

Do Mouse Models of Allergic Asthma Mimic Clinical Disease?

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Key Words

Allergic asthma, pathophysiology · Allergic asthma, pathology · Allergic asthma, therapy · Immunoglobulin E · Eosinophilic lung inflammation · Immunological memory · Th2 cells · Airway resistance · Allergens, administration and dosage · Mice, congenic

Abstract

Experimental mouse models of allergic asthma established almost 10 years ago offered new opportunities to study disease pathogenesis and to develop new therapeutics. These models focused on the factors governing the allergic immune response, on modeling clinical behavior of allergic asthma, and led to insights into pulmonary pathophysiology. Although mouse models rarely completely reproduce all the features of human disease, after sensitization and respiratory tract challenges with antigen, wild-type mice develop a clinical syndrome that closely resembles allergic asthma, characterized by eosinophilic lung inflammation, airway hyperresponsiveness (AHR), increased IgE, mucus hypersecretion, and eventually, airway remodeling. There are, however, differences between mouse and human physiology that threaten to limit the value of mouse models. Three exam-

ples of such differences relate to both clinical manifestations of disease and underlying pathogenesis. First, in contrast to patients who have increased methacholine-induced AHR even when they are symptom-free, mice exhibit only transient methacholine-induced AHR following allergen exposure. Second, chronic allergen exposure in patients leads to chronic allergic asthma, whereas repeated exposures in sensitized mice causes suppression of disease. Third, IgE and mast cells, in humans, mediate early- and late-phase allergic responses, though both are unnecessary for the generation of allergic asthma in mice. Taken together, these observations suggest that mouse models of allergic asthma are not exact replicas of human disease and thus, question the validity of these models. However, observations from mouse models of allergic asthma support many existing paradigms, although some novel discoveries in mice have yet to be verified in patients. This review presents an overview of the clinical aspects of disease in mouse models of allergic asthma emphasizing (1) the factors influencing the pathophysiological responses during the initiation and perpetuation of disease, (2) the utility of mouse models for studying clinical manifestations of disease, and (3) the applicability of mouse models for testing new treatments for allergic asthma.

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Introduction

Allergic asthma is a chronic inflammatory disease of the respiratory tract with rising incidence and prevalence in industrialized countries. According to the World Health Organization, asthma affects 150 million people worldwide and is the most prevalent chronic disease of childhood. It is estimated that there are 18 asthma-related deaths per million people and 180,000 deaths per year [1]. Increased school absenteeism, lost working days and productivity, and high medical care costs combine to constitute a substantial economic burden to patients, their families, and society [1]. Asthma-related morbidity and mortality continue to rise, although many effective drugs to alleviate signs and symptoms of asthma are available. This is thought, in part, to be due to increased exposure to indoor allergens, more pollution, fewer childhood viral illnesses, overuse of β_2 -agonists, treatment failure and serious adverse effects to treatment.

Although epidemiological studies and clinical investigation are powerful tools to study human disease, there are constraints that can be avoided when using animal models. First, ethical issues limit the study of allergen sensitization in humans. Second, there are few clinical indications for bronchoalveolar lavage (BAL) and lung biopsy in asthmatics, thus restricting access to lesions and reducing our capacity to study disease pathogenesis. Third, testing novel treatments in humans is difficult due to the tough regulations that need to be met before a drug is accepted for clinical phase trials. In 1994, the first mouse models resembling allergic asthma were published [2–5] and have since resulted in significant strides in our understanding of the pathophysiology of allergic disease. There are, however, differences between mouse and human physiology that threaten to limit the value of mouse models. This review will focus on the strengths and weaknesses of models of allergic asthma in mice.

Lessons from Mouse Models about Allergen Sensitization

Allergen Sensitization: Immunity versus Tolerance

The immune system is designed to either respond or tolerate foreign antigens. One rationale justifying immune tolerance is to avoid developing immune responses to harmless inhaled and ingested proteins, while at the same time protecting the host against pathogens. Failure of the regulatory mechanisms governing immunity and tolerance, either by generating an erroneous immune response

against perceived pathogens or failure to ignore innocuous proteins, could potentially result in immunity directed against innocuous proteins, like pollens or house dust mites. Pathogen recognition by the immune system elicits an immune response designed to eradicate the organism. Whether the class of the response is predominantly cell-mediated or humoral is dependent on the type of pathogens encountered, i.e. viruses, bacteria, or parasites. In atopic individuals, however, nonpathogenic foreign proteins elicit an allergic class of response.

Allergen sensitization begins when an individual encounters and responds to a foreign protein. This may occur at a variety of sites including, the skin, circulation, respiratory, gastrointestinal, and genitourinary tracts. Sensitization is initiated by specialized antigen-presenting cells, such as dendritic cells (DC), macrophages, and B cells, that take up foreign proteins, internalize them, and process them into small peptide fragments. MHC class II molecules within the cell bind to these peptide fragments and the class II:peptide complex migrates to the cell surface where it is displayed and can interact with the T cell receptor on CD4⁺ T lymphocytes. The consequence of antigen recognition is activation, differentiation, and clonal expansion of CD4⁺ T helper 2 (Th2) cells, alteration of the quantity and type of cell surface molecules, production and secretion of IL-4, IL-5, IL-6, IL-10, and IL-13 cytokines, and generation of cells that can provide help to B lymphocytes. The result is Th2-mediated recruitment and activation of eosinophils in the skin, respiratory and gastrointestinal tracts, as well as the promotion of their survival, and allergen-specific IgE production.

