

Insights into the Pathogenesis of Asthma Utilizing Murine Models

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Key Words

Asthma · Mouse model · Airway hyperresponsiveness

Abstract

Asthma is a common syndrome in children and adults. Despite the increasing prevalence and socioeconomic burden, the underlying pathophysiology remains poorly defined in a large percentage of asthmatics. Animal models and, in particular, murine models of allergic airway disease have helped to reveal some of the potential underlying mechanisms and have played an important role in identifying the importance of T cells and T_H2 cytokines in development of allergen-induced inflammation and airway hyperresponsiveness. In addition, other cell types including mast cells and eosinophils have been implicated in the development of some aspects of the disease. To further understand this complex syndrome, the development of animal models which mimic elements of this chronic airway disease is essential.

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Introduction

Over the last century, a steady increase in asthma prevalence in children as well as adults has been noted worldwide. The number of cases reported in the United States has increased by 75% since 1980 and the increase in children under the age of 5 surpasses 150% [1]. This escalation is also reflected in an increased socioeconomic bur-

den attributable to the disease [2]. Asthma is a complex syndrome with many clinical phenotypes. The disease is characterized as a chronic inflammatory disorder of the airways associated with airway obstruction and airway hyperresponsiveness (AHR) [3]. The etiology of asthma is not very well defined. Atopy, the genetic predisposition for the development of type I hypersensitivity reactions to common aeroallergens, is a major risk factor predisposing to the development of asthma [4]. Epidemiological studies suggest that certain environmental factors, such as viral infection [5], allergen exposure [6] and occupational exposures [7] are implicated, whereas other environmental influences, such as exposure to endotoxin [8, 9] or large amounts of cat dander [10] may even protect against the development of the disease. Reflecting the pathophysiology of the disease, the clinical manifestations of asthma are the direct extension of airway obstruction, intermittent or persistent, and which might be totally, partially or non-reversible. Airway inflammation is thought to play an important role in mediating airflow obstruction. The inflammatory changes associated with asthma are usually characterized by an accumulation of eosinophils, T lymphocytes, mast cells and neutrophils in the airway walls, lung tissue and airway lumen and are variably associated with changes in airway responsiveness to nonspecific bronchoconstrictors. Acutely or chronically, these changes may be accompanied by the development of airflow limitation [11]. To further understand the pathophysiological mechanism(s) underlying asthma, it is necessary to identify the factors, which initiate, worsen or modulate the disease, and to define how these immuno-

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logical and biological processes initiate the characteristic changes in the airways. This has an added dimension of difficulty with the increased recognition of the heterogeneity of the disease and the complexity of the gene-environment interactions. In the assessment of asthma, clinical investigations and epidemiological studies have been extremely useful. However, studying a human disease is limited by obvious ethical issues against repeated morphological assessments and in vivo experimentation. Modeling the disease in animals allows a more thorough in vivo assessment of changes in the airways and lung parenchyma incurred following allergen sensitization and challenge. A wide variety of animal models in different species, ranging from rats [12], guinea pigs [13], ferrets [14], monkeys [15] to horses [16], have been employed to study the inflammatory processes and alterations in airway function. Mice are the predominant species used as models of allergic airway disease. This is in large part due to a number of perceived advantages including short breeding periods, availability of inbred strains with known characteristics, good genetic markers, a well-described genome, a fully characterized immune system, the ability to readily induce genetic modifications and pragmatically, relatively low maintenance costs. But the reality is that there is likely no animal model of human asthma given the number of differences in the anatomy, development and physiology of the human airways and the nature of allergen exposure. Animal models have proven most useful in identifying potential contributors to the human condition, but their relevance is only established when directly tested in man.

Lessons Learned from Murine Models

If mice are the cornerstone of in vivo immunological experimentation of allergen-induced airway inflammation and hyperresponsiveness it is important to recognize what is shared. Recent sequencing of the genome revealed that to date only 300 genes or so appear to be unique to mice or humans [17]. Despite this remarkable similarity, several differences between the human and mouse immunological systems exist and have been recently reviewed [18]. Mice do not spontaneously develop asthma, but using different exposure models certain features concordant with asthma can be induced. Indeed, different models for allergic, occupational [19] or nonallergic asthma [20, 21] have been described but most commonly, mice have been used to model allergic airway inflammation and AHR. This is often achieved first by sensitization of

