

Allergic Asthma : What Have We Learned from the Mouse Model?

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ABSTRACT

Despite advances and introduction of new and effective anti-asthma therapies, including inhaled corticosteroids and leukotriene modifiers, the mortality and morbidity of asthma is still a worldwide concern. Moreover, asthma is a complex disease (more likely a syndrome) in terms of varied pathogenesis and a marked clinical and pathological heterogeneity. To overcome the clinical limitations in investigating and unraveling this complexity, animal models have been utilized. In many ways, these models have contributed to the clarification of some of the pathogenesis and identified new therapeutic targets for drug development. We have characterized a number of such mouse models of allergic airway inflammation and altered airway function, defining the role of various cells and factors such as mast cells, interleukin (IL)-13 and CD8+ T cells in the development of airway hyperresponsiveness (AHR) and allergic airway inflammation. In these models we demonstrated that mast cells play a role in the development of AHR through stimulation of immunoglobulin receptors (FcRs). Histamine, a potent chemical mediator secreted by mast cells, was shown to contribute to the development of AHR and airway allergic inflammation. In addition, IL-13 was shown to play a critical role in these allergic responses in the lung. To date, CD8+ T cells have not been recognized as much as CD4+ T cells in the pathogenesis of allergic disorders. However, we have recently shown that CD8+ T cells, particularly allergen-primed CD8+ T cells, were potent inducers of IL-13, AHR and allergic airway inflammation. Furthermore, a subset of CD8+ T cells (T effector ; T_{EFF}) was shown to be important in the development of AHR, allergic inflammation and IL-13. Although further investigations are necessary to fully understand the complex interactions between these inflammatory cells, mediators, cytokines and chemokines, these findings provide new insights into the initiation and persistence of asthma and hopefully will lead to new therapeutic strategies for the treatment of asthma.

KEY WORDS

allergy, asthma, interleukin-13, mast cell, mouse, T lymphocyte

INTRODUCTION

The mouse model of allergic airway disease is a useful tool to investigate potential mechanisms of bronchial asthma. In asthma, a number of different cell types, mediators and cytokines and chemokines have been identified, reflecting the complex heterogeneity of the disease. Although there are no exact animal models of human asthma, we have developed two distinct models of allergic airway inflammation and airway hyperresponsiveness (AHR) to examine the role of different pathways : in one which is IgE and mast cell-dependent, the animals are challenged with allergen, administered exclusively via the airways without systemic sensitization or adjuvant. In this model, AHR is detected by electrical field stimulation of tra-

cheal smooth muscle preparations, which in allergen exposed mice reflects an increase in acetylcholine release secondary to M2 receptor dysfunction.¹ In the second model, animals are systemically sensitized to allergen together with adjuvant and AHR is detected by measuring airway responsiveness to inhaled methacholine (MCh) following airway allergen challenge. Even in this commonly used approach to elicit AHR and airway inflammation, significant differences in terms of critical cellular elements and their products have been identified depending on whether there is a single series of challenges following sensitization (primary challenge) or whether the animals are re-challenged weeks after the primary challenge (secondary challenge).²

Using these different models, we have identified

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important roles for T-lymphocytes, eosinophils, mast cells, the high affinity receptor for IgE, IL-4, IL-5 and IL-13 in the development of AHR, depending on which approach is utilized. Even in terms of T lymphocytes, roles for both α/β and γ/δ T cells have been identified in the regulation of AHR and airway inflammation. As well as CD4+ T cells, we have also defined a role for CD8+ T cells in the development of allergic airway inflammation and AHR. We demonstrated that antigen-primed CD8+ T cells can produce IL-13 and restore AHR and airway eosinophilia following airway allergen challenge in sensitized CD8-deficient mice and enhance these responses in normal mice. To further define the specific subset of memory CD8+ T cells which was capable of mediating these responses, we generated antigen-specific, effector memory (T_{EFF}) and central memory (T_{CM}) CD8+ T cells and investigated the effects of these cells on AHR and airway inflammation in adoptive transfer experiments. T_{EFF} , but not T_{CM} , were shown to accumulate preferentially in the airways, produce IL-13 and mediate AHR and airway eosinophilia.