The IgE is, in turn, cross-linked as it binds to allergen and high-affinity Fc ϵ RI receptors on the surface of mast cells and leads to mast cell degranulation and the release of vasoactive mediators. In allergic asthma, the combination of Th2 cell- and mast cell-derived mediators in the lungs, along with factors derived from respiratory epithelium, smooth muscle, and the nervous system, cause eosinophilic inflammation, edema, smooth muscle contraction, increased mucus production, and airway obstruction [6].

The reason why susceptible individuals generate allergic responses to foreign antigen is unknown but appears to be dependent upon the context of the encounter. Host factors are linked to a family history of allergic disease (genetic susceptibility), allergen-specific IgE, and possibly, prenatal allergen exposure. Other factors contributing to the development of allergic asthma may also involve high aeroallergen exposures, viral infections early in life, vaccination programs, and a low socioeconomic status [1]. The

increased incidence of allergic disease associated with westernized countries with high standards of hygiene has led to the 'hygiene hypothesis', which states that improved hygiene and decreased infectious exposures are causally related to an increased incidence of asthma and allergy [7]. This has been substantiated by studies comparing rural and urban children showing that exposure to microbials associated with farm livestock influences the incidence of atopy [8]. Taken together, epidemiological data indicate that there are a multitude of host and environmental factors that affect the incidence of allergic disease. The promise of mouse models of allergic asthma lies in a further understanding of the effects of these factors on disease and the elucidation of the mechanisms underlying allergen sensitization.

How Are Mice Sensitized to Allergen?

Prerequisite for Allergic Asthma: Allergen-Specific T Cell Precursor Frequency

Mouse models of allergic asthma have provided important information about the conditions necessary for allergen sensitization. Induction of allergic asthma by systemic immunization leading to allergen-specific Th2 immunity followed by pulmonary allergen challenge is a common basis for a number of mouse models. Systemic allergen-specific Th2 immunity prior to lung challenge improves disease induction. Systemic priming can be achieved by intraperitoneal (i.p.) immunization with antigen precipitated in aluminum sulfate (alum) [9], small doses of soluble antigen without alum [10, 11], or repeated intranasal [12], or intratracheal [13] instillation for certain antigens, and i.p. or intratracheal administration of antigen-pulsed DC and macrophages [14–17]. In addition, coagulated egg white implanted subcutaneously will prime for chicken egg ovalbumin (OVA)-specific Th2 immune responses [18]. Mice exposed to aerosolized antigen in the absence of systemic immunity do not develop lung disease [19–23]. These observations suggest that a 'threshold' precursor frequency of activated antigen-specific Th2 cells in the circulation is necessary for respiratory tract antigen challenge to efficiently induce lung disease. The additional observation that systemic antigen boosting 1–4 weeks after primary immunization improves Th2 priming and the induction of disease corroborates the need for an adequate number of activated T cells.

Transgenic mice containing solely CD4⁺ T cells bearing the OVA-specific T cell receptor (DO11.10) develop allergic airway disease when administered repeated doses of aerosol OVA [24, 25]. Additionally, disease can be

induced in mice not previously sensitized, following repeated respiratory tract challenges with OVA, if preceded by adoptive transfer of fully activated and polarized Th2 cells from immunized wild-type [26–28] or DO11.10 [16, 29, 30] mice. This provides further support for the notion that circulating, activated antigen-specific T cells are a prerequisite for the initiation of allergic asthma (acute onset).

Once mice are primed and have sufficient numbers of activated Th2 cells, repeated respiratory tract antigen challenges by intratracheal, intranasal, or aerosol routes are necessary for establishing clinically manifest allergic asthma [10, 12, 31–33]. For reasons that are not entirely clear, a single pulmonary challenge with antigen is ineffective at inducing disease, a finding that supports the observation that increased exposure to aeroallergens is associated with allergic asthma.

Factors Influencing Allergen Sensitization: Allergenicity

Allergenicity is defined as an inherent ability of a protein to induce an allergic response. In mice, many antigens, allergenic and nonallergenic, have been successfully used to induce allergic asthma. The most commonly used antigen is OVA [2, 10, 11, 34], but others include bovine serum albumin [27], sheep red blood cells [35], β -lactoglobulin [36], schistosoma [37] and leishmania proteins [38–40], coagulated egg white implants [18], *Aspergillus fumigatus* [41], cockroach antigens [42], olive [43, 44], birch [45] and ragweed [46, 47] pollens, and house dust mite antigens [13, 48–53]. The induction of allergic asthma in mice in response to nonallergenic proteins suggests that allergenicity is not a necessary feature. This differs from current ideas about why particular proteins elicit allergic responses in human disease. The observation that patients are allergic to similar allergens suggests that inherent allergenic properties are important. Moreover, synthesis of allergens using the structural features of naturally occurring allergens has yielded proteins and polypeptides useful for diagnosis and allergen-based immunotherapy [54].

Factors Influencing Allergen Sensitization: Adjuvants

Though the ability to generate a Th2 response and subsequent disease in mice does not appear to depend on the type of allergen, the use of adjuvants can augment Th2 priming and increase allergic asthma severity. i.p. injection of alum without antigen induces a nonspecific Th2 cytokine environment [55]. When an antigen is added (precipitated) to alum and injected i.p., antigen-specific

Th2 responses are enhanced [55]. We find that nebulized OVA following i.p., but not subcutaneous injections of OVA precipitated in alum, induces allergic inflammation (unpubl. data). Despite the use of alum in most protocols, it is not necessary for inducing disease in mice. Mice administered two i.p. injections with OVA (10 µg in 200 µl PBS) 3 weeks apart develop Th2 immunity [56], which progresses to allergic asthma following OVA aerosol challenges 1 week later [10]. Thus, i.p. immunization of soluble antigen without alum effectively elicits Th2 immunity.