the animals to an allergen, which leads to the development of allergen-specific T cells and an immunoglobulin E (IgE)- and IgG1-dominated antibody response. Several different possibilities have been used to sensitize the animals to allergen. Systemic sensitization is often used, involving administration of the allergen, usually by intraperitoneal injection in combination with an adjuvant, e.g. aluminum hydroxide [22]. Sensitized animals can then be exposed to allergen, administered to the lung via inhalation, intranasal or intratracheal routes. Following airway allergen challenge, sensitized mice develop an initial but transient neutrophilic inflammatory response in the lung and airways, peaking early and disappearing [23, 24]. This is followed by a sustained eosinophil and lymphocyte accumulation, increase in T_H2 cytokine levels [interleukin-4 (IL-4), IL-5 and IL-13], goblet cell hyperplasia, mucus production and increased airway reactivity. The latter is detected in response to a nonspecific bronchoconstrictor such as methacholine (MCh) [22, 25]. Similar to the response of many asthmatics, antigen-specific early- and late-phase responses can be detected in sensitized mice following allergen challenge [26, 27]. The response to allergen sensitization in mice is critically dependent on age. Following systemic sensitization young (1 week of age) and somewhat older mice (8–20 weeks of age) develop a strong eosinophilic inflammatory response in the lung, AHR, allergen-specific IgE and IgG1 and a T cell-proliferative response to the antigen in vitro. These features are gradually lost if mice are initially sensitized at an older age. Importantly, it is the age of mice at the time of initial sensitization which is the critical determining factor for the response; the age at airway challenge of sensitized mice appears unimportant [28]. These findings may have an important bearing on the human disease as allergic asthma for the most part begins in infancy and early childhood and most adult asthmatics can trace the onset of their disease to these early exposures [29]. Further, sensitization of younger mice resulted in a much more florid T_H2 cytokine response on allergen challenge than observed when mice were sensitized at an older age, a skewing also observed in young infants [30, 31]. Systemic sensitization followed by airway allergen exposure induces an acute allergic response in the lungs and airways. At this stage, usually several challenges with brief exposure to nebulized allergen are necessary to fully establish the allergic response in the lung [32]. This might be due to the fact that the actual amount of allergen delivered to the lung by nebulization is very limited given the respiratory dynamics of mice (respiratory rate, tidal volume). By contrast, a single intranasal application of allergen in sensi-

tized mice can induce significant allergic airway disease [23]. Interestingly, once allergic airway disease has been established and after airway responsiveness and the numbers of eosinophils in BAL fluid and in the tissues were restored to baseline levels, reexposure to a single, brief challenge with aerosolized allergen is sufficient to induce AHR, airway inflammation and goblet cell hyperplasia, indicating the ability to trigger a rapid memory response to the allergen in the lung [26, 33, 34].

Development of allergic airway disease can also be induced by systemic administration of allergen alone without adjuvant followed by airway challenge [35–37] or, in the absence of systemic sensitization, by repeated administration of allergen directly into the lung either by inhalation [38, 39], intranasal [40, 41] or intratracheal [42] instillation. Administration of antigen-pulsed dendritic cells into the airways can also prime animals to develop airway disease following limited inhaled allergen exposure [43, 44]. Virtually all of the protocols, which avoid systemic sensitization with adjuvant, elicit less prominent airway inflammation and hyperresponsiveness when compared to sensitization with adjuvant.

CD4+ T Cells Contribute to the Development of Allergic Airway Disease

T cell populations can be subdivided based on their surface expression of either $\alpha\beta$ or $\gamma\delta$ T cell receptor. $\alpha\beta$ T cells can be further classified by the expression of surface CD4 or CD8. Murine models have been pivotal to our understanding of the involvement of T cells and specific subsets in inducing, orchestrating and regulating the inflammatory and physiological responses. These studies have convincingly demonstrated that the initiation and regulation of allergic disease and pulmonary involvement is absolutely dependent on antigen-specific $\alpha\beta$ + T cells. These findings were supported by the demonstration of activated $\alpha\beta$, CD4+ T cells in BAL fluid and bronchial biopsies of asthmatic subjects [45, 46]. In 1986, the concept of different subtypes of CD4+ T cells emerged based on the secreted cytokine profile [47]. T_H1 /CD4+ lymphocytes are characterized by the ability to express and secrete IFN- γ and IL-12 and are thought to be involved in host defense against viruses and bacteria. T_H2 /CD4+ lymphocytes, which express and secrete IL-4, IL-5, IL-9 and IL-13, have been shown to be critical for the induction of allergic airway disease. Animals which fail to develop T_H2 responses, e.g., mice lacking or depleted of CD4+ T cells [48–52], mice lacking the IL-4 receptor α chain [53] or the transcription factor signal transducer and activator of transcription (STAT)-6 [54, 55], both pivotal for the

