

IMMUNOLOGIC BASIS OF ANTIGEN-INDUCED AIRWAY HYPERRESPONSIVENESS

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ABSTRACT

The incidence, morbidity, and mortality of asthma has increased worldwide over the last two decades. Asthma is a complex inflammatory disease of the lung characterized by variable airflow obstruction, airway hyperresponsiveness (AHR), and airway inflammation. The inflammatory response in the asthmatic lung is characterized by infiltration of the airway wall with mast cells, lymphocytes, and eosinophils. Although asthma is multifactorial in origin, the inflammatory process in the most common form of the disease (extrinsic asthma) is believed to be a result of inappropriate immune responses to common aero-allergens in genetically susceptible individuals. As such, it has been hypothesized that CD4⁺ T cells that produce a Th2 pattern of cytokines play a pivotal role in the pathogenesis of this disease. Through the release of cytokines such as IL-4, IL-13, and IL-5, these cells orchestrate the recruitment and activation of the primary effector cells of the allergic response, the mast cell and the eosinophil. Activation of these cells results in the release of a plethora of inflammatory mediators that individually or in concert induce changes in airway wall geometry and produce the symptoms of the disease. The aim of this review is to discuss our current understanding of the pathophysiologic mechanisms by which Th2 cytokines induce airway disease, and the factors that predispose to the generation of these pathogenic cells in response to inhalation of ubiquitous aero-allergens. Elucidation of the exact immunological basis for allergic asthma may yield immunotherapeutic strategies to reverse the development of pathogenic Th2-mediated immune responses and reduce the morbidity and mortality associated with this disease.

INTRODUCTION

Asthma is a chronic inflammatory disease of the lung which has been increasing in prevalence, morbidity, and mortality over the last two decades. Despite this increase in the disease, considerable progress has been made in our understanding of the pathophysiology underlying the disease process. Although it was once thought that asthma was a disease of airway smooth muscle, over the last decade it has become widely accepted that asthma is primarily an inflammatory disease. Structurally, the airways of asthmatics are characterized by the presence of chronic inflammation with intense infiltration of the bronchial mucosa by lymphocytes, eosinophils, and mast cells, along with epithelial desquamation, goblet cell hyperplasia, and thickening of the submucosa (1, 2). These cellular findings have consistently been associated with the main physiologic abnormalities of the disease, including variable airflow obstruction and AHR (3–9).

Although asthma is multifactorial in origin, atopy, the genetic predisposition for the development of an IgE-mediated response to common aero-allergens, is the strongest identifiable predisposing factor for the development of asthma. Most childhood asthma is allergic in nature and referred to as extrinsic asthma (10, 11). In this form of the disease, the inflammatory process is thought to arise as a result of inappropriate immune responses to commonly inhaled allergens. After repeated low-dose exposure to allergens, atopic individuals develop specific IgE antibodies to the allergens. Subsequent exposure to allergens initiates a secondary humoral response. When the sequence of events following an allergen provocation are examined in allergic volunteers, the allergic reaction can be divided into both early (within minutes) bronchospastic responses and late (hours after exposure) inflammatory responses (Figure 1). The early (immediate) response is characterized by rapid onset of mucosal edema, increases in airway smooth muscle tone, and airway narrowing, associated with mast cell degranulation (12). Some allergic asthmatics also develop late-phase responses that begin three to six hours after antigen challenge and may persist for several days in the absence of therapy. In these responses, airway narrowing is associated with the migration of neutrophils, eosinophils, and lymphocytes from the blood into lung parenchyma and airway epithelium (12–15). This has led to the concept that the immediate response after allergen challenge is mediated by mast cells, whereas eosinophils are the predominant cells in the late asthmatic reaction.

It has recently become appreciated that as orchestrators of the inflammatory response, T lymphocytes, in particular CD4 T cells, play a pivotal role in the pathogenesis of asthma. This article reviews our current understanding of the pathogenesis of this disease with particular emphasis placed on the pathogenic

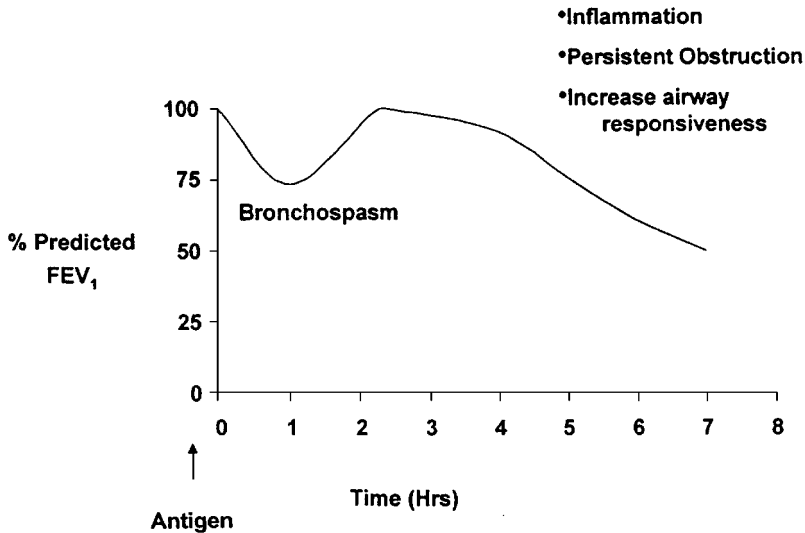


Figure 1 The early- and late-phase reactions to inhaled antigen and the consequence of these reactions on pulmonary physiology. Reprinted from Nadel and Busse, *Am. J. Respir. Crit. Care Med.* 157:S130–S138.

role of Th2 cytokine-producing cells. Because our fullest understanding of the mechanisms involved in the inflammatory response in the airways is of the allergen-driven asthmatic reaction, the primary focus is placed on extrinsic, or atopic asthma. Results from experiments on humans will be cited when available, although much of our current understanding of the disease has been derived from study of animal models, particularly murine models in which manipulation of immunological processes is possible.

CD4 T CELLS IN ASTHMA

As the primary orchestrator of the specific immune response, the T lymphocyte has been implicated in the pathogenesis of allergic airway disease (16–19). In support of a role for T lymphocytes in the pathogenesis of asthma, consistently elevated numbers of T lymphocytes have been found in the bronchoalveolar lavage (BAL) fluids and bronchial biopsies from asthmatics (16–19). These T cells are predominantly of the CD4 population, based on the fact that the numbers of CD8 cells are equivalent to those in normal individuals. Cell recruitment to the airways is suggested by the finding that increased numbers of CD4⁺ T cells in BAL are concurrent with decreased numbers of CD4⁺ T cells in peripheral blood following allergen challenge (20). Experimental data support a

generalized increase in T cell activation in asthmatics, with increased expression of interleukin-2 receptor (IL-2R), class II histocompatibility antigens (HLA-DR), and very late activation antigen-1 (VLA-1) in blood, BAL, and bronchial biopsies (19, 21).