There is growing evidence that other adjuvants can play a role in disease initiation. For instance, OVA protein (grade V from Sigma) contains substantial quantities of endotoxin. In certain lots of OVA, we measured 200 ng/ml of endotoxin (unpubl. data). It is known that bacterial lipopolysaccharides (LPS) are effective adjuvants [57]. This is further supported in experiments showing that OVA-induced allergic asthma is reduced or absent when LPS is eliminated from OVA and in Toll-like receptor-4-deficient mice that are unable to respond to LPS [58, 59]. Thus, generation of Th2 immunity appears to require endotoxin. Critics have argued that the adjuvant effect of endotoxin limits the usefulness of mouse models. However, since it is ubiquitous, it is most likely a clinically relevant adjuvant in allergen sensitization. Indeed, studies in children have shown that endotoxin exposure influences atopy [8].

In contrast to the adjuvant effect of endotoxin during disease initiation, added LPS during antigen lung challenge reduces disease [60, 61] and added to antigen during early life may decrease antigen sensitization in later life [62]. These data suggest a seemingly paradoxical influence of LPS on allergic disease that may reflect LPS dose and route of delivery [58, 63] and requires further investigation. In addition to endotoxin and alum, diesel exhaust particles [53, 64–66], cigarette smoke [67, 68], exogenous proteases [69], GM-CSF [47], and ozone [70–72] augment Th2 immune responses and enhance allergic asthma. Interestingly, adjuvants for allergic asthma are mainly environmental factors.

Factors Influencing Allergen Sensitization:

Downregulatory Factors

In contrast to the augmenting effects of adjuvants, several factors diminish disease initiation in mice. Factors that downregulate Th2 immunity include gender (male mice have less severe responses; unpubl. data) [73], oral feeding with OVA in OVA-induced models of allergic asthma [74, 75], and concurrent infections. Cholera toxin

[76], coinfection with worms [77], and *Mycoplasma pneumoniae* [78] decrease Th2 responses and reduce the induction of allergic asthma. Similarly, a powerful inhibitor of Th2 immunity is preexistent or coexistent Th1 immunity. An augmented Th1 response elicited by the addition of IL-12 [35, 79–81], IFN-γ [82, 83], and a Th1 response directed against BCG inhibits Th2-mediated allergic asthma [84, 85]. Immunization with CFA/OVA subcutaneously (unpubl. data) or OVA and CpG oligonucleotides [37, 86, 87] followed by OVA aerosol challenge reduces disease induction, suggesting that Th1 polarization diminishes Th2 immune responses. Moreover, the absence of Th1 cells in T-bet transcription factor (necessary for Th1 cell differentiation)-deficient mice suggests a regulatory role for Th1 cells [88]. Furthermore, clinical studies indicate that the incidence of allergy in children relates to enteric lactobacteria populations and can be reduced by supplementation with Th1-promoting probiotic lactobacilli and bifidobacteria [89]. These data suggesting that Th1 immunity curbs allergic sensitization are compelling. However, there are additional experiments showing contradictory results [29, 90–93] and suggest that a shift towards Th1 immunity may not be the mechanism underlying protection against allergen sensitization. Additionally, data demonstrating that Th2 immune responses directed against helminthes protect individuals and mice from developing allergies to environmental allergens suggest alternative mechanisms [94]. Taken together, these data indicate that concomitant or preexisting infections decrease Th2-mediated allergic disease and support the hygiene hypothesis.

Factors Influencing Allergen Tolerance

Exploring the mechanisms underlying allergen-mediated immune tolerance can also be achieved using mouse models. Unsensitized wild-type mice develop immune tolerance when they are nebulized with antigen without systemic priming. The mechanism involved in this type of inhalation-induced immune tolerance is not entirely understood but suppressor CD8+ T cells, B cells, T regulatory cells, and DC have been implicated [21–23, 95, 96]. One criticism of mouse models of asthma has been the inability to induce disease by respiratory exposure alone. This is based on a hypothesis that clinical asthma is the result of repeated inhalation of allergens. While there is epidemiological evidence indicating that increased exposure to indoor allergens is a risk factor for disease, there is no proof that priming to these allergens occurs by inhalation.

Evidence in mouse models shows that allergen sensitization occurs systemically and not during inhalation. Thus, it is tempting to speculate that sensitization by inhaled allergens may not be occurring in atopic individuals either. Two alternative possibilities explaining sensitization are: (1) that coincident factors, such as microbial products, interfere with immune tolerance and result in priming to innocuous antigens, or (2) that systemic priming to the allergens or cross-reactive antigens occurs elsewhere than the respiratory tree and once achieved, subsequent exposure to aerosol allergen leads to asthma. The latter possibility is supported by experiments in which allergen-specific Th2 immunity occurred in mice when fed allergen in combination with antacid medications [97]. It may also explain the enigmatic organ specificity in allergic disease. A possible paradigm is that systemic priming followed by subsequent exposure of antigen to the nose, eyes, and lungs might lead to allergic rhinitis, conjunctivitis, and asthma, respectively. Taken together, these data from mouse models illustrate that inhaled environmental allergens induce allergic asthma when preceded by priming in other organs. When applied to the clinical situation, these results suggest that immune tolerance to inhaled proteins can be overcome in susceptible individuals by systemic priming with allergen.

