating T-cell trafficking is chemokine–CKR interactions and adhesion molecules. The chemokine gradient and the recruitment of cells with the appropriate CKRs are the mechanisms underlying homing of cells to sites of inflammation. Up-regulation of CKR during T-cell activation will enable the cell to enter the lungs and airways. However, it is possible that cells may then down-regulate CKR in order to remain in the tissue. Memory Th cells are distinct subsets characterized by cell surface expression of CKR and adhesion molecules, such as CCR7, CD62L, and CD45RB, which correlate with effector function, capacity to home to lymphoid organs, and sites of tissue inflammation (Ahmadzadeh et al., 2001; Hogan et al., 2001; Roberts & Woodland, 2004).

5.5.1. Chemokine–chemokine receptor targets

The presence of allergen-specific Th2 memory cells from the lung infiltrates of mice recovered from asthma suggests that they are either continuously circulating to and from the lung or that they remain in situ. Allergen exposure would activate resting memory Th2 cells causing the up-regulation of CKR and migration of the cells to local draining lymph nodes and the circulation and then back to the lungs. There is evidence that during disease exacerbation, recruitment of effector T-cells is more important than the expansion of in situ lung and lymphoid T-cells lymphocytes (Harris et al., 2002; Reinhardt et al., 2003), and activated T-cells tend to migrate from the regional lymph nodes to the lung and airway, where they secrete IL-4 but do not proliferate (Harris et al., 2002).

During remission, memory Th2 cells appear to reside within the lungs and spleen rather than in draining lymph nodes (Mojtabavi et al., 2002), suggesting that allergen-specific memory Th2 cells circulate upon activation. Interestingly, lung CD4 T-cells from recovered mice are capable of adoptively transferring disease to naive recipients, whereas splenic CD4 T-cells do not (M. Epstein, unpublished observation). These data suggest that there is a particular characteristic, most likely a CKR, that enables lung memory Th2 cells but not spleen memory Th2 cells to home back to the lungs.

CKR of particular importance in allergic disease include CCR3, CCR4, CCR6, CCR7, and CCR8. Although there are a few CKR that are apparently selective for Th2 cells, data supporting specific roles for these receptors in asthma are limited. BAL Th cells derived from patients with allergic asthma express CCR2, CCR4, CCR5, CCR6, and CXCR3. Upon allergen challenge, the percentage of CCR4 and CCR7 positive cells increases, but that of CXCR3 cells decreases (Kallinich et al., 2005), suggesting a pathogenic role for these receptors in exacerbation. In addition to these CKR, allergic donors express chemokines, CCL17 (TARC), and CCL22 (MDC) that recruit CCR4 Th2 cells (Pilette et al., 2004), lending support to the importance of this ligand–receptor interaction in disease pathogenesis. However, administering anti-CCR4 antibodies does not alter the generation of allergic asthma in guinea pigs (Conroy et al., 2003) and allergic asthma can be generated in CCR4-deficient mice (Schuh et al., 2002), data that argue against a central role for CCR4 in allergic asthma. CCR8 is a chemokine receptor selective for Th2 cells, but CCR8-deficient mice develop normal Th2 immune responses and experimental lung eosinophilia, and the administration of antibody against the known ligand for CCR8, or the chemokine ligand TCA-3, does not alter the generation of airway allergic inflammation. (Chung et al., 2003). By contrast, CCR6 and its ligand MIP-3 α play a role in allergic asthma in mouse models (Lukacs et al., 2001; Lundy et al., 2005). CCR6-deficient mice develop less severe allergic asthma characterized by reduced AHR, airway eosinophils, IgE, and IL-5 compared with control mice, which is owing to a combined defect in T-cell activation and lung APC.