Functional subsets of CD4⁺ T cells have been distinguished at both clonal and population levels by the unique profiles of cytokines that they produce (22, 23). The differential presence of these cytokine phenotypes in a variety of allergic and infectious diseases both in mice and in humans has provided descriptive power and theoretical insight into disease pathogenesis (23–27). Th1 cells produce interleukin-2, TNF- β , and interferon gamma (IFN- γ), and are critical in the development of cell-mediated immunity (22, 23). On the other hand, Th2 cells produce IL-4, IL-13, IL-5, IL-9, IL-6, and IL-10 and are important in the stimulation of IgE production, mucosal mastocytosis, and eosinophilia (22, 23). A possible immunopathogenic role for Th2 cells in asthma has been postulated on the role that these cytokines play in IgE synthesis and eosinophil regulation. Several lines of evidence from experiments provide support for the involvement of these cytokines in the pathogenesis of asthma. Firstly, the T cells from asthmatic patients express a unique pattern of cytokines consistent with a Th2 pattern (21, 28, 29). Specifically, T cells from both the BAL fluids and bronchial biopsies of allergic asthmatics express elevated levels of mRNA for IL-4, IL-13, GM-CSF, and IL-5 (21, 28, 29). Secondly, it has been shown that successful steroid treatment increases IFN- γ levels in the BAL of asthmatics patients, and simultaneously decreases IL-4 and IL-5 levels (30). Thirdly, the increased numbers of activated T cells observed in asthmatics correlate with the numbers of activated eosinophils, the magnitude of the decrement in peak expiratory flow rates, and the severity of the disease (31, 32).

The production of this pattern of cytokines appears to be genetically controlled and established early during childhood (33). In an elegant longitudinal study of children, Martinez and colleagues (33) show that the propensity to develop asthma is associated with low stimulated levels of IFN- γ in children at 9 months of age, which suggests that a type-1 response is a protective factor. Furthermore, low stimulated levels of IL-2 and IFN- γ at 9 months of age were positively correlated with parental immediate skin test reactivity. In this regard, genetic linkage studies by multiple groups have shown, although not conclusively, that asthma is linked to a region of human chromosome 5q in which the genes for IL-4, IL-5, and IL-13 are localized (34, 35).

Additional support for this hypothesis as it relates to humans has been provided in a population-based study described by Shirakawa (36). The study found that among Japanese schoolchildren there was a strong inverse association between delayed-type hypersensitivity to *Bacillus Calmette-Guerin* (BCG) and atopy. Positive tuberculin responses predicted a lower incidence of asthma,

lower serum IgE levels, and cytokine profiles biased toward a type-1 profile. These results suggest that exposure and response to BCG may, by modification of immune cytokine profiles, inhibit atopic disorders.

Although considerable descriptive evidence suggests that CD4⁺ T lymphocytes and Th2 cytokines are important in the pathogenesis of airway hyperresponsiveness in asthmatic humans, definitive proof is difficult to obtain in human subjects. Therefore, experimental animal models have been extremely useful in contributing to a delineation of the role of CD4⁺ T cells and T-cell-derived cytokines in the pathogenesis of asthma. Direct evidence of a causal role for CD4⁺ T cells in the development of AHR was first provided in a murine model of allergen-induced AHR (37). In this model, ovalbumin sensitization and local lung challenge resulted in increases in airway responsiveness to acetylcholine challenge, and in an eosinophilic inflammatory response in the lung. Depletion of CD4⁺ T cells in sensitized mice prior to local lung antigen challenge with specific monoclonal antibodies prevented the development of allergen-induced allergic airway responses. Conversely, depletion of CD8 cells has been shown not to affect airway responses to allergen challenge in mice (38).

Definitive evidence of a pathogenic role for Th2 cytokines in allergen-driven pathophysiologic processes has been provided by studies in which IL-4 and IL-5 have been manipulated through either antibody blockade (39, 40) or gene targeting (41–43). Furthermore, AHR can be induced in naive mice by adoptive transfer of Th2 clones into their lungs (44). On the other hand, studies have shown that the administration of agents such as IL-12 and IFN- γ that inhibit Th2 cytokine production and stimulate Th1 pathways prevent the development of antigen-induced AHR and inflammation in murine models (44, 45). Along these lines, prior infection of sensitized mice with BCG (46) or administration of CpG oligodeoxynucleotides (47) have resulted in suppression of eosinophilic airway inflammation concomitant with a shift in cytokine production to a protective type-1 profile.

INDUCTION OF TH2 CELL DIFFERENTIATION IN ALLERGIC ASTHMA

Although considerable evidence supports a pathogenic role for Th2 cytokines in asthma, very little is known about the underlying cause(s) of the aberrant expansion of Th2 cells. The data from studies using T cell receptor transgenic mice (48, 49) suggest that naive T helper cells have the ability to differentiate into either Th1 or Th2 cells. Naive T cells generate IL-2 when activated by antigen presentation and costimulatory signals (i.e. B7 molecules, CD58, and CD40) and progress through a multipotential cell that generates a mixed spectrum of cytokines including IL-2, IL-4, and IFN- γ (50). Although many

immune responses are likely to retain the (Th0) phenotype, responses may be polarized to either the Th1 or Th2 type, a process markedly influenced by the nature and dose of the antigen and the cytokine milieu during initial priming by specific antigens (51). The elegant use of mice transgenic for a specific alpha/beta T cell receptor has provided details of this critical involvement of the cytokine environment on subset differentiation during naive lymphocyte priming (49, 50). In these models, IL-12 directly primes CD4⁺ T cells for Th1 differentiation (50). Th2 differentiation, in turn, is critically dependent upon IL-4 (49).

Role of Specific Allergens

Recent studies indicate that a number of allergens from diverse sources have enzymatic activity that may subvert the immune response toward the Th2 phenotype. For example, Der p1 is a 25 kDa cysteine protease which has been shown to cleave CD25, the 55 kDa alpha subunit of the IL-2R (52, 53). As a result of cleavage of CD25, peripheral blood T cells show markedly diminished proliferation and IFN- γ secretion in response to potent stimulation by anti-CD3 Ab. These findings indicate that Der p1 could upset the balance of Th1/Th2 subset distribution by decreasing growth and expansion of the Th1 subset and as a consequence augmenting expansion of the Th2 subset that favors a pro-allergic response. Der p1 may also contribute to the allergic phenotype by cleaving CD23 on B cells that would normally serve to inhibit IgE synthesis and thereby disrupt the IgE regulatory mechanism (54). Through its ability to disrupt epithelial architecture, Der p1 may also facilitate its own passage across the epithelium, thus enhancing its (own) access to immune cells. Although allergens such as Der p1 can potentially create a microenvironment conducive to Th2 cell expansion, normal individuals do not mount Th2 responses to these allergens, which suggests that despite the nature of these antigens other factors also contribute to the allergic outcome following inhalation of aero-allergens in susceptible individuals.

Antigen Presentation in the Asthmatic Lung

A pivotal step in induction of a T cell immune response is the uptake, processing, and presentation of Ag by professional antigen-presenting cells (APCs). In the lung, several potential APCs exist, including alveolar macrophages (AMs), epithelial cells, and dendritic cells DCs. Early studies focused on the accessory cell capacities of AMs, but numerous studies have shown that AMs are poor accessory cells (55). In fact, AMs have been shown to inhibit mitogen-stimulated T cell proliferation.