Lessons from Mouse Models about Clinical Disease

What Are the Clinical Manifestations of Allergic Asthma?

Validation of mouse models of allergic asthma requires that they resemble clinical disease. Allergic asthma is a relapsing and remitting clinical syndrome induced by allergen exposure and is characterized by episodic reversible airway obstruction [6]. The hallmarks of disease include eosinophilia, lung inflammation, elevated serum IgE, mucus hypersecretion, and hyperreactive airways [6]. The clinical course of disease depends on the type of allergen exposure. Encounter with seasonal allergens, such as pollens, leads to disease relapses, while ubiquitous allergens, such as those from house dust mites, cause a more chronic, clinically manifest or 'overt' disease. Mast cell products mediate the initial response to allergen exposure, which peaks within 20 min, and is characterized by bronchial constriction, airway edema, and mucus plugging. Within 24 h, lymphocytes and eosinophils are recruited to the lungs. The resulting symptoms include shortness of breath, difficulty breathing, wheezing, chest

discomfort, and coughing, sometimes leading to respiratory failure requiring intubation and ventilation or status asthmaticus. Periods of disease remission may be symptom-free, yet are associated with abnormal pulmonary function tests, lung inflammation, positive immediate-type hypersensitivity skin tests, and elevated serum IgE. Eventually, patients may develop permanent lung tissue changes and damage resulting in irreversible airway obstruction.

Do mouse models of allergic asthma recapitulate clinical disease? There are many established mouse models of asthma currently in use that are characterized by eosinophilic lung inflammation, mucus hypersecretion, airway hyperresponsiveness (AHR), and elevated production of IgE. A wide variety of experimental protocols in wild-type mice differing with respect to inciting antigen, route of antigen administration, use of adjuvants, and dosing schedule induce disease. Furthermore, models of cellular adoptive transfer, transgenic and knockout models also generate allergic asthma. Interestingly, there is also mouse strain variability, a feature that implicates genetic factors [12, 33, 51, 52, 98–103]. Collectively, these models demonstrate a tremendous clinical diversity that is potentially more representative of the clinical syndrome in humans. The following sections describe the manifestations and clinical course of allergic asthma in mice and confirm that they are viable clinical models.

What Do Mouse Models Teach Us about Acute Onset Disease?

Lung Inflammation

BAL and histological lung sections are used to evaluate lung inflammation in mice. Total BAL cell counts in naïve mice are normally about 20,000 cells/ml of BAL and contain almost exclusively macrophages. Immunization with OVA (25 µg) precipitated in alum injections on days 0 and 5, followed by repeated aerosol challenges 1 week later, increases BAL infiltration to up to 1.5×10^6 cells/ml of BAL and peaks between 24 and 48 h after the last nebulization [34]. The resulting airway inflammation is predominantly eosinophilic (70–80%), which over the following 11 days completely resolves (fig. 1). Lymphocytes, macrophages, and neutrophils are also increased with some variations dependent on the type of experimental protocol. Eosinophilia in lavage fluid is higher with alum protocols compared to protocols without alum [10], following OVA-pulsed DC priming [14], and egg white implantation (unpubl. data). Importantly, evaluation of lavage fluid must be based on both absolute cell numbers/milliliter and percentages of each cell type

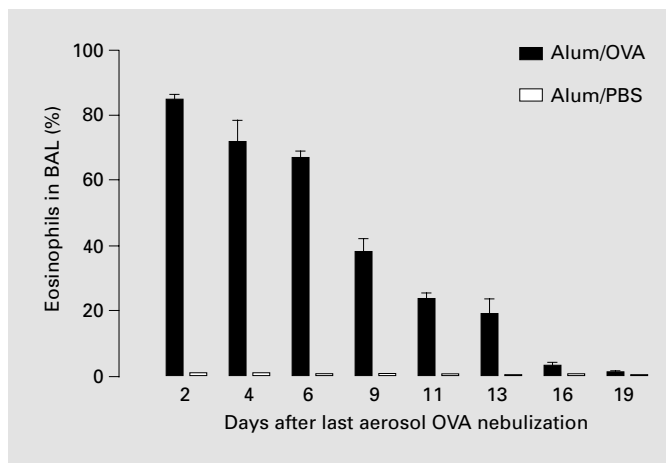


Fig. 1. The kinetics of BAL eosinophilia in mouse allergic asthma induced with OVA. BALB/c mice were immunized with OVA (Sigma, grade V) 25 µg precipitated in 4 mg of alum i.p. on days 0 and 5. One week later, mice were nebulized with OVA 1% for 60 min twice a day on 2 consecutive days. Mice were intubated and lavaged 3 times with 1 ml of PBS on the days indicated. Cells were counted and cell differential was done on May-Grünwald-stained cytopsin slides. These data are expressed as percentages of eosinophils in the BAL.

present in the BAL. The percentage of BAL cells yields information about the relative cell contribution to inflammation and, for example, shows that the percentage of macrophages is invariably lower in diseased mice compared to normal controls, despite an increase in absolute numbers. Conversely, absolute numbers of neutrophils significantly increase during disease, but they comprise only 1% of BAL cells in mice with allergic asthma and, hence, suggest a less important role in pathogenesis [34].