Given the heterogeneity of the human disease, these newly identified pathways and cellular functions may all contribute to the development and/or persistence of airway inflammation and AHR in patients and help in understanding and guiding new and more effective therapeutic interventions. It has not been easy to simply translate the findings in mice to asthmatics. Although these limitations have been argued against the use of such models, it is more than likely that the limitations may also lie in the heterogeneity of the human disease, the chronicity of asthma, the years of repeated allergen exposure, and the complex genetics and gene-environment interactions. Further, the development, anatomy and innervation of the airways are quite different in the two species. Why then use mouse models? The answers are straight-forward: studies in man are necessarily limited and the availability of reagents and genetically manipulated strains of mice provide unique investigative opportunities. At all times, identification of potential targets in mice must be validated in well-defined clinical trials in man, and to define their applicability to asthma pathophysiology. In several instances, when studies in mice were translated to man, the failures, for example targeting eosinophils,³ were used to discredit the mouse models despite the fact that the clinical trial design may have been at fault.⁴

ASTHMA-THE HUMAN DISEASE

In spite of the considerable advances in pharmacotherapy, bronchial asthma remains a serious life threatening respiratory condition with considerable morbidity and mortality worldwide. Numerous studies have been carried out to explore the pathogenesis of the disease and have defined a dysregulation of immune function, structural changes in the airways, al-

tered neurogenic control of airway function, and respiratory tract infections. Based on these insights, various therapies have been designed and examined in asthmatics to provide better control of the disease as well as proof-of-concept. However, asthma remains a complex syndrome with the potential for multiple etiologies, and there is a considerable heterogeneity among asthma patients both in terms of the pathophysiology as well as the response to therapy. The phenotype of the disease may vary depending on several factors including atopy, inflammatory characteristics such as airway eosinophilia, age of the patient at the clinical onset of asthma, gender and the environment in which they spent their early years.⁵ To carry out reliable clinical studies, a remarkable number of participating subjects and relatively complex procedures are sometimes necessary. As a result, clinical trials of asthma have become more difficult to perform because of costs, time and ethical issues. For ethical reasons, it is virtually impossible to directly investigate all mechanisms potentially involved in the disease in human subjects, and to perform repeated morphological and physiological assessments of function *in vivo*. Moreover, it is difficult to compare new therapies to placebo (Helsinki Doctrine), so that patients are already on a variety of medications, complicating interpretation. *In vitro* and *ex vivo* assessments of cellular and molecular functions using patient clinical samples are helpful but they may not always reflect the *in vivo* aspects of the disease. The last decade has witnessed a long list of failed interventions or interventions with only limited success. In many instances these interventions were not based on animal studies but were the consequences of clinical investigation alone.

ALLERGEN-INDUCED AHR AND INFLAMMATION IN THE MOUSE

There is no counter argument to the statement that "man is the best model of human disease". Despite their limitations, several animal species have been utilized to model some pathophysiological aspects of asthma in order to better define the mechanisms underlying the disease and to initially evaluate potential therapeutic strategies for asthma. Among these animal species, mice have been the most widely used because of the many advantages including a well described genome, a fully characterized immune system, and availability of immunological reagents and genetically modified strains to assess particular pathways and functions.

In mice, the immune system has been well characterized in terms of inflammatory cell subsets, cell surface markers, cytokines, chemokines and cell signaling pathways.⁶⁻⁸ In addition, various genetically engineered strains of mice are available, providing additional tools for investigating cellular and molecular pathways potentially involved in human disease.^{9,10}