Airway epithelial cells have also been thought to have antigen-presenting capabilities. Because epithelial cells line the respiratory tract, they are ideally

located to encounter antigens and present them to T cells. A role for airway epithelium as APCs has been suggested by the observation that APCs express many of the costimulatory molecules important in antigen presentation such as MHCII, CD40, B7 molecules, and ICAM-1 (55a). However, to date there is no definite evidence of their role in antigen presentation in the lung.

The most important professional APC in the lung is probably DC. It has been shown that DCs form an extensive network above the basement membrane of the airway epithelium that ensures accessibility to inhaled antigens (56). It is hypothesized that upon encountering inhaled antigen, airway DCs migrate to the draining lymph nodes of the lung, upregulate expression of costimulatory ligands, and interact with naive T lymphocytes, initiating a primary immune response. Lambrecht et al (57) have demonstrated the important role of DC in the lung for development of allergic airways responses in an elegant study of mice. In the study, the herpes simplex virus type-1 thymidine kinase suicide gene was overexpressed in cells of DC myeloid lineage. Activation of this gene was accomplished by treating mice with the nucleotide analogue ganciclovir prior to lung challenge with allergen. In sensitized thymidine kinase transgenic mice, the infiltration of lymphocytes and eosinophils into the airways following local antigen challenge was almost completely abolished, which illustrated the reliance of antigen presentation in the lung on DCs.

Several lines of evidence suggest that DC function in the asthmatic lung may be altered. For example, the number of DCs recovered in the BAL is greater in asthmatic patients than in normal controls (58). Moreover, van den Heuvel (59) demonstrated that when stimulated *in vitro* under the same conditions, circulating monocytes (MOs) from atopic subjects develop into more potent accessory cells than those from normal control subjects. Although the mechanism(s) behind the increased accessory function are currently unknown, it is possible that there are intrinsic differences in monocyte derived dendritic cell populations between atopic and normal individuals, or that perhaps they respond differently to the cytokines IL-4 and GM-SCF used to expand them *in vitro*. Further evidence for altered DC function in asthmatics is provided by the demonstration that the proportion of DCs expressing the alpha subunit of FC epsilon RI (Fc ϵ RI) is significantly increased in asthmatics as compared to non-atopic controls (60). This increase in Fc ϵ RI on DCs may also be the result of higher endogenous levels of IL-4, a known modulator of immunoglobulin Fc receptors. Although the role of this receptor on DCs is not known, it is assumed that Fc ϵ RI-alpha expression by DCs may facilitate the capture and internalization of allergens, which can then be processed and presented in the context of MHCII to CD4 T lymphocytes. If this mechanism is used in an asthmatic individual already sensitized to a particular allergen, Fc ϵ RI⁺ DCs may exacerbate the chronic allergic inflammatory response by ensuring efficient accumulation

of allergen-specific T cells. Another observation that suggests DC may contribute to the allergic phenotype is that systemic steroid treatment dramatically inhibits the recruitment of DC into the respiratory tract epithelium during acute inflammation (61).

Although collectively these studies suggest a role for DC in augmented presentation of allergens to T cells, they do not explain the preferential expansion of CD4 T cells into Th2-producing cells in the asthmatic lung. In this regard, murine DCs have been shown to produce IL-12 in response to CD40/CD40L interactions, with a resulting differentiation of naive T cells toward a Th1 phenotype (62). However, to my knowledge murine DCs do not produce IL-4. DC cells are, however, capable of driving differentiation of CD4 T cells toward a Th2 phenotype when inhibitors of IL-12 such as IL-4, IL-10, and PGE2 have been exogenously added to cultures, which suggests that alterations in the production of these mediators in the microenvironment are more likely than an intrinsic defect in the DC to be the primary defect in asthma. However, since the study of DC function is in its infancy, it remains to be seen whether DCs exert a primary influence over expansion of Th2 cells in the asthmatic lung.

Cytokine Regulation of Th2 Differentiation in the Asthmatic Lung

Because IL-4 and IL-12 are known regulators of T cell differentiation, alterations in either production of or responsiveness to these cytokines could result in the polarization of T cell responses to allergens observed in the asthmatic lung.

The differentiation of uncommitted T cell precursors into Th2 cells is largely driven by IL-4. Although IL-4's role in the development of acute Th2 responses and maintenance of the response has been recently disputed (63), it is still likely that IL-4 plays an important role in the initial priming of T cells. Indeed, the results of several studies suggest that perhaps genes regulating IL-4 production may be altered by asthma. Firstly, a specific polymorphism in the IL-4 gene has been shown to correlate with high serum IgE levels and enhanced IL-4 gene expression (64). Secondly, Hershey et al (65) have demonstrated that expression of a mutant form of the IL-4 receptor α chain in allergic patients is associated with increased IL-4 signalling. Lastly, studies in mice have shown that alterations in a number of the family members of the NFAT protein family may result in altered IL-4 gene expression and polarization of T cell responses toward the type-2 pattern. NFAT proteins are expressed in T cells, B cells, and mast cells, and control the transcription of a number of genes relevant to asthma, including IL-4. Rao and colleagues (66) recently demonstrated that NFAT-p (NFAT-1)-deficient mice have an increased number of eosinophils in the bone marrow and blood concomitant with increased production of Th2

cytokines. These experiments suggest that deficiencies in this transcription factor may lead to the development of the allergic phenotype. It has also been shown that other NFAT family members, including NF-ATC1 (NF-ATc), are important in differentiation of T cells. Several groups have shown that loss of NF-ATc activity results in impaired T lymphocyte activity and secretion of IL-4 in mice (67, 68). Taken together, these studies suggest that altered gene expression of the NFAT proteins may result in an imbalance in the regulation of genes important for Th2 differentiation. Collectively, these results suggest that the known genetic predisposition of allergic asthma in humans may involve dysregulation of the genes controlling IL-4 production or the IL-4 receptor.

Although Th2 cell polarization in asthma can clearly arise as a result of aberrant expression of the genes important in Th2 differentiation, alterations in factors controlling expansion of the opposing Th1 pathways may also play an important role. In this regard, interleukin-12, a product of MAs and other APCs, is the primary determinant of T cell differentiation to a Th1 pattern (69). Studies of IL-12 production in the lungs of human asthmatics do indeed suggest that impaired IL-12 production occurs, but it is not known at the present time whether this is a primary or secondary event. Naseer and colleagues (70) demonstrated that the number of cells positive for IL-12 p40 mRNA is significantly lower in asthmatic patients than in normal controls. Furthermore, successful steroid treatment was characterized by a significant increase in the numbers of cells expressing IL-12 mRNA, whereas steroid therapy in steroid-resistant patients did not result in an increase in IL-12-expressing cells. Additionally, another group has shown that *Staphylococcus aureus* (SAC)-induced production of IL-12 p70 in whole blood cultures from asthmatic patients was significantly less in comparison with non-atopic control subjects (71). Further support for the importance of IL-12 in prevention of antigen-induced allergic airway responses has been provided by the observation that blockade of endogenous production of IL-12 in naturally resistant murine strains (C3H/HeJ) renders them susceptible to the development of allergen-induced AHR and eosinophilic inflammation (72). Taken together, these studies suggest that dysregulation of endogenous IL-12 levels may be an important mechanism governing the pathogenesis of allergic airway disorders.