The quantity of infiltrating cells in the airways appears to correlate with the extent of inflammation in the lung parenchyma. Inflammation noted on H&E-stained sections is predominantly peribronchial and perivascular (fig. 2). Immunization with OVA precipitated in alum followed by OVA nebulization leads to cell infiltrates that tend to be large, diffuse, and located near large airways [34]. They contain predominantly eosinophils, as well as many macrophages and lymphocytes > plasma cells > multinucleated giant cells > neutrophils. In contrast, nebulization of mice immunized with soluble OVA results in infiltrates containing similar numbers of lymphocytes and macrophages but fewer eosinophils, neutrophils and multinucleated giant cells [10]. Furthermore, soluble OVA-induced infiltrates are smaller, denser, occur predominantly in the lung periphery around smaller airways, and are consistent with inflammation observed in bronchial and autopsy specimens from humans [104]. Other experi-

mentally induced disease models elicit different infiltration patterns. For example, i.p. injection of OVA-pulsed DC followed by OVA nebulization leads to a striking increase in multinucleated giant cells and large, activated macrophages within alveoli, in addition to eosinophils, lymphocytes, and macrophages within infiltrates [14]. The variation of cellular composition, extent, and location of lung inflammatory infiltrates seems to be due both to differences between immunizing protocols, as well as reflecting genetic diversity among mouse strains [102] (unpubl. data). The clinical significance for the diversity of lung inflammation patterns is currently unclear.

Airway Hyperresponsiveness

AHR is measured using invasive and noninvasive techniques including contraction of isolated airway segments, tracheal pressure determination during mechanical ventilation, occlusion mechanics, pulmonary resistance, dynamic lung compliance versus dual chamber, and whole-body plethysmography [105]. In the last few years, the use of whole-body plethysmography has become popular, in particular, because it is noninvasive, mice survive the procedure and can be reevaluated, and it is technically easier to do than many of the other methods. However, some investigators suggest that the noninvasive whole-body dimensionless measurement, Penh, is not an absolute value and needs to be compared with a more accurate and meaningful measurement [106–110]. Nevertheless, we and others measure AHR in response to cholinergic [34, 99] and allergenic [111] stimuli using noninvasive total body plethysmography equipment manufactured by Buxco Electronics (Troy, N.Y., USA) and observe AHR in sensitized mice compared to normal unsensitized control mice. Methacholine- and OVA-induced AHR is detectable 24 h after the last nebulized OVA challenge, before peak lung eosinophilia and remains elevated compared to naïve control mice for 11 days [111] (fig. 3). Methacholine-induced AHR in mouse models correlates with an antigen-specific Th2 immune response [99, 102, 103, 112–115] and a contribution of genetic factors [99, 102, 114, 116] but not with severity of eosinophilic lung inflammation [99, 117]. In contrast, when measuring AHR following OVA challenge instead of methacholine challenge, a correlation with the intensity of eosinophilic inflammation is seen [111]. One important distinction between human and mouse AHR is the transient increase in methacholine-induced hyperresponsiveness in mouse models. This differs from asthmatic patients who respond to methacholine even during clinical remission of symptoms and have what is termed ‘twitchy airways’. This dis-