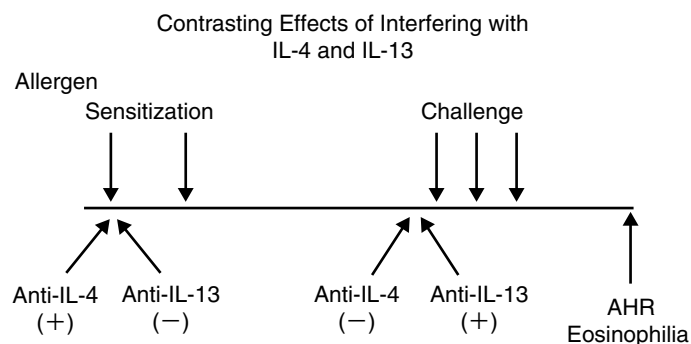


Fig. 1 The role of IL-4 and IL-13 in the development of AHR and eosinophilic airway inflammation. The development of AHR and airway eosinophilia is abolished by neutralization of IL-4 but not IL-13 during the sensitization phase, and by neutralization of IL-13 but not IL-4 during the airway allergen challenge phase. (+) : positive effect; (–) : no effect.

Most importantly, measuring airway function in mice is no longer a challenge owing to recent technological advances acquired in the field of lung physiology.¹¹

Notwithstanding the many and important anatomical differences between mouse and human lung (7–8 generations of airways in mice versus 23–25 in human), well-established murine models can now provide assessments of morphological, immunological and physiological changes in the airways that mimic some of the pathophysiological features of asthma. Mice, like many other laboratory animals, do not spontaneously develop asthma. However, if sensitized to an allergen and subsequently exposed via the airways to the same allergen they develop allergic airway inflammation and AHR to inhaled MCh, hallmarks of clinical asthma. Various protocols have been developed and used to produce allergic airway inflammation and AHR in mice. Most often, mice are systemically sensitized by intraperitoneal administration of an allergen (e.g., ovalbumin (OVA), ragweed or house dust mite), mixed with adjuvant (aluminum hydroxide), and subsequently challenged via the airways with the allergen, administered by inhalation, or by intranasal or intratracheal instillation. Following these protocols, sensitized and challenged mice develop increased levels of antigen-specific IgE in the serum, a highly polarized Th2 cytokine response in the lung and AHR without the requirement for B cells, mast cells or IgE.^{12,13} During the sensitization phase IL-4 appears to be required as well as CD4+ α/β T cells.¹⁴ During the challenge phase IL-4 and T cells may be less important and IL-5, IL-13, and eosinophils have been implicated,^{15–17} but not in all studies.¹⁸ In the absence of systemic sensitization with adjuvant, repeated airway allergen challenge leads to the development of low levels of antigen-specific IgE with discrete inflammatory changes in

the airways.¹⁹ AHR to inhaled MCh in this model has been difficult to demonstrate except under specific conditions.²⁰ Altered airway responsiveness in allergen-exposed mice can be detected monitoring contractile responses of isolated tracheal smooth muscle strips to electrical field stimulation.²¹ A third approach is to passively sensitize mice with allergen-specific IgE (or IgG1) and then challenge the recipients with allergen via the airways.²² Since AHR is a common feature to all asthmatics, models in which AHR develops have been perceived as more “relevant” to human disease. However, it is important to recognize that the AHR that develops may be very different in terms of the pathophysiology between species. Further, in several studies bronchoconstrictor agents were administered via the intravenous route and not by inhalation, the conventional route to assess disease activity in man.²³

THE ROLE OF MAST CELLS, FC ϵ RI, IL-13 AND MAST CELL-DERIVED MEDIATORS IN ALLERGIC AIRWAY INFLAMMATION AND AHR

Numerous mediators and cytokines released from activated mast cells, following crosslinking of IgE on the surface of these cells with allergen, are thought to play a major role in the pathogenesis of allergic airway diseases such as atopic asthma.^{20,24} However, the importance of such pathways in non-atopic asthma is less clear. For example, there are some asthmatics with no detectable serum level of allergen-specific antibodies and we also reported that mast cells may not be necessary to develop AHR when there are other inflammatory cells in the lung.²⁵ IL-4 and IL-13 are important cytokines that regulate the development of allergic AHR and mucus production through goblet cell hyperplasia. Both cytokines share the same receptor unit (IL-4R α chain) and signal

through STAT-6.^{26,27} However, they appear to play somewhat distinct roles and are involved at different phases in the development of allergic airway responsiveness. IL-4 appears to be essential during the sensitization phase, whereas IL-13 plays a dominant role in regulating AHR during the allergen challenge phase (Fig. 1).^{16,17}