CRTH2 is a PGD2 CKR-homologous molecule expressed on Th2 cells, eosinophils, and basophils (Nagata et al., 1999a, 1999b; Cosmi et al., 2000; Spik et al., 2005). All CRTH2+ CD4+ T-cells have a Th2 phenotype and are attracted by PGD2 and TARC (Iwasaki et al., 2002). PGD2 is a specific chemoattractant for Th2 cells (Hirai et al., 2001). Several CRTH2 agonists are available and have been shown to recruit eosinophils and Th2 cells (Shiraishi et al., 2005). The newest of the agonists is L-888,607; it is highly selective for CRTH2 (Gervais et al., 2005). There are also several CRTH2 antagonists. Ramatroban and a newly modified form of the drug are orally active antagonists for CRTH2 that inhibit PGD2-induced migration (Shichijo et

al., 2003; Sugimoto et al., 2003; Ulven & Kostenis, 2005). In addition, there is a highly specific 4-aminotetrahydroquinoline derivative-CRTH2 antagonist that has recently been developed (Mimura et al., 2005). Antagonizing both Th2 cells and eosinophils has added advantages for the treatment of asthma in mice.

The role of Th1-type chemokines in asthma is controversial. CXCL10 (IP-10) is a chemokine induced by IFN γ , which preferentially recruits activated Th1 cells, but is up-regulated in asthmatic airways. In a series of experiments, IP-10 was up-regulated in the lung in mice with allergic asthma. AHR, lung eosinophilia, and IL-4-secreting Th cells were increased in transgenic mice that overexpressed lung IP-10, whereas IP-10-deficient mice had less severe disease (Medoff et al., 2002). Taken together, these results suggest a role for this polarized Th1-type chemokine in allergic asthma.

5.5.2. Arrestins

Arrestins are G protein-coupled receptor regulatory proteins that affect CKR function. T-cells lacking β -arrestin-2 have impaired MDC-mediated migration, a finding observed in β -arrestin-2-deficient mice that are unable to recruit T-cells in the airways and do not develop allergic asthma upon immunization and lung challenge (Walker et al., 2003). The specificity of these findings has not yet been confirmed.

5.5.3. Adhesion molecules

Cell-adhesion molecules are essential in allergic inflammation (Bochner, 2004). T-cell infiltration into the tissue involves an initial step of rolling on vascular endothelial cells, a process which is mediated via adhesion molecules. In allergic asthma, the recruitment of T-cells to lungs has been shown, by using blocking antibodies and genetically deficient transgenic mice, to be dependent upon adhesion molecules. The specific molecules include E-selectin, P-selectin, very late activation Ag-4 (VLA-4), and lymphocyte function associated antigen-1 (LFA-1; Kanwar et al., 1997; Abraham et al., 2000; McCafferty et al., 2000; Fiscus et al., 2001; Tang & Fiscus, 2001; Curtis et al., 2002; Issekutz & Issekutz, 2002; Lukacs et al., 2002; Stephens & Chaplin, 2002; Pitchford et al., 2005). In an experimental allergic asthma model in rabbits, treatment with blocking mAbs against CD49d (alpha (4) integrin), CD18 (β 2-integrin), and the alpha subunits of β 2-integrin CD11a (LFA-1 integrin) and CD11b (Mac-1 integrin) demonstrates that lymphocyte recruitment appears to be mediated by a combination of LFA-1 (CD18/CD11a) and Mac-1 (CD18/CD11b; Gascoigne et al., 2003).

In a small clinical study, Efalizumab, the humanized mAb against the LFA-1 alpha chain, inhibited allergic infiltration in induced sputum (Gauvreau et al., 2003). No Th2-cell-specific adhesion molecules have been identified as yet.

5.5.4. Migration inhibitors

FTY720 is a chemical derivative of myriocin, a metabolite of the ascomycete *Isaria sinclairii*. The phosphorylated derivative of FTY720 is a sphingosine 1-phosphate receptor agonist capable of sequestering lymphocytes in secondary lymphoid organs and preventing migration to sites of inflammation. Oral FTY720 suppresses AHR and Th2 cell-induced T-cell and eosinophilia recruitment into lungs in a model generated by adoptive transfer of in vitro differentiated antigen-specific T-cells. However, it does not impair T-cell activation, proliferation, or recall responses to antigen and evokes no selective expression of the receptor between Th1 and Th2 cells. It may provide an interesting opportunity for the treatment of asthma, but its lack of specificity and selectivity could perhaps result in a more generalized immunosuppression (Sawicka et al., 2003).