induction of T_H2 cells, do not develop allergic airway inflammation and AHR following systemic sensitization and airway allergen challenge. In contrast, mice deficient in the transcription factor t-bet, which are not able to mount a T_H1 response, develop spontaneous eosinophilic airway inflammation, AHR and goblet cell hyperplasia [56]. The ability of developing T_H2 cells or at least a T_H2 cytokine response may not only be necessary during the sensitization period but also during the airway challenge phase. This has been suggested by studies in transgenic mice expressing a mutant for GATA-3, a transcription factor which is important in the differentiation of T_H2 cells following T cell activation [57]. Expressing a dominant-negative transgene in an inducible T cell-dependent manner showed that inhibition of GATA-3 following sensitization but prior to allergen challenge still reduced eosinophilia and AHR [58]. Similarly, administration of a GATA-3 antisense nucleotide to sensitized mice before allergen challenge decreased airway inflammation and AHR [59]. The important role of CD4+ T cells in the development of allergic airway disease is further supported by the findings that CD4-deficient mice show fewer eosinophil numbers in the lung following sensitization and challenge compared to the wild-type mice [49]. Depletion of CD4+ cells before airway allergen challenge in sensitized mice also reduces allergic airway inflammation [60] and adoptive transfer of CD4+ T lymphocytes from sensitized and challenged mice to naïve mice is capable of inducing AHR and airway inflammation in the challenged recipient [61, 62]. Transfer of T_H2 cells leads to induction of goblet cell metaplasia and mucus production [53, 63]. The mechanisms by which T_H2 cells initiate, induce and maintain lung inflammatory responses have been attributed to elaboration of T_H2 cytokines [64], interactions between resident pulmonary leukocytes and local induction of chemokines in the lung [49, 65, 66].

Allergen-Specific Effector CD8+ T Cells Contribute to Allergic Airway Disease

In contrast to the role of CD4+ T cells in allergic airway disease, the contribution of $\alpha\beta$ CD8+ T cells to the development of allergic airway disease is more controversial and has been more difficult to define. No role or even active suppression of allergic airway disease has been associated with CD8+ T cells [67, 68]. This is in contrast to other studies which have demonstrated that CD8+ T cells are required for the induction of allergic airway dysfunction [39]. A recent study showed that following sensitization and allergen exposure, mice deficient in the CD8 α chain developed less airway inflammation and AHR

compared to wild-type mice [69]. This was associated with decreased levels of the T_H2 cytokine IL-13 in BAL fluid. Interestingly, CD8+ T cells isolated from the lung of sensitized and challenged wild-type mice were shown to stain positively for IL-13, suggesting that CD8+ T cells are a source of IL-13 production in the lung in allergic airway disease. CD8+ cells have been shown to possess the capacity to differentiate into type 2 cytokine-producing (T_C2) cells and produce T_H2 cytokines [70–73]. Transfer of CD8+ T cell from sensitized, but not from naïve donors, prior to allergen challenge fully reconstituted the development of airway inflammation and AHR in sensitized CD8-deficient mice, emphasizing that ‘allergen priming’ is necessary for the supportive function of CD8+ T cells, similar to that shown with CD4+ T cells [53]. However, transfer of ‘primed’ CD8+ T cells not capable of producing IL-13 (from IL-13-deficient mice) failed to reconstitute airway inflammation and AHR, further confirming that IL-13-producing CD8+ T cells play an important role in the development of AHR in this model. Recent focus has been on defining different subtypes of memory CD8+ T cells, especially following allergen priming. Antigen-experienced populations of memory CD8+ T cells can be distinguished by surface expression of the CD62L ligand (CD62L) and the chemokine receptor CCR7. Different functional and migratory properties have been recently described [74–76]. Antigen-experienced central memory CD8+ T cells (T_{CM}) are CD62L^{hi}/CCR7^{hi} and home preferentially to lymph nodes. Effector CD8+ T cells (T_{EFF}) are CD62L^{lo}/CCR7^{lo} and traffic more efficiently to non-lymphoid tissues and to sites of tissue inflammation. In vitro, CD8+ T cells can be differentiated in culture to provide either of these subtypes. When cultured in the presence of IL-15, antigen-specific CD8+ T cells acquire the phenotypic and functional characteristics of CD8+ T_{CM} , whereas CD8+ T cells cultured in the presence of IL-2 show characteristics of CD8+ T_{EFF} cells [76, 77]. Previous studies have shown that migration of T_{EFF} cells can be initiated by mast cell activation and that this migratory response can be regulated by the leukotriene B₄ receptor 1 (BLT1) expressed on T_{EFF} cells under in vitro conditions [78]. When transferred to sensitized CD8-deficient mice, T_{EFF} were found in increasing numbers in the lung following airway allergen challenge, unlike transferred T_{CM} , which migrated to lymph nodes [79]. Importantly, transfer of T_{EFF} fully reconstituted airway inflammation as well as AHR in CD8-deficient mice whereas transfer of T_{CM} had no effect on airway inflammation or AHR. In addition, transfer of these allergen-specific T_{EFF} cells to sensitized wild-type mice further increased the degree of

AHR and airway inflammation following airway allergen challenge. These findings implicate a specific subgroup of CD8+ T cells, which participates in the development of allergic airway disease. How these CD8+ T cells differ from ‘suppressor CD8+ T cells’ is an important question for understanding the regulation and maintenance or curtailment of allergic lung inflammation and AHR.