Because mast cells can produce IL-13,²⁸ we investigated the role of mast cells, the high affinity receptor for IgE (FcεRI) and IL-13 in the development of allergic airway inflammation and AHR in the allergen challenge model without adjuvant.²⁹ These studies were carried out using mast cell-deficient mice, IL-13-deficient mice (IL-13^{-/-}) and mice lacking the high affinity IgE receptor I alpha subunit (FcεRI^{-/-}). The mice were challenged with aerosolized OVA on 10 consecutive days. Tracheas were isolated after completion of airway challenge and contractile responses to increasing frequencies of electrical field stimulation (EFS) were monitored *in vitro*. The data are expressed as the frequency of electrical stimulation causing 50% of the maximal contractile response (ES₅₀). The results showed that in contrast to wild-type mice (WT), which developed contractile responses at lower electrical frequencies, *i.e.*, lower ES₅₀, neither FcεRI^{-/-} mice nor mast cell-deficient mice developed AHR. The levels of IL-13 detected in homogenized lung tissue significantly increased following repeated airway allergen challenge in WT mice but did not increase in FcεRI^{-/-} mice or in mast cell-deficient mice. These data suggested that the levels of IL-13 in the lung were closely related to both mast cell activation and development of AHR. To directly address the role of IL-13 in the development of AHR in this model, we used IL-13^{-/-} mice and neutralization of IL-13 in WT mice during allergen challenge. Following 10 days of airway allergen exposure, IL-13^{-/-} and anti-IL-13-treated mice failed to develop AHR, indicating a central role for IL-13 in this response. To further elucidate the role of mast cells in the development of this altered airway responsiveness, bone marrow-derived mast cells (BMMC) were derived from WT, FcεRI^{-/-} and IL-13^{-/-} mice. These BMMC were transferred (intravenously) into recipient mice before the daily OVA exposures were initiated. Transfer of mast cells did not induce AHR in the absence of allergen challenge in any of the recipients. Interestingly, transfer of mast cells from WT mice or from IL-13^{-/-} mice restored AHR following allergen challenge in FcεRI^{-/-} recipients but not in IL-13^{-/-} recipient mice, indicating that although IL-13 was required, it was not necessarily mast cell derived for the development of AHR.

Histamine is an important mediator of allergic reactions, and is secreted mainly by mast cells and basophils. Histamine triggers vasodilation, edema, mucus hypersecretion, and smooth muscle contraction, all

considered to be important pathologic responses in asthma.^{30,31} Although anti-histamine treatment is generally not considered as an effective therapy for asthma, interesting data have emerged using H1 antagonists in the early prevention or delay in onset of asthma in infants.³² We and others have shown that, treatment of sensitized and challenged mice with antagonists of the histamine H1 receptor, effectively reduced AHR and airway inflammation despite a low density of H1 receptors in the mouse lung.^{33,34} These findings may be explained by an effect of histamine on various inflammatory and non-inflammatory cells and the mediators they release. Dendritic cells are the most potent antigen-presenting cells³⁵ and are capable of releasing numerous cytokines and chemokines.^{36,37} Histamine induces the expression of CD86, an important accessory molecule, on dendritic cells in a dose-dependent fashion. Similarly, histamine enhances the expression of the key adhesion molecules E-selectin, intercellular adhesion molecule-1 and leukocyte function-associated molecule-1 in a dose-dependent manner on epithelial cells, endothelial cells, eosinophils and fibroblasts.³⁹⁻⁴² Furthermore, histamine triggers IL-6 and IL-8 secretion in endothelial cells^{43,44} and induces IL-1, IL-6, IL-18, and IFN-γ production but suppresses IL-12 production by peripheral blood mononuclear cells.⁴⁵⁻⁴⁹ Histamine induces the production of IL-10 and IL-6 from macrophages,^{50,51} and IL-1, IL-6, IL-8, and MCP-1 from dendritic cells.³⁸ Histamine can also stimulate CD8 T cells, inhibiting antibody synthesis, T-cell proliferation, and T cell-mediated cytotoxicity.^{50,52,53} Collectively, these immunoregulatory effects of histamine appear to deviate the immune response towards a Th2-like response.