The mechanisms which give rise to alterations in IL-12 production are unclear at the present time; however, several potential mechanisms exist, such as altered expression of the genes encoding either one or both of the individual subunits of the functional cytokine, or alterations in receptor signalling pathways. Although specific polymorphisms in the genes encoding p40 and p70 have not been reported, the gene encoding the p40 subunit of the functional heterodimer is located in the region of human chromosome 5q that has previously been linked to human asthma. In addition, recent studies in mice have implied

that loss of IL-12 responsiveness and subsequent inability of mice to mount Th1 responses is due to altered expression of the IL-12 receptor B2 subunit (73). IL-12-dependent signalling in human Th1 cells was shown to correlate with the selective expression of the transcripts encoding the signalling component of the IL-12 receptor B2 and with the presence of high-affinity IL-12 binding sites selectively located on Th1 cells (74). Thus it is proposed that not only is IL-12RB2 a marker of Th1 cells, but that lack of expression of this receptor subunit may lead to a Th2-polarized immune response. To my knowledge, IL-12 receptor expression has not been examined in T cells from human asthmatics. As IL-12 production is deficient in the lungs of human asthmatics, we might propose that selective expression and regulation of the IL-12RB2 subunit may be a potential mechanism by which aberrant Th2 cell differentiation occurs in allergic diseases.

Alternatively, the deficient production of IL-12 in asthma may occur as a result of altered regulation of IL-12 production by mediators and cytokines, which either positively (IFN- γ) (75), or negatively (IL-4, PGE2) (76, 77) regulate its production. Clearly, IL-4 and PGE2 levels are elevated in allergic diseases, whereas IFN- γ levels are reduced. Thus a better understanding of the mechanisms underlying dysregulation of IL-12 production and responsiveness awaits studies designed to examine IL-12 receptor expression and modulation of IL-12 production in asthma.

MECHANISMS OF TH2 CYTOKINE-INDUCED EOSINOPHILIC INFLAMMATION AND AHR

Although an immunopathogenic role for Th2 cells is suggested by the role of IL-4, IL-13, and IL-5 in IgE synthesis and eosinophil differentiation and activation, the exact mechanisms by which Th2 cytokines mediate eosinophilic inflammation and subsequent AHR are still not entirely clear. Th2 cytokines may potentially induce AHR through direct or indirect effects on B cells, mast cells, and eosinophils (Figure 2). To my knowledge, no one has examined the direct effects of IL-4, IL-13, or IL-5 on airway reactivity in *in vitro* systems that are relatively devoid of cellular, neural, and humoral components.

Of the uniquely Th2 cytokines, IL-4 likely plays a particularly important role in the allergic diathesis. In addition to its pivotal role in Th2 differentiation of naive T cells into Th2-producing cells, IL-4 also induces a plethora of cellular responses that are potentially important in the development of allergic airway diseases including: (a) its role in B cell-class switching to IgE production (78); (b) in conjunction with IL-3, it is a growth factor for mast cells (79); and (c) its ability to upregulate vascular cell adhesion molecule-1 (VCAM-1) expression which leads to preferential migration of eosinophils into tissues (80).

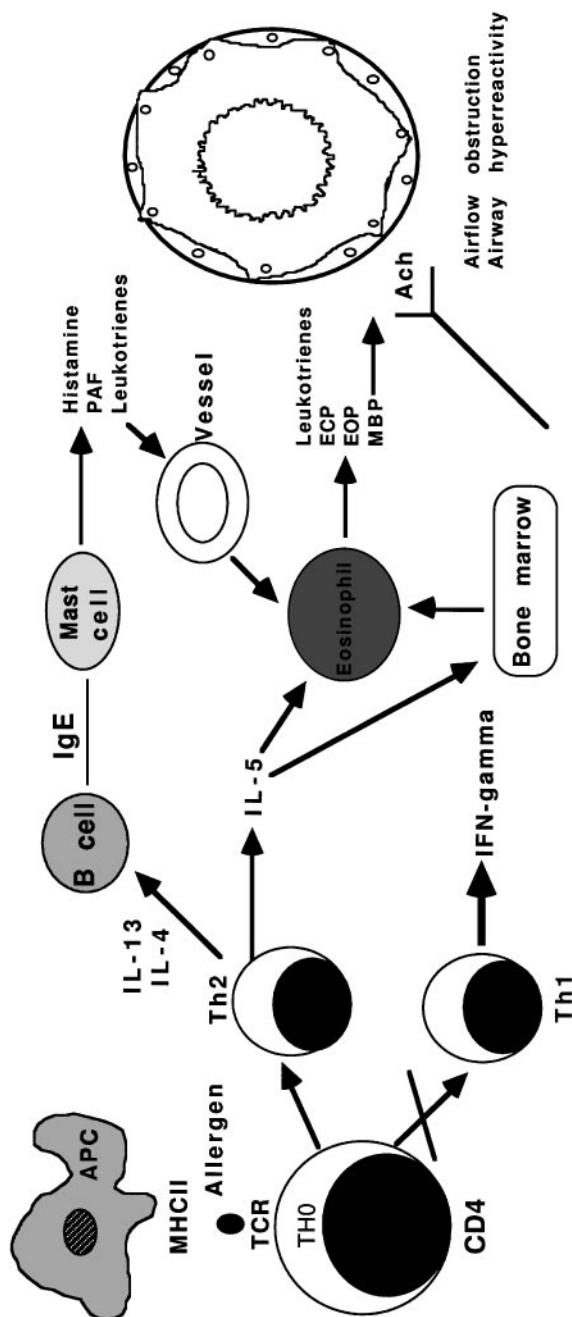


Figure 2 Proposed mechanism of the role of CD4⁺ T cells in the pathogenesis of asthma.

Initial studies in IL-4 mice supported a pivotal role for IL-4 in both the early- and late-phase allergic response. However, recent studies have shown that blockade of IL-4 prior to antigen challenge in sensitized mice is not effective in attenuating AHR or eosinophilic inflammation (40). Although these results were initially puzzling, the interpretation at the time was that perhaps IL-4 was essential for the initial expansion of Th2 cytokine-producing cells, but was not required for maintenance of this response. In contrast, studies conducted by Gavett et al (81) demonstrated that blockade of the IL-4 receptor prior to antigen provocation in sensitized mice effectively inhibited both AHR and eosinophilic accumulation. Although these discrepant results were initially difficult to resolve, recent studies in Stat6-deficient animals have provided a potential explanation. Kuperman et al (82) showed that a deficiency in the Stat6 molecule, which mediates most of the cellular actions of both IL-4 and IL-13, abolished antigen-induced eosinophilic inflammation and AHR. These results indicated that perhaps the effectiveness of IL-4 receptor blockade was due to inhibition of IL-13-mediated processes and not to those mediated by IL-4. Although these results suggest that IL-13 rather than IL-4 plays the central role in driving allergic responses, direct proof of this hypothesis is not available at this time. Because IL-4 and IL-13 have many overlapping actions, further studies will be necessary to determine the exact contributions of each of these mediators to the allergic response. Therefore, in the following discussion regarding the role of IL-4 in allergic responses, I point out the instances of IL-13 implication.