Differential Role of $\gamma\delta$ T Cell Subsets in Regulating AHR

In contrast to $\alpha\beta$ T cells, which are pivotal for the induction of allergen-induced airway inflammation and AHR, the role of $\gamma\delta$ T cells is more obscure. Responsive $\gamma\delta$ T cells have been detected in increasing numbers in the lung following repeated airway allergen challenges. $\gamma\delta$ T cells capable of suppressing primary IgE responses to the allergen following transfer to naïve recipients have been demonstrated, in parallel to the loss of responsiveness to this antigen in challenged cell donors [80, 81]. In sensitized mice, depletion of $\gamma\delta$ T cells before airway challenge leads to an increase in AHR, suggesting a regulatory role for these cells [82]. These regulatory effects of $\gamma\delta$ T cells were dependent on TNF- α [83] and signaling through the TNF receptor 2 (p75) [84], an observation consistent with the finding that $\gamma\delta$ T cells preferentially respond to TNF- α signals mediated by this receptor [85]. There are three major intrapulmonary subsets of $\gamma\delta$ T cells, which can be identified by the V γ chains expressed [86, 87]. Studies measuring airway responsiveness following airway allergen challenge in mice without systemic sensitization have suggested that the (negative) regulatory population is part of the V γ 4+ subpopulation of pulmonary lymphocytes [87]. In sensitized and allergen-challenged mice an increase in the V γ 4+ population in the lung can be observed. Furthermore, depletion of V γ 4+ cells in sensitized mice before allergen challenge resulted in increased AHR, whereas depletion before sensitization had no effect on airway function [88]. In addition, transfer of isolated lung V γ 4+ cells from sensitized and challenged donors to sensitized recipients prior to airway challenge suppressed the development of AHR [89]. In contrast, depletion of V γ 1+ cells before challenge did not affect the development of AHR. However, when V γ 1+ cells were depleted before sensitization, the development of AHR, airway inflammation and T_H2 cytokine production was significantly attenuated [88]. Overall, these results indicate that the V γ 4+ subpopulation of $\gamma\delta$ T cells suppresses the development of AHR during allergen exposure in the lung. In contrast, it appears that V γ 1+ cells contribute to the development of allergic AHR primarily during the sensitization

period. These results illustrate the complexity of functionally different T cell subtypes, which can be only elicited and characterized in in vivo experimental approaches. These approaches emphasize the importance of detailed subtype analyses of cell populations involved in the allergic response.

IL-4 and IL-13: Similar T_H2 Cytokines with Distinct Effector Functions

In addition to revealing the network of T cells involved in allergic airway disease, mouse models have been central in defining the importance and different function of cytokines in the induction and maintenance of these disease processes. However, the complexity of the cytokine network in these responses is no less than the cells themselves. Results from murine studies have identified the central role of the expression of the T_H2 cytokines IL-4, IL-5, IL-9 and IL-13 in the development of allergic airway disease. It is quite clear that allergic sensitization is critically dependent on IL-4 production. IL-4-deficient mice or mice deficient in IL-4 receptor α chain, which cannot signal following exposure to IL-4 or IL-13, fail to develop sensitization, T_H2 polarization, immunoglobulin isotype switching and IgE production [90, 91]. Type 2 immune responses in the lung have been linked to the orchestrated recruitment of cells committed to IL-4 production into the lung [92]. However, it seems that once sensitization has occurred, IL-4 is less critical for the induction of airway inflammation and AHR following airway exposure to allergen. Indeed, administration of a neutralizing antibody to IL-4 or a soluble IL-4 receptor during the challenge phase alone, only partially reduces the development of allergic airway responsiveness in previously sensitized mice, suggesting an alternate mechanism other than IL-4 in inducing an allergic phenotype in the lung [93, 94]. Further, adoptively transferred T_H2 polarized T cells from IL-4-deficient mice are capable of inducing AHR in naïve recipient mice following allergen challenge [95]. Interestingly, treatment of sensitized mice with an IL-4 receptor antagonist, which blocks signaling through the IL-4 receptor α chain, during allergen challenge of sensitized mice has proven effective in impairing the development of AHR and airway inflammation [96]. This suggested that cytokines other than IL-4, which signal through the IL-4 receptor α chain, could be involved in the regulation of airway inflammation and AHR. Indeed, IL-13 signals through a receptor composed of a heterodimer of the IL-4 receptor α chain and the IL-13 receptor α 1 chain [97]. In contrast to IL-4, IL-13 is not required for the development of T_H2 cells, but it plays a role in T_H2 cell activation