5.6. Th2 effector function targets

The cytokines produced by Th2 cells are critical to the pathogenesis of allergic asthma. Memory Th2 cells within the lungs are activated and up-regulate IL-4, IL-5, IL-6, IL-10, and IL-13 within 1 hr of aeroallergen challenge (Mojtabavi et al., 2002). The rapid response to allergen initiates a cascade, which recruits inflammatory cells into the lungs, induces AHR, and evokes goblet cell mucus hypersecretion. Inhibiting the effector arm of memory Th2 cells would effectively prevent the cascade. Inhibiting Th2 effector cytokines would serve to treat disease but may not cure it. However, Th2 cytokines, in particular IL-4, are autocrine regulatory factors. It is possible that growth and differentiation of memory Th2 cells would be inhibited in the absence of any Th2 cytokine growth factors, thereby reducing Th2-mediated responses.

5.6.1. Interleukin-5

IL-5 has been the favoured Th2 cytokine to inhibit because it plays a central role in eosinophil differentiation, maturation, recruitment, and survival. Eosinophils are required for protection against parasitic infections but have little influence in other aspects of immunity. A great number of studies have shown indisputable evidence that IL-5 is essential for the initiation and perpetuation of allergic asthma (Leckie et al., 2000; Leckie, 2003), along with some conflicting data regarding the role of IL-5 in airway remodeling (Cho et al., 2004; Leigh et al., 2004a). IL-5 neutralizing mAbs in mice clearly show abrogation of both acute and chronic eosinophilia (Hessel et al., 1997; Mathur et al., 1999a; Kumar et al., 2004) but less of an effect on AHR and mucus secretion (Hessel et al., 1997). A role for IL-5 in airway remodeling in humans has been shown (Flood-Page et al., 2003a). Mepolizumab, a humanized mAb specific for human IL-5, inhibits eosinophilia in monkeys without affecting AHR (Hart et al., 2001). In humans, it decreases mature eosinophil numbers in the

bone marrow and bronchial mucosa (Menzies-Gow et al., 2003), reduces peripheral blood eosinophils (Buttner et al., 2003), decreases the expression of proteins important for airway remodeling in bronchial mucosa, decreases airway eosinophils expressing mRNA for TGF β , and reduces the concentration of TGF β in BAL (Flood-Page et al., 2003a) and of eosinophil infiltration into the skin (Phipps et al., 2004). Similarly, blood and sputum eosinophils have been reduced without inhibiting AHR (Flood-Page et al., 2003b). Despite these results, blocking IL-5 in patients has not been as beneficial as expected, possibly because eosinophils play a smaller role in AHR and mucus hypersecretion in humans, although this has yet to be elucidated. Galectin-3 is a member of a β -galactoside-binding animal lectin family that is expressed at high levels in BAL macrophages and overexpressed in peribronchial inflammatory cells that increase during active disease (Zuberi et al., 2004). Galectin-3-deficient mice develop fewer eosinophils and less goblet cell metaplasia, less AHR, lower Th2 responses, but higher Th1 responses, suggesting that galectin-3 regulates the Th1/Th2 response (Zuberi et al., 2004). Plasmid DNA encoding galectin-3 reduces eosinophil and T-cell counts in BAL, inhibits IL-5 mRNA in lungs, and reduces AHR in allergic asthma in mice (del Pozo et al., 2002; Del Pozo et al., 2005).