One of the potential effector mechanisms by which IL-4 may induce allergic inflammation and airway function changes is in its role as the primary inducer of immunoglobulin-class switching in B cells that leads to the synthesis and secretion of IgE (78). The combination of IL-4's effects on IgE synthesis and mast cell growth suggests that its role in AHR may be mediated primarily through mast cell activation. As will be discussed in more detail, IgE activation of mast cells leads to the synthesis and release of a number of inflammatory mediators that may contribute to the bronchoconstriction, vascular changes, and mucus changes observed in the early-phase response to allergen challenge.

Excessive production of airway mucus glycoproteins is found consistently in the lungs of asthmatics, and in particular in those patients who die in *status asthmatics* (83). Evidence of a role for IL-4 in mucus production is provided by studies conducted in IL-4 transgenic mice that exhibit profound increases in mucus-containing cells in the airway epithelium (84). This fact is supported by the finding that antigen-induced increases in mucus-containing cells is ablated by blockade of the IL-4 receptor (81). Also consistent with this hypothesis is the demonstration that IL-4 induces expression of the *MUC5* gene in the airways of IL-4 transgenic mice (84). The effects of IL-4 on goblet-cell hyperplasia may be mediated through IL-4's involvement in mast cell activation and eosinophil

recruitment since both of these effector cells release the potent mucus secretagogues LT, 15-HETES, and PAF. Interestingly, Cohn et al (85) demonstrated that although mucus changes could be induced via transfer of Th2 clones into the airways, clones derived from IL-4-deficient mice could still confer mucus production, which suggests that Th2 cells are important for mucus production but that IL-4 is not essential. Taken together with the finding that the airways of Stat6-deficient animals do not show evidence of mucus production following allergen provocation, we may conclude that IL-13 is the type-2 cytokine mediating mucus changes in the airways. This speculation is supported by studies in *N. brasiliensis*-infected IL-13^{-/-} mice in which the authors demonstrate that IL-13 mediates parasite-induced goblet-cell hyperplasia (86). The relative contribution of IL-4 and IL-13 to allergen-driven mucus hypersecretion in asthma remains to be determined.

IL-4 may induce AHR by way of another mechanism—its potential role in eosinophil recruitment. Numerous studies have shown that IL-4 blockade eliminates antigen-induced increases in eosinophils (42, 43, 81). Although the exact mechanisms involved in IL-4's ability to induce tissue eosinophilia are unknown, there are several potential mechanisms. IL-4 may mediate pulmonary eosinophilia through its role in Th2 cell differentiation and the subsequent production of IL-5. In addition, IL-4 may regulate eosinophil influx by regulating VCAM-1 expression on the endothelium and/or stimulating release of specific chemokines from resident airway cells (87). In support of a role for IL-4/IL-13-mediated VCAM-1 in pulmonary eosinophilia, numerous studies have shown that VCAM-1 is necessary for eosinophil recruitment into the lung in response to antigen provocation in mice (88).

Because interleukin-5 has been shown to be the primary determinant of eosinophil differentiation, activation, and survival, it is a likely candidate in the development of AHR (89). In bone marrow, IL-5 is important for stimulation of eosinophilopoiesis and promotion of the terminal differentiation of myeloid precursors into eosinophils. IL-5 also increases eosinophil adhesion to vascular endothelial cells, promotes the migration of eosinophils from the blood into tissues, prolongs eosinophil survival in tissues, and augments the cytotoxic activity of eosinophils. Furthermore, IL-5 may activate pulmonary eosinophils and cause them to release cytotoxic products. As stated above, although IL-5 is derived from CD4 T cells, more recent studies show that it is released by mast cells and by eosinophils themselves (90). The importance of IL-5 in antigen-induced eosinophilia has been well established in several species (39, 41, 91). For example, blockade of endogenous IL-5 levels in antigen-sensitized guinea pigs (91) and in mice (39) has resulted in significant suppression of both BAL and tissue eosinophilia. Consistent with these observations is the demonstration that intratracheal administration of IL-5 induces eosinophil accumulation in the

guinea pig lung in vivo (91). More definitely, mice in which the *IL-5* gene has been disrupted do not develop aero-allergen-induced pulmonary eosinophilia (41). Reconstitution of these mice with IL-5 by administration of recombinant vaccinia viruses engineered to express IL-5 completely restored aero-allergen-induced eosinophilia. If taken at face value, this result would suggest that IL-5 alone is sufficient for restoration of tissue eosinophilia. However, it has been shown that overexpression of IL-5 in mice results in a marked elevation of circulating eosinophils, but that the tissue levels of eosinophils remain similar to their wildtype controls (92). These results suggest that other processes also contribute in the recruitment of eosinophils to sites of inflammation in the lung.

Chemokines, or chemotactic cytokines, attract and activate lymphocytes, granulocytes, and monocytes. They are 7–16 kDa proteins with four conserved cysteines. Based on the position of the first two cysteines, these chemotactic cytokines are divided into two subfamilies which differ in terms of what cells they attract. The C-C chemokines (including eotaxin, RANTES, MCP-3, and MCP-1) are particularly relevant to allergic inflammation. Elevations in a number of chemokines including MCP-1, RANTES, and eotaxin have been shown in the lungs of asthmatic patients (93). One chemokine of particular importance in airway disease is eotaxin because it has been shown to be exquisitely selective for eosinophil recruitment (94). Studies show that increases in eotaxin mRNA-positive cells are inversely correlated to the dose of histamine, and induce a 20% drop in forced expiratory volume in one second in asthmatic patients (93). Furthermore, eotaxin and IL-5 have been shown to cooperate in the orchestration of eosinophil accumulation in tissues because IL-5 increases responsiveness to eotaxin (95). Although eotaxin probably plays an important role in lung eosinophilia, studies have shown that neutralization of this cytokine results only in partial suppression of antigen-driven eosinophil migration (96). In turn, these results suggest that other chemokines may also be involved in controlling eosinophil chemotaxis in the lung. In this regard, mMCP-5, a chemokine produced by lung MAs and smooth muscle cells, has also been implicated in eosinophil recruitment into the murine lung (97).

Several lines of evidence suggest that Th2 cells regulate chemokine expression in the lung following antigen challenge. Specifically, MacLean and colleagues (98) have demonstrated that anti-CD3 mAb administration prior to antigen challenge in sensitized mice was associated with a reduction in eotaxin mRNA expression. The demonstration that IL-4 stimulates eotaxin production in fibroblasts provides support for this conclusion (87). Similarly, mMCP-5 expression in mast cells has been shown to be lymphocyte-dependent. Thus we may postulate that eotaxin, mMCP-5, and other chemokines yet to be defined may act in a sequential manner to regulate migration of eosinophils to the lung during an asthmatic response.