and negatively regulates T_H1 differentiation indirectly through downregulatory effects on the production of proinflammatory cytokines, e.g., IL-12 [98]. As well, T_H2 cells generated in culture using T cells or splenocytes from IL-13 gene-deficient mice produce less IL-4, IL-5 and IL-10 than their counterparts from wild-type mice [99]. Interestingly, IL-13 has been found to be a central regulatory cytokine in the development of allergic airway disease. In particular, and unlike inhibition of IL-4 [100–102], inhibition of IL-13 during the challenge phase in previously sensitized mice abolishes the development of AHR and goblet cell hyperplasia, with less effect on airway (eosinophilic) inflammation [35, 103]. Similarly, studies in IL-13-deficient mice show decreases in AHR and goblet cell hyperplasia but more variable effects on airway inflammation [104]. However, in gene-manipulated mice the reduction in airway responsiveness has been described to be strain dependent [105] and appears to be linked to the isoform of the IL-4 receptor α [106]. Other studies reported that induction of AHR through IL-13 is not critically dependent on expression of the IL-4 receptor α chain but on STAT-6 [107]. Similar to the studies on acute responses, neutralization of IL-13 has also been effective following rechallenge of mice with established airway disease. In this secondary challenge protocol, previously sensitized and challenged mice are rechallenged several weeks later, after all acute changes normalize, with a single inhalational exposure to aerosolized allergen. This leads to a memory response in the lung with development of an early and late airway response and the rapid development of AHR to MCh and airway inflammation [26]. Neutralizing IL-13 during this secondary challenge phase also inhibits the development of a late airway response, development of AHR, goblet cell hyperplasia and T_H2 cytokine production in the lung, with less effects on airway inflammation [34]. These findings suggest an important role for IL-13 in established airway disease.

Other strategies used to investigate the role of IL-13 in development of allergic airway disease include the overexpression of the IL-13 gene in the lung. This can be achieved by expression of IL-13 under the control of a Clara cell 10-kD protein (CC10) gene promoter. This promoter can be used to target gene expression in the lung, because it is selectively expressed by the Clara cell, which makes up about 40% of the epithelium in murine airways [108]. Overexpression of IL-13 was associated with a strong inflammatory response in the lung, predominated by eosinophils, lymphocytes and macrophages. In addition, IL-13 overexpression resulted in goblet cell hyper-

plasia, AHR, airway fibrosis, and ultimately led to the development of emphysematous changes [108]. Using this approach, several proteins were identified which may have contributed to IL-13-induced changes in the lung, e.g., adenosine, VEGF, TGF- β 1 MMPs, different chemokines and chitinase [109–112].

Similar to the results in IL-13-overexpressing mice, transgenic mice overexpressing IL-9 develop airway inflammation with eosinophils and lymphocytes, mast cell hyperplasia, goblet cell hyperplasia and AHR, suggesting the direct involvement of IL-9 in the induction of allergic airway disease [113]. Interestingly, recent studies have shown that IL-9 mediates these effects through the induction of IL-13 [114].

Another cytokine, which has been closely linked to the development of allergic airway disease and eosinophil inflammation, is IL-5. IL-5 production by CD4⁺ T lymphocytes appears necessary for the proliferation of eosinophil progenitors in the bone marrow [115], amplifies eotaxin-induced eosinophil chemotaxis in the airways and regulates eosinophil recruitment to the lung [116]. IL-5-secreting CD4⁺ T_H2-type cells have been shown to play a pivotal role in generating blood and airways eosinophilia and in the subsequent development of bronchial hyperreactivity and lung damage that occurs in response to allergen exposure [62]. Treatment of normal mice with an IL-5-neutralizing antibody also decreased airway eosinophilia as well as the development of AHR [117, 118]. Further, administration of IL-5 to IL-4-deficient mice reconstituted their ability to develop AHR, implicating a direct effect of IL-5 in the airways [119]. Interestingly, mice overexpressing IL-5 in the lung show increased mucus production, an effect which is dependent on CD4⁺ T cells and IL-4 receptor α chain [120]. This implies that IL-13 is involved in the IL-5-induced airway changes as the absence of allergic AHR in IL-5/eotaxin-deficient mice has been linked to the inability of T cells to produce IL-13 in these mice [121].

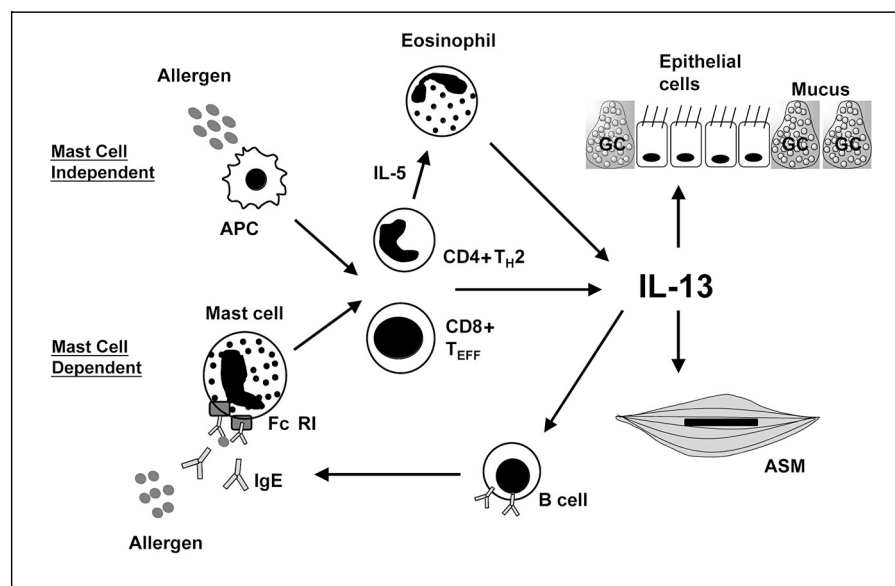
Overall these results demonstrate a central role for IL-4 in the allergen sensitization phase, whereas there is strong evidence that IL-13 is an important effector cytokine during the challenge or maintenance phase of allergic airway disease and might serve as the final common pathway for T_H2-mediated allergic airway disease. The source of IL-13 for the induction of allergic airway disease is likely complex as well and may vary during various phases of the disease. One major source is the T_H2-committed CD4⁺ T cell. Other cells that possess the potential to produce and secrete IL-13 include smooth muscle cells [122], basophils, mast cells [123–125] and natural killer cells [126].