5.6.2. Interleukin-4

IL-4 is a central cytokine in asthma as has been shown in animal models (Brusselle et al., 1994; Shimoda et al., 1996; Gavett et al., 1997; Justice et al., 2002; Leigh et al., 2004a). Although blocking IL-4 in some experimental systems has little effect on allergic asthma (Hessel et al., 1997), in humans and mice, IL-4 antagonists are effective (Henderson et al., 2000; Steinke & Borish, 2001; Tomkinson et al., 2001; Steinke, 2004). Soluble IL-4 receptors have been shown to improve lung function, stabilize asthma symptom scores, reduce β -agonist use, and reduce exhaled nitric oxide (Borish et al., 1999; Borish et al., 2001). Approaches for inhibiting IL-4 include soluble human IL-4 receptor, humanized anti-IL-4, IL-4 mutein, and human anti-IL-4 receptor, all of which are available and are summarized in a recent review (Steinke, 2004). The results from clinical studies have documented their effectiveness, but they are not as promising as expected, for reasons that are not entirely obvious. Additional approaches inhibiting IL-4 more proximally include blocking IL-4 transcription factors and signaling pathways with agents such as Stat6 (Foster, 1999) or SOCS-1, an endogenous inhibitor of STATs (Losman et al., 1999). It is possible that a more proximal interruption of IL-4 production would be more effective than blocking ligand–receptor interactions. Although IL-4 is a key growth factor for Th2 cells, it has been shown to be unnecessary for the maintenance of memory Th2 cells. Memory Th2 cells maintained Th2-specific remodeled chromatin in both the IL-4 and IL-13 gene loci independent of IL-4 (Yamashita et al., 2004a),

implying that IL-4 is not necessary for memory Th2 cell survival. This might explain the less than optimal efficacy in clinical studies with anti-IL-4.

Several Th2 cytokine inhibitors are available that inhibit both IL-4 and IL-5 production. M50367 is an oral benzimidazole derivative that decreases IL-4 and IL-5 production, increases IFN γ production, suppresses Th2 differentiation, and down-regulates Th2 response in a murine model of atopic asthma (Kato et al., 1999, 2003, 2005). Suplatast tosilate is a drug that selectively inhibits IL-4 and IL-5 production and prevents allergic asthma in mouse models (Oda et al., 1999). Oral suplatast tosilate therapy in patients with mild and moderate asthma, decreased blood and sputum eosinophils, exhaled nitric oxide level, and AHR (Yoshida et al., 2002a) improved lung function, symptom score, and eosinophilia (Sano et al., 2003). Histone deacetylase inhibitor, Trichostatin A, reduces allergic airway inflammation by decreasing the expression of the Th2 cytokines, IL-4 and IL-5, and IgE (Choi et al., 2005). The Japanese herbal medicine, Sho-seiryu-to (Xiao-Qing-Long-Tang in Chinese), inhibits cytokine production and allergic asthma in mice (Nagai et al., 2004) and Chung-Yeul-Gue-Soup-Sa-Gan-Tang, a traditional Korea herbal medicine, increases Th1 and decreases Th2 specific cytokine production (Ko et al., 2004a). Taken together, IL-4 is an essential cytokine in allergic disease and, although blocking IL-4 studies have not yet shown the effectiveness that one would expect, IL-4 blockers may still prove effective, either by themselves or in combination with other anti-Th2 cytokines.

5.6.3. Interleukin-13

IL-13 is 1 of the 3 Th2 cytokines important for the initiation and perpetuation of allergic asthma. IL-13 induces the pathophysiological features of asthma independent of IgE and eosinophils (Wills-Karp et al., 1998). It mediates AHR, mucus hypersecretion, and eosinophilia and is particularly important for sustained AHR and airway remodeling (Leigh et al., 2004a). Pulmonary expression of IL-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, and eotaxin production (Zhu et al., 1999). IL-13 shares a receptor and signaling pathway with IL-4, but may function via alternative intracellular pathways. Anti-IL-13 effectively suppresses lung inflammation and partially suppresses airway remodeling, goblet cell hyperplasia, and subepithelial fibrosis. Anti-IL-13 has had little effect on AHR (Kumar et al., 2004) in 1 study, but in another study has been found to be effective (Yang et al., 2004). Soluble humanized IL-13 receptor acts as a decoy that may be a therapeutic agent for atopic disease. The results from clinical trials are pending. IL-4 receptor antagonist also inhibits IL-13 in a mouse model (Tomkinson et al., 2001). An adenosine–IL-13 amplification pathway operates in IL-13 transgenic mice (Blackburn et al., 2003), suggesting a role for adenosine modulators as therapy for allergic asthma. Acidic mammalian chitinase

induces IL-13 and may be a novel target for the therapy of allergic disease (Zhu et al., 2004).