Based on the independent roles of IL-4 and IL-5 discussed above, we would envision the following paradigm for regulation of allergen-induced pulmonary eosinophilia by CD4 Th2 cytokines: Following antigen-specific induction of Th2 cytokine production, IL-5 would rapidly induce differentiation of eosinophils from myeloid precursors in the bone marrow and stimulate their release into the bloodstream. IL-4 and/or IL-13 would promote eosinophil egress from the vascular compartment by upregulating VCAM-1 expression on vascular endothelial cells and by stimulating chemokine production (eotaxin and MCP-5) by endothelial cells, epithelial cells, and smooth muscle cells in the lung. Once the cells accumulate in the tissues, locally produced IL-5 would promote their actions by prolonging their survival in tissues. Within this paradigm, allergen-driven eosinophil recruitment into the lung is coordinately regulated by the Th2 cytokines, IL-4, IL-13, and IL-5.

ROLE OF EFFECTOR CELLS IN AHR

It is considered that the principal effector cells of the allergic airway response are mast cells and eosinophils. Although these two cells have unique functions and release a unique profile of mediators, they also produce an overlapping array of mediators which are known to contribute to the allergic diathesis. It has long been thought that mast cells contribute to the early-phase response and that eosinophils mediate the late-phase response and the structural changes, regardless of the fact that the recent discovery of mast-cell production of a number of chemokines and cytokines may dispel this notion. Next we explore the individual role of each of these effector cells in the pathophysiology of allergic asthma.

Mast Cells

Although an increase in mast cell numbers in the lungs of asthmatics has not been demonstrated, numerous studies have shown evidence of mucosal mast-cell activation in the lungs of asthmatics (99). Mast cells are thought to contribute to the pathogenesis of allergic airway responses through IgE-dependent mechanisms. It is hypothesized that IgE produced by allergen-reactive B cells binds to FcεR receptors present on the surface of mast cells and basophils, and that when challenged with allergen, these cells release vasoactive mediators as well as chemotactic factors and cytokines that promote leukocyte infiltration and exacerbate the inflammatory response. Through the production and release of these proinflammatory molecules, mast cells set in motion a series of events that result in the immediate response to inhaled antigens and may also contribute to the late-phase allergic response (LPR).

Mast cell release of pre-formed mediators such as histamine, PGD₂, LTC₄, and PAF within minutes after inhalation of antigens together produce the

symptoms of the early response to allergen challenge. Each of these mediators is found in increased levels in the lungs of asthmatics, and can enhance activation of inflammatory cells, cause microvascular leakage, increase mucus production, and induce bronchoconstriction (99).

Although it has long been thought that mast cells only contribute to the early acute response (EAR), recent evidence demonstrates their potential contribution to the late-phase response. Through the elaboration of the cytokines TNF- α , IL-1, IL-3, GM-CSF, IFN- γ , IL-4, IL-5, IL-6, and IL-8, IL-16 and the chemokines MCP-1, MIP-1B, MIP-1a, and RANTES, mast cells may contribute to the cellular component of the late-phase response. They may also perpetuate or amplify the eosinophilic inflammatory process by preferentially expressing IL-4. Indeed, immunohistochemical studies of bronchial biopsies of allergic patients suggest that mast cells are the primary source of IL-4 protein (100). Thus, mast cells may favor the acquisition of the Th2 phenotype by providing a continuously high local concentration of IL-4.

Mast cell mediators may also contribute to the airway wall remodeling observed in the asthmatic lung since many of the mediators they release influence connective tissue turnover. Specifically, histamine and tryptase have been shown to stimulate fibroblast growth and collagen synthesis *in vitro* and *in vivo* (101).

Support of a role for mast cells in the LPR comes from studies in which mast cell stabilizers such as sodium nedocromil effectively inhibit both the EAR and the late-phase response to allergen exposure. Further support of this tenet is provided by the finding that anti-IgE pre-treatment does not elicit AHR in mast-cell deficient mice (102). Recent clinical trials utilizing a monoclonal antibody against IgE resulted in modest improvements in the lung function of asthmatics (103). However, IgE-deficient mice can develop antigen-induced AHR, which indicates that although mast cells are capable of inducing AHR, mechanisms other than IgE-mediated mast cell degranulation are also important in the development of AHR (104).

Eosinophils

Eosinophilic inflammation is clearly a hallmark of both allergic and non-allergic asthma, and considerable evidence suggests that there is an association between pulmonary eosinophil infiltration and AHR in human asthma (105). Eosinophils release a myriad of mediators and cytokines that are potentially important in the allergic response including: (a) the eosinophil-specific proteins, eosinophilic cationic protein (ECP), and major basic protein (MBP), and eosinophil-derived neurotoxin (EDN); (b) a number of cytokines (IL-2, IL-3, IL-4, IL-5, GM-CSF, IL-6, IL-10, IFN- γ , IL-12, TGF- α , and TGF- β); and (c) lipid mediators such as LTs, PGE₂, and PAF (90).

Eosinophils are postulated to induce AHR through the actions of these basic proteins on the airway wall. In fact, ECP and MBP have been detected in the BAL of patients with asthma (106). These proteins are cytotoxic to the airway epithelium. Damage to the airway epithelium may lead to AHR by removing enzymes important in the degradation of neuropeptides and/or in the loss of epithelial-derived relaxing factor. In addition, these proteins may have direct effects since MBP has been shown to potentiate contraction of airway tissues *in vitro* without showing evidence of epithelial damage (107). Further evidence for this hypothesis is provided by the fact that administration of MBP to primates induces AHR (108). MBP may induce AHR through its demonstrated ability to competitively inhibit binding of M2 receptors to acetylcholine autoreceptors on parasympathetic nerves that may result in increased release of acetylcholine (109). The importance of eosinophils in M2-receptor dysfunction has been shown in a guinea pig model of asthma. For example, blockade of inflammatory cells, especially eosinophils, prevents M2-receptor dysfunction in antigen-challenged guinea pigs (110). Further support for this hypothesis is provided by the fact that blockade of IL-5 in antigen-sensitized animals also protects the M2-muscarinic receptor function (111). Thus the demonstrated presence of eosinophil-derived proteins in the asthmatic lung, coupled with their potent actions on airway epithelial cells and/or neural receptors, strongly suggests a role for these mediators in the development of allergen-induced AHR and airway obstruction.

Although there is a substantial body of evidence supporting the role of eosinophils in asthma, a number of studies on humans have not confirmed a correlation between eosinophils and disease severity (112). A similar conflict also exists in mouse studies of allergic asthma. Studies in *IL-5* gene knockout mice show that both eosinophilia and AHR are IL-5-dependent (41). Results from a study in which IL-5 transgenic mice over-express IL-5 in the lung also support the role of IL-5 and eosinophils in the development of AHR (113). In contrast, Corry et al (114) demonstrated that anti-IL-4 treatment of mice prevented AHR in spite of the presence of eosinophils in the lung. Also, the development of AHR in that model was not affected by an anti-IL-5 treatment that abrogated eosinophilia. Conflicting results in murine models may be explained by the recent finding that eosinophils in the lungs of certain strains of mice do not degranulate following antigen challenge. In addition, since the role of eosinophils in AHR has mostly been based on IL-5 depletion, it is conceivable that IL-5 has other actions that induce AHR in addition to the recruitment of eosinophils. In support of this hypothesis, Kraneveld et al (115) have shown that IL-5 induces AHR through release of tachykinins in the guinea pig airway, an action which is independent of its effect on eosinophils.