Studies of murine models of allergic airway disease have shown that IL-13 production by CD8⁺ T cells [69] and natural killer T cells [127] in vivo may also be critical to the induction of allergic airway disease, at least under certain circumstances.

Mast Cells and High-Affinity IgE Receptor (Fc ϵ RI)-Dependent and -Independent Pathways of AHR

A variety of responses and pathways have been described in murine models with sometimes varying results. Some of this variation can probably be attributed, at least in part, to differences in mouse strains and the protocol used to induce sensitization. One example, which is highly dependent on the protocol used, is in the demonstration of the relevance of mast cells and Fc ϵ RI in the development of allergic airway disease. Tissue mast cells are highly abundant in the body and have the potential to respond to low doses of allergen, amplifying the response through release of different potent inflammatory mediators in an antibody-dependent mechanism [128]. In mouse lung, mast cells are most easily detected in the peritracheal tissue. However, they can also be found in the peribronchial tissue in increased numbers following prolonged allergen exposure of sensitized mice [129]. The role and contribution of mast cells to the development of allergic airway disease appear to be highly dependent on the sensitization and allergen exposure protocol. When studying mechanisms of allergic airway disease, variable decreases in eosinophil numbers have been reported in mast cell-deficient mice compared to wild-type mice [130, 131]. Systemically sensitized and challenged mast cell-deficient mice are capable of fully developing a T_H2 response, airway inflammation, and AHR, similar to wild-type control mice [22, 90, 132]. In an analogous fashion, it has been shown that B cells [133] or IgE [118, 134] are not required for the development of allergic airway disease. In most of these studies, animals were sensitized systemically by injection of allergen in combination with an adjuvant and subsequently challenged via the airways, which leads to marked inflammatory changes in the lung, increased serum levels of allergen-specific antibodies, increased airway reactivity and sensitivity to inhaled MCh, and prominent goblet cell hyperplasia. When 'less potent' protocols are followed, a prominent role for mast cells in the development of allergic airway disease can be unmasked. In a study by Williams and Galli [36], mast cell-deficient mice exhibited weaker airway responsiveness and lung tissue eosinophil inflammation following systemic sensitization without adjuvant and repeated allergen exposure com-

Fig. 1. Potential mechanism of mast cell-dependent and mast cell-independent development of allergic airway disease in sensitized hosts following allergen exposure. Following allergen exposure, activation of mast cells via the high affinity IgE receptor (FcεRI) leads to mast cell activation and recruitment of allergen-specific CD4⁺ T_H2 cells and allergen-specific CD8⁺ T effector cells (T_{EFF}). In the mast cell-independent pathway allergen exposure leads, likely through antigen-presenting cells (APC), to recruitment of T_H2 and T_{EFF} cells. Both cell types are capable of producing T_H2 cytokines, especially IL-5 and IL-13. IL-5 is important for eosinophil migration into the lung, which are also another source of IL-13. IL-13 then acts on airway epithelial cells to induce goblet cell hyperplasia and on airway smooth muscle (ASM) to induce increased responsiveness.



pared to similarly sensitized and challenged control mice. Similarly, Mayr et al. [135] described the inability of FcεRI-deficient mice to develop allergic airway inflammation and AHR after systemic sensitization with a single injection of ovalbumin (with adjuvant) followed by repeated intranasal challenges. In rodents, FcεRI appears to be expressed only on mast cells and basophils, so these findings suggest the importance of mast cells and their activation through IgE cross-linking. Recently, it has been shown that following repeated inhalational exposures to allergen in the absence of systemic sensitization, mast cell-deficient mice and mice lacking FcεRI develop less airway responsiveness to electrical field stimulation compared to similarly exposed wild-type mice [136]. In addition, airway inflammation, goblet cell metaplasia and levels of IL-13 in the lung were decreased in mast cell- and FcεRI-deficient mice. This defect could be reconstituted after transfer of FcεRI-expressing mast cells into FcεRI-deficient recipients following allergen exposure. These results underscore the importance of which sensitization and challenge model is selected to assess airway function and airway inflammation. As a rule it appears that in less 'potent' allergen sensitization and exposure models, mast cells are needed to amplify the allergic response in the airways, whereas more 'potent' sensitization and exposure approaches bypass these requirements (fig. 1).