5.6.4. Interleukin-6, interleukin-9, and interleukin-25

IL-6, IL-9, and IL-25 are cytokines produced by Th2 cells that may lead to novel targets for the treatment of allergic asthma. The observation that asthmatic patients have increased levels of the soluble IL-6 receptor has led to studies showing that interrupting IL-6 signaling with soluble IL-6 receptor inhibits mouse allergic asthma via T reg cells (Doganci et al., 2005). IL-9 is an important inflammatory mediator in asthma that can be blocked with anti-IL-9 antibody and reduces airway inflammation and AHR (Temann et al., 1998; Levitt et al., 1999; Bhathena et al., 2000; Soussi-Gounni et al., 2001; Cheng et al., 2002; McMillan et al., 2002; Temann et al., 2002; Thomas & Heywood, 2002). IL-25 is a cytokine that induces IL-4, IL-5, and IL-13 gene expression, as well as increasing IgE, IgG1, and IgA levels, blood eosinophilia, eosinophilic lung infiltrates, mucus production, and epithelial cell hyperplasia and hypertrophy; it appears to function via APC (Fort et al., 2001).

5.6.5. Arginase

Arginases I and arginase II are cationic amino acid transporters involved in the metabolism of basic amino acids that are prominently expressed in submucosal inflammatory lesions of patients with allergic asthma (Zimmermann et al., 2003; King et al., 2004). They are up-regulated during allergen challenge and regulate several important functions in the airway, including nitric oxide generation, cell growth, and collagen deposition (Shi et al., 2001; Iyer et al., 2002; Zimmermann et al., 2003). Arginase gene expression and enzyme activity is enhanced by IL-4 and IL-13. It has been suggested that arginase is an effector of Th2 cells (Zimmermann et al., 2003), which may provide an effective target for the treatment of allergic disease.

5.6.6. Th2 cell help for B-cell IgE production targets

Cooperation between Th2 cells and B-cells is necessary for B-cell differentiation, productive isotype switching, and IgE production. Animals that have recovered from allergic asthma have B-cells within their lungs, which, upon subsequent allergen exposure, differentiate to plasma cells producing high levels of IgG1 and IgE in BAL (Jungsuwadee et al., 2002; Mojtavavi et al., 2002). Clinical trials indicate that Omalizumab, an anti-IgE mAb that reduces inflammation, provides symptomatic relief and reduces steroid use in patients with allergic asthma (Buhl, 2005; Holgate et al., 2005). Furthermore, the effects of anti-IgE include a reduction in Fc receptor expression that, in turn, reduces antigen presentation affecting Th2 cells. Thus, interfering with IgE production by B-cells may create an autoregulatory loop by initially reducing IgE and then decreasing Th2 stimulation.