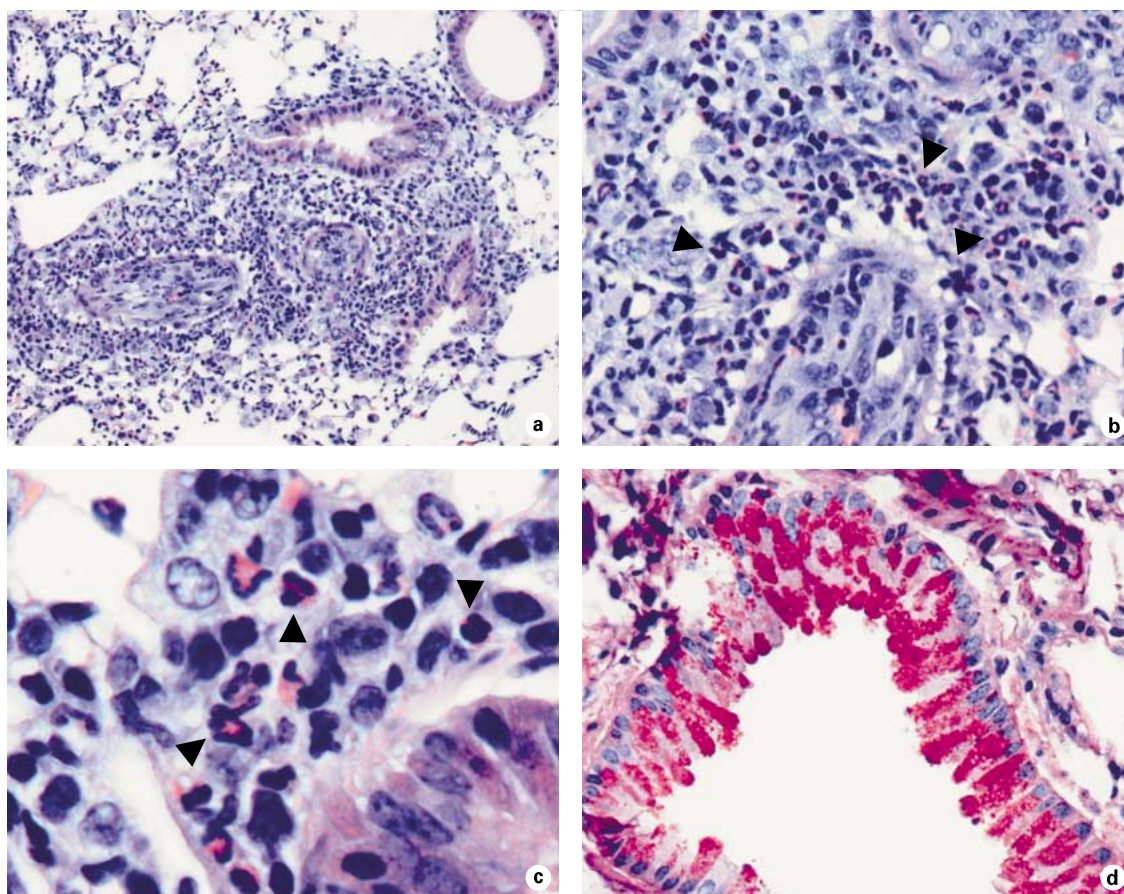


Fig. 2. Lung histology from BALB/c mice with acute-onset allergic asthma. BALB/c mice were immunized with OVA 25 μ g precipitated in 4 mg of alum i.p. on days 0 and 5, and nebulized with OVA 1% for 60 min twice a day on 2 consecutive days, 1 week later. Mice were then intubated, and lungs were lavaged and perfused. Paraffin-embedded lungs were stained with H&E or PAS. Representative photomicrographs of lung sections from mice 48 h after the last aerosol OVA challenge demonstrate extensive perivascular and peribron-

chial inflammatory infiltrate containing eosinophils, lymphocytes, and macrophages (low power field, objective lens, 100 $\times$) (**a**), and numerous eosinophils, macrophages and lymphocytes surrounding airways (high power field, objective lens, 400 $\times$, 600 $\times$) (**b**, **c**). Arrowheads indicate eosinophils. Intense pink-colored staining reveals mucopolysaccharide globules within respiratory epithelial goblet cells on PAS-stained section (objective lens; 400 $\times$) (**d**).

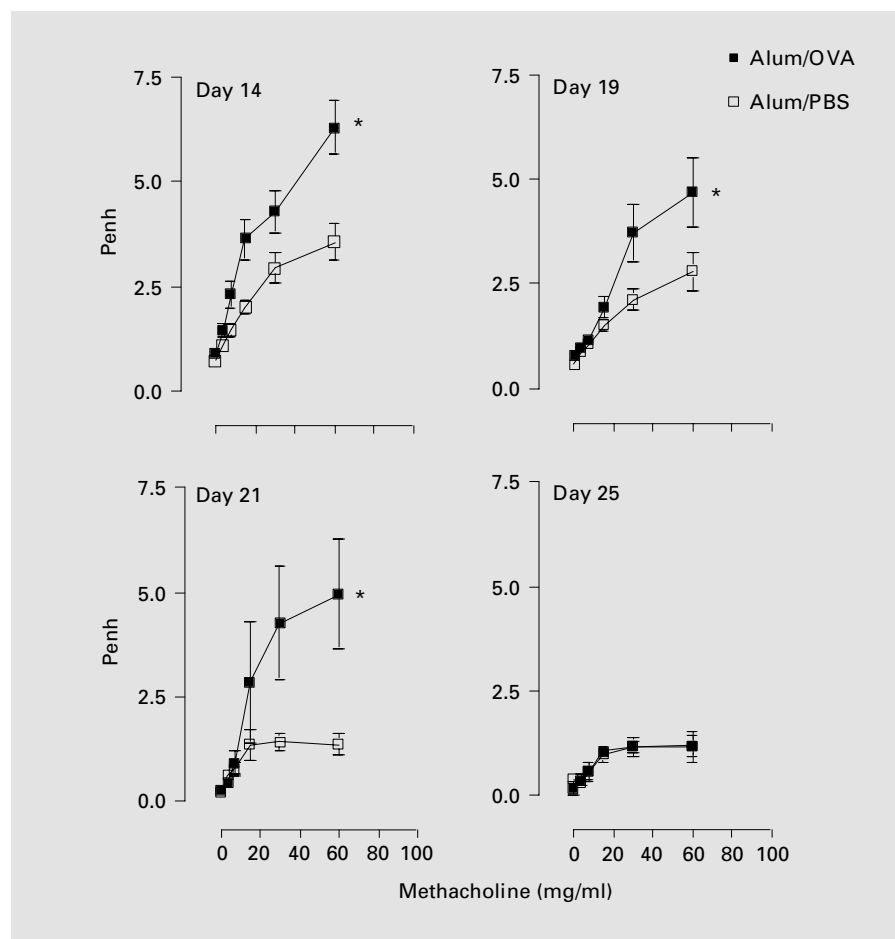
crepancy indicates that airway physiology is somewhat different in mice compared to humans. Furthermore, the extent to which components like smooth muscle contraction, edema, and mucus hypersecretion leads to airway obstruction and consequent changes in AHR measurements in mice may differ from humans.

Mucus Hypersecretion

Mucus hypersecretion is evaluated in mice on periodic acid-Schiff (PAS)-stained lung sections. In normal mice, there are few mucus-stained respiratory goblet cells, indicating low production of mucus [34]. However, during the onset of acute disease, goblet cells in the respiratory epi-

thelium produce abundant mucus, peaking 48 h after the last aerosol challenge [10, 34] (fig. 2c). Depending on disease severity, mucus may be present in goblet cells in the large and/or small airways and may even be evident in airway lumina. Mucus within goblet cells decreases gradually following acute onset of disease and returns to baseline levels within 30 days [10, 34]. Increased production of mucus correlates with antigen and IL-13 [118], and is consistent with human physiology [93, 118–121]. However, whether genetic control is similar to humans, how mice react physiologically to excess mucus, and whether mucus obstruction plays a significant role in AHR remains unclear in mouse models.