Mast cells can contribute through different mechanisms to the development of AHR, by secreting mediators and cytokines (e.g., IL-13 [123]) which can directly induce AHR or by secreting chemoattractants and chemokines,

which lead to the recruitment of other cell populations, such as effector T cells [78]. Transfer of mast cells expressing FcεRI but not able to express IL-13 to FcεRI-deficient recipients nonetheless reconstituted their ability to develop increased airway responsiveness, suggesting that IL-13 production from mast cells does not play a primary role in the development of allergic airway disease. In addition, transfer of FcεRI- and IL-13-expressing mast cells to IL-13-deficient recipients failed to reconstitute the ability of the IL-13-deficient mice to develop AHR [136]. Together these data suggest that mast cell activation through FcεRI is critical in IL-13-dependent induction of AHR in this model. However, these mast cells do not appear to be the major source of IL-13, suggesting that other cell populations may contribute to the production of IL-13 in the lung (fig. 1).

It is very apparent that it is critical which model is chosen to investigate allergic airway disease. Depending on the mode of sensitization and challenge, different cell populations and mediators might be involved in the allergic responses in the lung. Careful investigation and characterization of these different methodological approaches are important if we are to mimic certain traits of a complex human disease [137]. As asthma is a very heterogeneous syndrome, it is unlikely that one model will mimic all of the different aspects of the disease. Therefore a careful selection and consideration of the different sensitization and exposure models are critical to adequately study the spectrum of asthma.

Murine Models of 'Chronic' Allergic Airway Disease

Typical features of asthma are recurrent episodes of airway inflammation and airway obstruction, and some patients experience a gradual decline in lung function and fixed obstruction. These fixed alterations have been attributed to structural changes in the airways, which are apparent on histologic sections and have been termed 'airway remodeling'. The features of airway remodeling include airway wall thickening, fibrosis in the lamina reticularis and adventitia, mucus metaplasia, cartilage degradation, airway smooth muscle cell hypertrophy and hyperplasia, and neovascularization [138, 139]. The functional consequences of these morphological changes are not entirely clear, but it is likely that these structural changes contribute to the clinical phenotypes of the disease. It is important to recognize that most murine models of allergic airway disease have been used to primarily understand and investigate those changes related to acute responses, often the initial response to the very first exposure to allergen in the lung. It is therefore not always clear if and how these acute models translate to human disease. In particular, evaluating the effect of any new therapy in these models must be viewed cautiously, given the fact that patients with asthma are usually treated not during the initiation of the disease, but much later, when the disease has been well-established. Following the initial development of allergic airway disease, sensitized mice can be reexposed to allergen after the initial functional and inflammatory changes have subsided. This rechallenge or secondary challenge leads to a rapid memory response in the lung which is associated with early- and late-phase airway responses, rapid accumulation of airway inflammatory cells and development of AHR [26]. Although AHR and goblet cell hyperplasia are dependent on IL-13 in this model [34], it has been shown that the effectiveness of certain pharmacological interventions varies between an acute and this secondary response [33]. Unfortunately, in this secondary model, similar to acute models, few morphological changes can be detected on tissue sections. Following allergen exposure, goblet cell hyperplasia can be detected, but thickening of the airway wall or smooth muscle hypertrophy have been not been described. To create a chronic model with features of airway remodeling, different approaches have been tried, mainly by sustaining airway challenges over weeks to months following the initial acute response. One of the problems encountered in mice with repeated challenges over a prolonged period of time is that the phenotype, AHR and airway inflammation, become attenuated [40, 140, 141]. This phenomenon, however, seems to be closely related to con-