Since mast cells may not be the critical source of IL-13 for the development of AHR as described above, perhaps a mast cell-derived mediator can contribute to the induction of IL-13 by another cell type residing or recruited to the lung (Fig. 2). In this context, activation of mast cells by antigen-mediated crosslinking of IgE and critical signaling through FcεRI can lead to the release of mediators involved in the initiation of an allergic inflammatory response following allergen exposure. Mast cells capable of secreting mediators such as histamine may well influence the activation, recruitment and cytokine production by other cells in the lung environment.

THE ROLE OF CD8+ T CELLS AND IL-13 IN ALLERGIC AIRWAY INFLAMMATION AND AHR

T cells are thought to play a key role in orchestrating the disease process through the release of various cytokines in asthma.⁵⁴ Most attention has been focused on the role of Th2 subset of α/β+ CD4+ T cells, which produce IL-4, IL-5, and IL-13, in the development of allergic airway inflammation and AHR.⁵⁵⁻⁵⁸

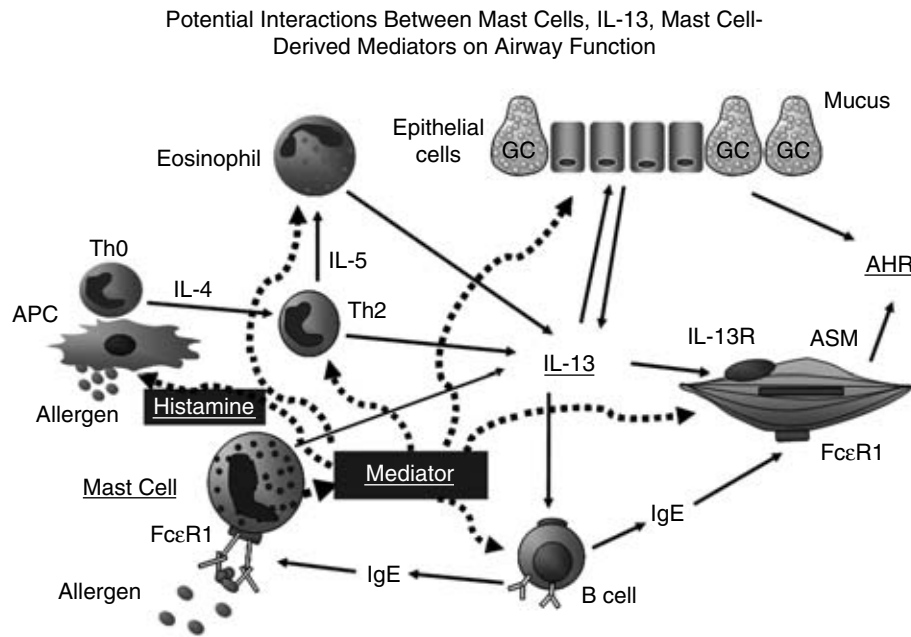


Fig. 2 Potential interactions linking mast cells, IL-13 and altered airway function. Allergens activate IgE-sensitized mast cells triggering release of mediators (e.g., histamine, lipid mediators) that promote Th2-type responses including increased IL-13 production, goblet cell hyperplasia, mucus production and AHR. APC, antigen presenting cells; FcεR1, high affinity Fc receptor for IgE; Th0, undifferentiated helper T cell; Th2, type-2 helper T cell; ASM, airway smooth muscle; GC, goblet cell.