5.7. Th2 survival targets

The maintenance and perpetuation of memory responses against allergens is an undesirable consequence of immunological memory. It leads to the perpetuation of allergic immunity upon repeated exposure to allergen. Although the memory response is long lived, it is not clear whether memory cells have a unique ability to sustain themselves and self-perpetuate or are continuously regenerated and restimulated. The factors promoting the longevity of immunological memory, death, turnover, circulation, and differentiation have not been elucidated. To maintain homeostasis in the immune system, cells are continuously being generated and eliminated, using mechanisms that both induce and protect against apoptosis. The induction of apoptosis for the elimination of Th2 cells is a potential strategy for the treatment of allergic asthma. It has been achieved to some extent with cyclosporin A, which induces apoptosis and correlates with decreased expression of the antiapoptotic Bcl-2 protein (Ying et al., 2003). An additional strategy is the use of recombinant adenovirus expressing murine FasL administered intratracheally, which reduces allergic asthma in mice (Chuang et al., 2004). Furthermore, there is evidence suggesting that Th2 dominance in allergic asthma correlates with susceptibility to apoptosis. T-cells from p53-deficient mice have decreased apoptosis and increased Th2 differentiation (Ohkusu-Tsukada et al., 1999). CTLA-4 has been shown to promote T-cell apoptosis (Lee et al., 1998; Oosterwegel et al., 1999a; da Rocha Dias & Rudd, 2001), and CTLA-4-deficient Th cells are directed towards Th2 differentiation (Oosterwegel et al., 1999b; Ubaldi et al., 2003). Moreover, Th2 cells are more resistant to apoptosis than Th1 cells (Ramsdell et al., 1994; Zhang et al., 1997b), favouring Th2 immunity. Th1-derived IFN γ induces Fas and FasL expression, leading to apoptosis of Th cells from asthmatic patients (Duez et al., 2001; De Rose et al., 2004). Recently, the broad spectrum caspase inhibitor benzyloxycarbonyl-Val-Ala-Asp-fluoromethylketone was shown to increase IL-4 and Th2 cell differentiation mediated by Bcl-2 (Sehra et al., 2005). However, in another study, treatment with the same caspase inhibitor inhibited allergic asthma in

Table 1

Promising target candidates for the inhibition of memory Th2 cells in allergic asthma

Adenosine modulators
Allergen peptide immunotherapy
Calcineurin inhibitors
Cell adhesion molecule inhibitors
Cell signaling modulators
Chemokine antagonists
Cytokine modulators
ISS-ODNs
PDE4 inhibitors
Prostaglandin modulators
TLR agonists
Th cell transcription modulators

a mouse model (Iwata et al., 2003). It can be expected that the resolution of lung inflammation following disease exacerbation would be regulated by Fas–Fas ligand-mediated apoptosis that would preferentially eliminate activated effector Th2 cells, leaving memory Th2 cells intact. In turn, this suggests the possibility of memory cell-specific antiapoptotic mechanisms that may be a potential target for eliminating memory Th2 cells.

A basic strategy for reducing survival is to increase apoptosis in an allergen-specific fashion. In a recent study, restimulation of memory cells with antigen was shown to influence Th cell longevity (Patke & Farber, 2005). Rechallenge with higher avidity antigens lead to a vigorous proliferative response and decreased survival, whereas low avidity antigen caused reduced proliferation and increased survival. These results support the concept behind the latest trends in immunotherapy, which are to treat with high avidity allergens or allergoids that would potentially increase apoptosis and decrease Th2 cell survival.

Additional targets that influence the homeostasis and survival of T-cells include OX40 and CD30, which are expressed on Th2 cells and, when deficient (OX40 and CD30 double-deficient mice), cause reduced Th cells survival (Gaspal et al., 2005). IL-7 is also a regulator of survival and homeostasis of naive and memory T-cells but influences CD4⁺ and CD8⁺ T-cells and, thus, is not a specific target (Bradley et al., 2005).

6. Summary

Clinical studies and animal models have further elucidated a central role for Th2 memory cells in allergic asthma. The identification of functional pathways and genes associated with allergic asthma has had tremendous impact on the understanding of disease pathogenesis and drug discovery. The result has been specific strategies that exploit the unique properties of Th2 cells without concomitantly producing harmful immune responses or broad immunosuppression. A list of the most promising target candidates is summarized in Table 1. Aggressive use of anti-Th2 cell therapies in addition to combination therapies aimed at B-cells and mast cells may also improve clinical outcome. Additionally important will be the tailoring of treatment approaches for mild, moderate, and severe patients and for patients during remission versus exacerbation. New approaches using antisense DNA and siRNA will add to the armamentarium.

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