tinuous allergen exposure, which leads to a form of 'tolerance'. Interestingly, this 'tolerance' to repeated exposure demonstrated a bystander suppression of reactions to unrelated antigens associated with the production of anti-inflammatory factors, e.g., IL-10 [142]. Despite these issues, a wide variety of methodological approaches have been attempted to mimic morphological changes seen in human airways. These vary from controlled exposure to low-mass concentrations of aerosolized antigen [143], to nasal allergen exposure in previously sensitized [144–148] or nonsensitized mice [40, 149], to sustained overexpression of certain cytokines, e.g. IL-13, in the lung [150], sensitization and challenge with *Aspergillus fumigatus* [151] and sustained AHR following virus infection in the lung [152]. Strain differences, which are also apparent following the acute responses to inhaled allergen [153], may also be important in the susceptibility to chronic airway exposure. A/J mice have been reported to be more susceptible to chronic intranasal allergen exposure compared to BALB/c mice, where morphological and inflammatory changes are attenuated following chronic exposure [40]. Differences in the ability of different allergens to induce a chronic response have been described. Continuous exposure to house dust mite extract for 5 days a week for up to 7 weeks in BALB/c mice elicited persistent eosinophilic airway inflammation, in vitro T_H2 cytokine production, AHR, increased peribronchial contractile elements and airway collagen deposition whereas continuous exposure to ovalbumin failed to induce any significant airway disease [154]. Long-term overexpression of IL-13 in the lung leads to certain morphological changes which culminate in emphysematous changes in the lung [150]. In humans these changes are more characteristic of patients with chronic obstructive pulmonary disease than those with asthma [155]. Different factors contributing to the chronic morphological changes in the lung include cysteinyl leukotrienes [145] and platelets [156]. IL-5 has been identified as important for the development of allergen-induced chronic changes in mice [147]. However, other studies suggested that IL-4 and IL-13, but not IL-5, contribute to allergen-induced chronic changes in the lungs of these animals [148]. The former observations may lie in the fact that IL-5-deficient mice were used, so that the deficiency was present from the beginning of the exposures. Assessment of chronic changes, for example smooth muscle hypertrophy or collagen deposition, cannot be quantitated without very careful tissue preparation and morphometry. Too many reports rely on simple provision of limited photomicrographs, tangential cuts of airways and blood vessels, and collagen deposition that is not peri-

bronchiolar, contributing to a great deal of mis- and over-interpretation. Given these discrepancies, problems of tolerance induction, issues related to differences among certain strains and allergens and the wide variability in methodological approaches, more thorough studies of 'chronic' models are needed to critically judge their applicability and usefulness. However, once these issues have been resolved, chronic models might then contribute significantly to the understanding of asthma pathophysiology and to the impact of morphological changes on airway function and disease phenotype. Furthermore, they would allow a more relevant approach to test new therapeutics.

Implication of Murine Models for Understanding Human Disease

As asthma prevalence increases throughout the world [157], new therapeutic options and intervention strategies are needed. Despite considerable progress in understanding the pathophysiology of asthma, little progress has been achieved in actual development of therapeutic options, which sustain any benefit in the clinic. Several cellular and molecular targets have been identified [158], sometimes with the help of animal models and the usefulness of some of these therapeutic options, including the neutralization of IL-13, is still being evaluated [159]. Unfortunately, some recent therapeutic approaches such as targeting the leukotriene pathway, IgE or targets within the cytokine network (e.g., IL-4, IL-5, IL-12) have provided little or only modest benefit for the treatment of asthma [160, 161], despite initial promise in certain mouse models. While some of these clinical trials have been criticized for intrinsic design flaws [162], the results have nevertheless fueled negative views as to the usefulness of the animal model in asthma research [163]. Some of the discrepancies in the data on efficacy in murine models and human asthma may be explained by, e.g., the extent of eosinophil depletion and their persistence in certain lung tissue sites. More likely, the effects described in the murine model were obtained in a primary sensitization and challenge model or in gene-manipulated mice. We have repeatedly shown different requirements for airway inflammation and AHR, using different models, in particular comparing primary versus secondary challenge models [84]. Therefore, it is not surprising that simple translation from mouse to man is not sustainable in the clinic. Similar to human asthma heterogeneity, the different mouse models also reveal a complex heterogeneity. In neither species have the cumulative results identified a single or exclusive pathway underlying allergic asthma or development of AHR.

As outlined above, models of acute allergic airway responses are often used in the evaluation of new therapeutic strategies, overemphasizing results in an acute response protocol, which underlies some of the failure to translate findings to human disease. This includes systemic sensitization of the animal by systemic administration of the allergen, together with adjuvant, and less often exclusive allergen exposure through the airways, more in line with human exposures. Murine airways differ significantly from human airways. Murine AHR is not asthma, which is a human condition. However, despite the criticism, it is important to point out that the spectrum of murine models have had a large impact in defining the immunological mechanisms underlying allergic asthma and have provided critical understanding of the disease. Murine models have allowed direct investigation of complex in vivo mechanisms, cell and cytokine or mediator interactions, something that is difficult or impossible to achieve using in vitro or ex vivo systems. For these reasons murine or other animal models will remain an essential part in further identifying the complex in vivo mechanisms and the sequence of events leading to asthma pathophysiology and they have a necessary role in the integrative research of allergic asthma. It is clear that insights gained through the use of animal models cannot be simply translated into human disease in a noncritical manner. Newly discovered pathways or potential therapeutic targets need to be carefully explored and validated in human subjects. Because of the vast heterogeneity in asthma phenotypes, it is clear that there is not one model which is capable of modeling all of the different traits of the human disease. Rather, it is likely necessary to use a number of different models. A variety of murine models are available to define eosinophil-dependent and eosinophil-independent pathways, as well as mast cell-dependent and mast cell-independent components, leading to AHR and airway inflammation. Genetically manipulated mice provide a unique approach to defining novel and important components of the disease, including airway remodeling. When evaluated within the context of their known limitations, murine models will continue to drive asthma research and the development of novel and hopefully effective therapies.

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