MECHANISMS OF INFLAMMATION-INDUCED AHR

Although the studies to date have not elucidated the precise mechanisms by which Th2 cytokines mediate AHR, they have suggested that either eosinophils, mast cells, or both are likely to play a role in this process. These cells are thought to induce the physiologic changes of asthma by means of the secretion of an array of inflammation mediators including histamine, leukotrienes, PAF, eosinophil-derived basic proteins, and various proteases. Because mast cells and eosinophils both produce many of these mediators, the importance of each cell type has been difficult to assess. This uncertainty is highlighted by the fact that the murine studies discussed in this review show that AHR can be induced in the absence of either one or the other cell type. Since these cell types release an overlapping array of mediators, it may be that development of AHR is achieved through separate pathways that result in the release of a common mediator such as leukotrienes. Alternately, AHR can be induced by two or more independent processes that both result in AHR by means of different mechanisms. Perhaps the varying severity of the disease is due to involvement of multiple independent processes working in concert and resulting in a more severe phenotype. The existence of two separable clinical phenotypes would support this conclusion. For example, extrinsic asthma, possibly in concert with IL-5-mediated eosinophil responses, is most certainly an IgE-dependent process. On the other hand, although the inciting agent in intrinsic patients is unknown, this pathway is probably not IgE-dependent, but rather IL-5- and eosinophil-dependent. Genetic linkage studies conducted to date would also support the hypothesis that multiple independent or additive pathways contribute to the asthmatic phenotype because asthma has clearly been shown to be a multigenic disease. Thus, in the next sections, I explore the potential mechanisms by which many of the above-mentioned mediators induce AHR without explicit regard for the origin of the mediators.

Bronchoconstrictor Effects of Inflammatory Mediators

A variety of inflammatory mediators released by mast cells and eosinophils directly constrict airways. Among these are histamine, leukotrienes, PAF, endothelin, and eosinophil granule proteins. The contractile effects of these agents are mediated via direct stimulation of specific receptors on smooth muscle and through mechanisms including induction of the release of other contractile agonists. Because each of these mediators is increased in the airways of asthmatics, one could envision airway smooth muscle bathed in a virtual soup of bronchoconstrictors, resulting in hyperresponsiveness. However, blockade of each mediator independently has not convincingly shown that they contribute to AHR, with one exception: leukotrienes. A more likely explanation

for these observations is that inflammation induces alterations in the airway wall geometry that convey stimuli to an already thickened airway wall, conceivably resulting in an even greater narrowing of the airway for a given stimulus. These airway changes include: (a) altered neural regulation of airway tone; (b) increases in muscle content; (c) increased mucus secretion; (d) airway wall edema; and/or (e) airway epithelial desquamation. Evidence exists to support each of these possibilities.

Altered Neural Regulation of Airway Tone

The neuronal pathways controlling airway tone include the sympathetic, parasympathetic, and peripheral sensory nerves. A number of inflammatory mediators are known to alter function of these neural pathways. First, the eosinophil-derived MBP has been shown to alter release of the neurotransmitter acetylcholine from the parasympathetic nerve terminal by binding the M2 autoreceptor on the nerve ending (109). This leads to an increase in the release of acetylcholine and an enhanced narrowing of the airway wall for any given stimulus. In addition, responsiveness to sympathetic input regulating relaxation of the airway via β -adrenergic receptors on airway smooth muscle has also been shown to be impaired in asthmatics (116). One hypothesis regarding the mechanism of this defect is that the cytokines IL-1 and TNF- α alter β -adrenoceptor signalling mechanisms and lead to impaired ability of the smooth muscle to relax (117). In this regard, polymorphisms in the β -adrenergic receptor have been shown to correlate with the severity of the disease (118). Inflammation is also associated with enhanced release of a number of sensory neuropeptides including substance P and neurokinins that possibly augment reflex constriction of airway smooth muscle.

Airway Smooth Muscle Hypertrophy

Another alteration in airway smooth muscle noted in the lungs of asthmatics and experimental animals is an increase in airway smooth muscle mass (ASM). Recent evidence suggests a role for several inflammatory mediators in the induction of muscle growth through hyperplasia. In Brown-Norway rats for example, ovalbumin sensitization and repeated challenge induced an increase in the quantity of ASM in the airways (119). This increase was inhibited by administration of nedocromil sodium prior to antigen challenge. Nedocromil sodium may inhibit airway smooth muscle hyperplasia by inhibiting a variety of mediators released by mast cells including histamine, leukotrienes, 15-HETES, and endothelin that have each been shown to increase growth of ASM. In addition, IL-1 and IL-6, both present in the asthmatic airway, can potentially increase the proliferation of ASM cells (120). Thus, numerous mediators of the asthmatic response may contribute to the increased muscle mass observed in patients

with asthma. This increase in muscle mass may theoretically contribute to an exaggerated narrowing of the airways to a variety of environmental stimuli.

Mucus Hypersecretion

Excessive production of airway mucus glycoproteins is a consistent finding in the lungs of asthmatics, in particular in those patients who die in *status asthmaticus*. There is evidence for hyperplasia of submucosal glands and increased numbers of epithelial goblet cells in the lungs of asthmatics. The increased secretory response is most likely due to a combination of effects of inflammatory mediators (i.e. IL-4, IL-13, LT, PAF) on submucosal glands, neural elements controlling submucosal glands and/or on goblet cells (121). Although the exact contribution of mucus plugging of the airways to AHR has not been determined experimentally, the fact that mucus plugging is a consistent feature of fatal asthma suggest that it plays an important role in the pathogenesis of asthma.

Airway Wall Edema

Airway edema is thought to be a result of inflammatory-induced increases in airway microvascular permeability. Inflammatory mediators released in the airway wall during the asthmatic response including histamine, PAF, and leukotrienes are potent inducers of increased bronchial permeability and are thus promoters of bronchial edema and airway wall swelling. It is hypothesized that thicker airway walls due to edema would theoretically result in greater airway narrowing for the same degree of airway smooth muscle contraction. However, studies designed to examine the direct effects of airway wall edema in the absence of inflammation have shown that edema in and of itself does not induce AHR (122). Thus the correlation of airway reactivity in asthmatics with airway wall edema may more accurately reflect the increase in inflammatory cells and mediators which accompany inflammation-induced microvascular leakage rather than a physical effect on airway smooth shortening.