Until recently, only few studies have attempted to define the exact role of CD8⁺ T cells in AHR. A number of studies have reported that CD8⁺ T cells play a protective role in allergic airway disease.⁵⁹⁻⁶³ We previously showed that depletion of CD8⁺ T cells in fact abrogated AHR and airway inflammation in mice exposed to allergen exclusively via the airways, in the absence of adjuvant.⁶⁴ These apparent discrepancies between studies may be due to the (functional) heterogeneity of CD8⁺ T cell subpopulations involved in different phases of the development of an allergic airway response. At least two distinct functional subsets of CD8⁺ T cells have been identified;⁶⁵⁻⁶⁷ a Th1-type cytokine-producing subset (Tc1) and a Th2-type cytokine-producing subset (Tc2). A potential role for CD8⁺ T cells in the pathogenesis of asthma is supported by the findings of increased numbers of activated CD8⁺ T cells in fatal asthma⁶⁸ and by the demonstration of circulating IL-4-producing CD8⁺ T cells in the peripheral blood of atopic patients with mild asthma⁶⁹ as well as in the peribronchial tissue.⁶⁸

To further define the role of CD8⁺ T cells in the development of airway inflammation and AHR, we first investigated these responses in sensitized and challenged CD8-deficient (CD8^{-/-}) mice.⁷⁰ CD8^{-/-} mice developed reduced AHR and airway inflammation and lower levels of IL-13 in the BAL fluid when compared with WT mice. To confirm these findings,

we carried out adoptive transfer experiments in CD8^{-/-} mice and demonstrated that OVA-primed CD8⁺ T cells but not naive CD8⁺ T cells or non-CD8⁺ T cells restored the full development of AHR and airway inflammation. In recipient mice, the levels of IL-13, measured in BAL fluid, were restored after transfer of OVA-primed CD8⁺ T cells but not naive CD8⁺ or non-CD8⁺ T cells. When mice received CD8⁺ T cells from IL-13-deficient mice, they failed to develop AHR and allergic airway inflammation. Intracellular cytokine staining revealed that IL-13 was expressed in the lung by CD8⁺ T cells transferred from allergen sensitized and challenged WT mice. These results suggested that antigen primed (memory) CD8⁺ T cells contribute to the development of AHR by secreting IL-13 in the lung.

Memory CD8⁺ T cell subsets can now be distinguished. One subset, the central memory CD8⁺ T cells (T_{CM}), is CD62L^{hi}CCR7^{hi} and can be generated following culture with IL-15 after a short stimulation with allergen. Effector memory CD8⁺ T cells (T_{EFF}) are CD62L^{lo}CCR7^{lo} and are generated in culture with IL-2.⁷¹⁻⁷³ These two distinct populations of memory CD8⁺ T cells also differ in their functional and migratory properties: T_{CM} are localized mainly in the lymphoid tissue, whereas T_{EFF} are recruited efficiently to non-lymphoid tissues and to sites of inflammation.