Fig. 3. Methacholine-induced AHR in sensitized mice decreases to normal 11 days after aerosol OVA challenge. BALB/c mice were immunized with OVA 25 µg precipitated in 4 mg of alum i.p. on days 0 and 5. One week later, mice were nebulized with OVA 1% for 60 min twice a day on 2 consecutive days. Beginning 24 h after the last nebulization and on the days indicated, mice were challenged with gradually increasing doses of methacholine and were tested for AHR by total body plethysmography (Buxco Electronics). These data are expressed as the Penh, a dimensionless unit representing airway resistance. * $p < 0.05$ is considered significant.



Serum Allergen-Specific Immunoglobulin

Serum antigen-specific IgE and IgG1 are elevated following almost all immunization protocols, with IgG1 titers several magnitudes higher than IgE [34, 56]. Serum antibody titers are much higher than BAL titers at acute-onset allergic asthma [34]. Kinetics experiments indicate that serum antigen-specific IgE decreases over several months, in contrast with the maintenance of consistently elevated levels of IgG1 [10]. Despite the obvious presence of antigen-specific IgE and IgG1 and the capacity to induce immediate-type hypersensitivity in mice [122], IgE and IgG1 are not necessary for disease. There is no difference in the induction or severity of allergic asthma in mast cell [3, 123, 124]-, B cell [9, 112, 125–127]-, and IgE [41]-deficient mice [128]. These observations seriously limit the generalizability of mouse models. However, data illustrating that mast cells [124], IgE [129–131] and mast cell mediators do play roles in mouse allergic

asthma, and that there are genetic differences in IgE production [103] are useful results. These latter findings indicate that IgE biology in mouse models is consistent with human physiology and that perhaps, mast cell- and immunoglobulin-deficient mice resemble a subpopulation of asthmatic patients without elevated serum IgE.

What Do Mouse Models Tell Us about Chronic Disease?

Repeated encounters with allergen in sensitized individuals cause a continuation of symptoms with exacerbations and eventual permanent organ damage with irreversible airway compromise. It is, therefore, essential to develop mouse models resembling clinically manifest and chronic allergic asthma to study the pathophysiology and discover new treatments for ongoing disease. We investi-

gated mouse models, which reflect the clinical course of allergic asthma [34]. We found that acute-onset and ongoing/overt allergic asthma in mice are distinct. To induce acute disease, BALB/c or C57Bl/6 mice are immunized with i.p. injections of OVA/alum (days 0 and 5) and subsequently nebulized with OVA. Mice treated with repeated aerosol (OVA 1%, 1 h b.i.d., once per week) developed overt, ongoing disease evident during the first 3 weeks of weekly nebulization with OVA. Weekly aerosol OVA caused a progressive increase in serum and BAL immunoglobulin, mucus production, and inflammatory infiltrates containing fewer eosinophils but with more plasma cells, macrophages, and lymphocytes as compared to that seen in acute onset disease. Both disease phases are characterized by AHR. Eventually, repeated weekly OVA aerosol challenges suppress lung inflammation and mucus secretion by 6 weeks without affecting IgE and IgG1 production or AHR [34]. Inhibition of inflammation and mucus hypersecretion was also evident in B cell-deficient mice, indicating that B cells and antibody are not essential for repeated aerosol antigen-induced suppression of inflammation [9]. The mechanism underlying suppression of this type is unknown [132–134]. Although chronic allergic asthma similar to that seen in patients does not develop in mice, it is possible that this is a protective, desensitizing mechanism resembling allergen immunotherapy.

Chronic allergic asthma is associated with airway remodeling and lung damage [104]. Established mouse models reveal that chronic antigen exposure leads to subepithelial fibrosis, hypertrophy and hyperplasia of smooth muscle, microvascular remodeling, and neovascularization [9, 37, 132, 135–139]. Furthermore, these models demonstrate an apparent correlation between the resolution of inflammation and presence of airway remodeling, which suggests that the process occurs after inflammation resolves and supports the concept that remodeling in asthmatic airways is caused by abnormal injury and repair responses [140].

Taken together, these observations demonstrate that studying chronic forms of disease is possible in mice particularly when studying airway remodeling. In contrast, evaluating overt disease is a more difficult prospect in currently used models because of antigen-induced suppression. Developing a mouse model of chronic, overt disease similar to patients proves to be challenging, but may be solved by modifying current experimental protocols to generate conditions comparable to chronic exposure with allergens like house dust mite.

What Do the Mouse Models Tell Us about Disease Relapse and Memory?

Seasonal exposure to ragweed, grass, and tree pollens induces symptoms in sensitized allergic patients. Additionally, cat-allergic individuals who only intermittently encounter cats/cat allergens develop allergic symptoms immediately upon exposure. The reactions to these allergens illustrate the relapsing nature of allergic disease. Moreover, the rapid and vigorous response to allergens in sensitized individuals is consistent with immunological memory. While immunological memory is effective and beneficial for protection against pathogens and is the basis of vaccine development, when directed against allergens, it is harmful and can be fatal.