Airway Epithelial Desquamation

Airway epithelial damage correlates with airway hyperreactivity and appears to be mediated by inflammation of the airways, particularly due to the presence of eosinophils. In atopic asthmatics, positive correlations were observed between the concentrations of MBP, the numbers of desquamated epithelial cells in BAL fluid, and the degree of bronchial hyperresponsiveness (123). Loss of airway epithelium may contribute to allergen-induced obstruction of airways by exposing irritant receptors of nerves, which may increase the response of the airways to various stimuli, or by inducing the release of a variety of inflammatory mediators PGs, 15-lipoxygenase products, ET-1, nitric oxide, and several cytokines (IL-6, GM-SCF, TNF- α and RANTES) which either directly induce changes in the airway or elicit the recruitment of inflammatory cells. Furthermore, loss of

the epithelial barrier may provide greater access of allergens to dendritic cells and specific immune cells such as T and B cells, serving to amplify the inflammatory process. Thus the disruption or loss of the airway epithelium through a variety of mechanisms may play a pivotal role in the development of AHR, airway inflammation, and airflow obstruction.

CONCLUSION

In summary, asthma is a complex inflammatory lung disease which is multifactorial in origin. However, it likely originates from an abnormal specific immune response to inhaled antigens. The presentation of inhaled allergens to CD4 T cells in the lungs of susceptible individuals results in the production of the Th2 cytokines, IL-4, IL-13, and IL-5, which orchestrate the differentiation, recruitment, and activation of mast cells and eosinophils in the airway mucosa. Such effector cells release a plethora of inflammatory mediators (e.g. histamine, LTs, PAF, eosinophil-derived basic proteins, and proteases) which have overlapping effects on the airway wall. Individually or in concert, these mediators cause acute bronchoconstriction, disruption of the airway epithelial layer, alterations in neural control of airway tone, increased mucus production, and increased smooth muscle mass. Each of these consequences of the inflammatory process could conceivably induce AHR, but it is more likely that they occur in combination. Although the mechanisms underlying the aberrant production are unclear, current studies suggest that perhaps altered regulation of genes controlling either IL-4/IL-13 or IL-12 production may lead to the expansion of deleterious Th2 cells in response to otherwise harmless inhaled antigens. It is hoped that as we gain a better understanding of the underlying pathophysiology of the disease, this knowledge will lead to the development of immunotherapeutics strategies to reduce or prevent the morbidity and mortality associated with this disease.

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CONTENTS

Discovering the Origins of Immunological Competence, <i>Jacques F. A. P. Miller</i>	1
Multifaceted Regulation of IL-15 Expression and Its Role in NK Cell Differentiation & Host Response to Intracellular Pathogens, <i>T. A. Waldmann, Y. Tagaya</i>	19
Immunodominance in Major Histocompatibility Complex Class I-Restricted T Lymphocyte Responses, <i>Jonathan W. Yewdell, Jack R. Bennink</i>	51
Integration of TCR-Dependent Signaling Pathways by Adapter Proteins, <i>James L. Clements, Nancy J. Boerth, Jong Ran Lee, Gary A. Koretzky</i>	89
Evolution of Antigen Binding Receptors, <i>Gary W. Litman, Michele K. Anderson, Jonathan P. Rast</i>	109
Transcriptional Regulation of T Lymphocyte Development and Function, <i>Chay T. Kuo, Jeffrey M. Leiden</i>	149
Natural Killer Cells in Antiviral Defense: Function and Regulation by Innate Cytokines, <i>Christine A. Biron, Khuong B. Nguyen, Gary C. Pien, Leslie P. Cousens, Thais P. Salazar-Mather</i>	189
Mature T Lymphocyte Apoptosis--Immune Regulation in a Dynamic and Unpredictable Antigenic Environment, <i>Michael Lenardo, Francis Ka-Ming Chan, Felicita Hornung, Hugh McFarland, Richard Siegel, Jin Wang, Lixin Zheng</i>	221
Immunologic Basis of Antigen-Induced Airway Hyperresponsiveness, <i>Marsha Wills-Karp</i>	255
Regulation of T Cell Fate by Notch, <i>Ellen Robey</i>	283
The CD1 System: Antigen Presenting Molecules for T Cell Recognition of Lipids and Glycolipids, <i>Steven A. Porcelli, Robert L. Modlin</i>	297
Tumor Necrosis Factor Receptor and Fas Signaling Mechanisms, <i>D. Wallach, E. E. Varfolomeev, N. L. Malinin, Yuri V. Goltsev, A. V. Kovalenko, M. P. Boldin</i>	331
Structural Basis of T Cell Recognition, <i>K. Christopher Garcia, Luc Teyton, Ian A. Wilson</i>	369
Development and Maturation of Secondary Lymphoid Tissues, <i>Yang-Xin Fu, David D. Chaplin</i>	399
The Structural Basis of T Cell Activation by Superantigens, <i>Hongmin Li, Andrea Llera, Emilio L. Malchiodi, Roy A. Mariuzza</i>	435
The Dynamics of T Cell Receptor Signaling: Complex Orchestration and the Key Roles of Tempo and Cooperation, <i>Ronald N. Germain, Irena Stefanová</i>	467
The Regulation of CD4 and CD8 Coreceptor Gene Expression During T Cell Development, <i>Wilfried Ellmeier, Shinichiro Sawada, Dan R. Littman</i>	523
Genetic Analysis of B Cell Antigen Receptor Signaling, <i>Tomohiro Kurosaki</i>	555
Mechanisms of Phagocytosis in Macrophages, <i>Alan Aderem, David M. Underhill</i>	593
Population Biology of HIV-1 Infection: Viral and CD4 ⁺ T Cell Demographics and Dynamics in Lymphatic Tissues, <i>A. T. Haase</i>	625

Chemokine Receptors as HIV-1 Coreceptors: Roles in Viral Entry, Tropism, and Disease, <i>Edward A. Berger, Philip M. Murphy, Joshua M. Farber</i>	657
The IL-4 Receptor: Signaling Mechanisms and Biologic Functions, <i>Keats Nelms, Achsah D. Keegan, José Zamorano, John J. Ryan, William E. Paul</i>	701
Degradation of Cell Proteins and the Generation of MHC Class I-Presented Peptides, <i>Kenneth L. Rock, Alfred L. Goldberg</i>	739
The Central Effectors of Cell Death in the Immune System, <i>Jeffrey C. Rathmell, Craig B. Thompson</i>	781
Selection of the T Cell Repertoire, <i>Eric Sebzda, Sanjeev Mariathasan, Toshiaki Ohteki, Russell Jones, Martin F. Bachmann, Pamela S. Ohashi</i>	829
Regulation of Immune Responses Through Inhibitory Receptors, <i>Eric O. Long</i>	875
The Wiskott-Aldrich Syndrome Protein (WASP): Roles in Signaling and Cytoskeletal Organization, <i>Scott B. Snapper, Fred S. Rosen</i>	905
The High Affinity IgE Receptor (Fc Epsilon RI): From Physiology to Pathology, <i>Jean-Pierre Kinet</i>	931
THE CRYSTAL STRUCTURE OF THE HUMAN HIGH-AFFINITY IgE RECEPTOR (Fc epsilon RI alpha), <i>Scott C. Garman, Jean-Pierre Kinet, Theodore S. Jardetzky</i>	973