To define potential functional differences between

Potential Interactions Between Mast Cells, IL-13, CD8 and Development of AHR

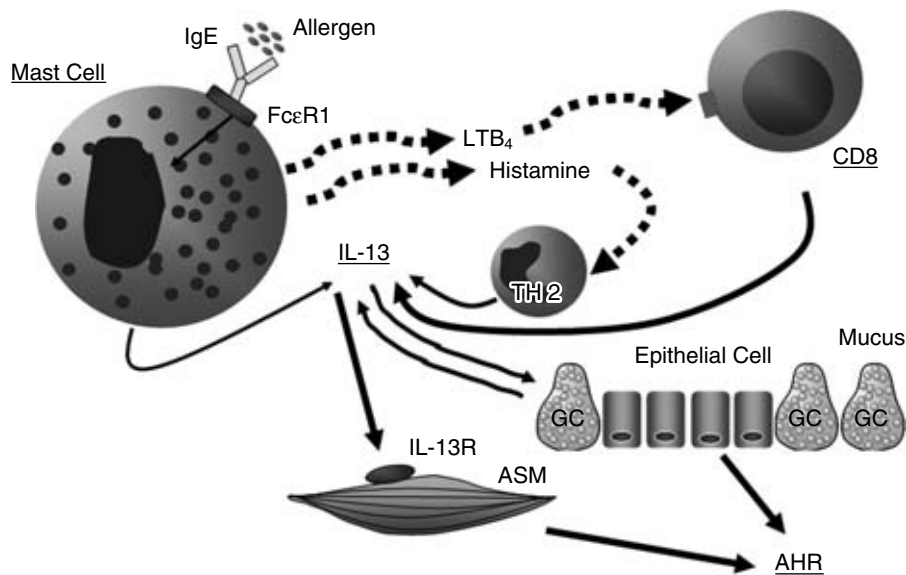


Fig. 3 Potential interactions linking CD8⁺ T cells, mast cells, IL-13 and AHR. Mast cells, activated by allergen through IgE and FcεRI, release mediators (e.g., histamine, LTB₄) that promote Th2 function and mediate recruitment/activation of CD8⁺ (T_{EFF}) cells, resulting in enhanced IL-13 production, mucus hypersecretion and AHR. LTB₄, leukotriene B₄.

these two subsets of CD8⁺ memory T cells, we studied the role of antigen-specific, memory CD8⁺ T cells in allergic airway inflammation and AHR.⁷⁴ We generated OVA₂₅₇₋₂₆₄(SIINFEKL) peptide-specific T_{EFF} and T_{CM} from OVA₂₅₇₋₂₆₄ peptide-specific TCR transgenic OT-1 mice (C57BL/6 strain B6.PL-*Thy1a*/Cy) congenic for the Thy1.1 cell surface marker. T_{EFF} and T_{CM} CD8⁺ T cells were adoptively transferred into OVA-sensitized CD8^{-/-} recipients, just before airway challenge with aerosolized OVA. Transfer of T_{EFF} but not T_{CM} restored AHR, airway eosinophilia and goblet cell hyperplasia in recipient CD8^{-/-} mice. Tracking of transferred CD8⁺ cells was by analysis of the cell surface marker Thy1.1 in recipient mice. This revealed that T_{EFF} were predominantly localized in airway tissue, whereas T_{CM} were mainly present in regional lymphoid tissue. The development of AHR and airway eosinophilia was also accompanied by increased levels of IL-13 in the BAL of CD8^{-/-} mice following adoptive transfer of T_{EFF}. Following cell transfer and allergen challenge, significant numbers of T_{EFF} expressing the Th2 cytokines IL-4, IL-5 and IL-13 were detected in the lungs of sensitized CD8^{-/-} recipient mice. Even when transferred into normal (CD8⁺) mice, T_{EFF} enhanced AHR and allergic airway inflammation. These results indicate that antigen-specific memory CD8⁺ T_{EFF} cells can play a critical role in the development AHR and allergic air-

way inflammation by producing IL-13 in the lung. The mechanisms leading to the differentiation, recruitment and activation of effector memory (T_{EFF}) CD8⁺ T cells *in vivo* needs to be further investigated. One possibility, based on recent *in vitro* studies, is that activated lung mast cells induce the recruitment of leukotriene B₄ (LTB₄) receptor-expressing T_{EFF}, but not T_{CM}, through the production of LTB₄.⁷⁵ Thus, as illustrated in Figure 3, activation of mast cells through IgE and FcεRI can lead to the release of a lipid mediator, resulting in the recruitment of IL-13-producing CD8⁺ T_{EFF}.

CONCLUSIONS

The pathogenesis of allergic asthma is complex, involving multiple pathways, cells and mediators. Unraveling this heterogeneity in humans is limited by ethical considerations and somewhat sparse tools. Animal models have been established to help resolve some of these issues, but they are no less complex with a similar heterogeneity of culprits depending on the particular model used. Here, we have summarized some of the general issues confronted in using different mouse models of allergen-induced AHR and airway inflammation. These studies reveal an important regulatory role for mast cells in the maintenance of airway dysfunction that goes beyond early responses mediated by mast cell degranulation and the

release of acute phase mediators. In these ways, the mast cell release of histamine or LTB₄ may play a major role in the recruitment of specific receptor bearing T cells (especially CD8⁺ T cells) that are capable of releasing significant amounts of IL-13, a pivotal cytokine in the development of AHR, airway eosinophilia and goblet cell hyperplasia. Studies directly assessing these pathways in asthmatics will determine if they are important in the human condition and whether therapeutic targeting will be feasible.

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