We took the opportunity to study immunological memory using a mouse allergic asthma model. The model was designed to generate acute disease followed by remission. At various times mice were rechallenged during remission with a second aerosol antigen exposure to evaluate disease relapse [10]. We eliminated a potential source of continuous and available antigen by generating disease with soluble and nebulized OVA (without alum). The experimental protocol for acute onset disease is immunization with 10 µg of OVA in PBS on days 0 and 21 and nebulization with OVA 1% (1-hour b.i.d., on 2 consecutive days) 1 week later. Acute disease was less severe without the use of alum, but all features of allergic asthma were apparent. One month after acute-onset allergic asthma, BAL inflammation, mucus hypersecretion, and methacholine-induced AHR completely resolved, similar to naïve control mice. Four months later, serum OVA-specific IgE titers were comparable to naïve controls. Although the mice recuperated from acute allergic asthma, evidence that they recovered from an immune response included elevated serum OVA-specific IgG1 and persistent inflammatory infiltrates in their lungs up to 3 years after acute-onset allergic asthma (unpubl. data). The association between remission and elevated immunoglobulin and nitric oxide breath levels is also apparent in asthmatics [141]. While recovered mice no longer had eosinophils in lung infiltrates, lung inflammation consisting of lymphocytes and macrophages persisted. Immunohistochemical staining of lung infiltrates in recovered mice revealed a large number of CD4+ T cells (unpubl. data), which can be purified and upon restimulation with OVA *in vitro* produced Th2 cytokines [10], indicating that OVA-specific Th2 memory cells are harbored in the lungs of mice that have recovered from allergic asthma.

Recovered mice develop eosinophilic lung inflammation, mucus hypersecretion, AHR, and high titers of OVA-specific IgG1 and IgE upon secondary aerosol OVA (1%, 1 h b.i.d. on 2 consecutive days) at any time, up to 800 days after acute onset disease [10] (unpubl. data). Recall challenge also induces the differentiation of plasma cells within lung infiltrates and increases the titers of BAL immunoglobulin. Secondary responses leading to disease are not apparent in age-matched naïve mice or in mice systemically sensitized but not aerosolized with OVA (no acute onset disease). Secondary responses leading to disease relapse in recovered mice are rapid. Upregulation of Th2 cytokine RNA in lungs occurs within 1 h of nebulization with OVA. Furthermore, disease relapse can be induced with 10-fold lower doses of nebulized OVA (0.1%, 1 h b.i.d. on 2 consecutive days), a dose unable to induce acute onset disease (unpubl. data). Taken together, these results demonstrate a fast and more effective response to secondary allergen encounter, and demonstrate characteristic features of immunological memory.

These observations suggest that immunological memory leading to allergic asthma upon allergen exposure in sensitized mice is due to the persistence of CD4⁺ T cells within the lung infiltrates. In addition, lung plasma cells and increased BAL immunoglobulin implicate a role for lung memory B cells. Collectively, these observations support the notion that the lungs play a crucial role in maintaining Th2 memory responses by generating a microenvironment encouraging memory cell residence and possibly survival. Whether patients with allergic asthma have persistent memory cells within their lungs is not yet known. However, if allergen-specific Th2 cells reside within the lungs of asthmatics, then developing topical (inhaled) therapy aimed at inhibiting memory cells would be a reasonable treatment approach.

What Do the Mouse Models Teach Us about Treating Allergic Disease?

To determine whether mouse models are useful for screening new compounds and testing efficacy of new therapeutics, it is important to ascertain the effectiveness of drugs known to be useful. This would enable them to be used as reference standards for comparison with novel pharmacological strategies. For example, one of the most effective treatments for patients with allergic asthma and particularly for those with chronic disease is glucocorticoids. They have been used in mouse models to test effects on lung inflammation, airway remodeling, AHR,

and immunoglobulin production [137, 142–146]. We chose to test effectiveness of corticosteroids on established experimental allergic asthma in mice [147]. Upon treatment with inhaled Dex, we observed the elimination of AHR before relapse and during overt disease. Dexamethasone (Dex) more efficiently reduced airway inflammation, mucus production, and OVA-specific IgG1 and IgE upon relapse compared to overt disease. However, during overt disease, parenchymal inflammatory infiltrates were more effectively eliminated. These data illustrate that inhaled corticosteroids attenuate disease relapses and overt disease differentially and suggest that both airway and parenchymal inflammation should be evaluated for treatment efficacy.

Testing therapy in mouse-allergic asthma models has yielded information about disease pathogenesis, but it remains uncertain whether this is a suitable treatment model. To ensure the validity of mouse allergic asthma as a treatment model, more studies confirming known effective drugs need to be done. In addition, it is important to test treatment efficacy on established disease. Many studies have focused on interfering with disease induction at time points prior to immunization and between sensitization and airway challenge. Evaluating treatment effects on initiation of lung disease may be misleading when one considers that inflammatory cells, including effector and/or memory lymphocytes, persist in lungs during remission and during overt disease. These cells might alter the lung microenvironment, by secreting chemokines, inducing the upregulation of chemokine, adhesion, and homing receptors, which undoubtedly lead to changes in infiltration patterns of inflammatory cells and consequently, differential responses to treatment.

Lessons from Mouse Models about Pathogenesis

Studies in mouse models have established that mouse CD4⁺ Th2 cells, IL-4, IL-5, and IL-13 [4, 30, 112, 148–150], and products upstream, such as Stat 6 [151–154] and GATA-3 [155–157], mediate AHR, eosinophilic lung inflammation, mucus hypersecretion, and elevated IgE that are all hallmarks of allergic asthma [4, 30, 158, 159]. Th1 cells and their secreted products play a somewhat controversial role in disease [29, 90–93]. The development of asthma in mice deficient in IFN- γ [160, 161] and in T-bet transcription factor [88] clearly underscores the importance of Th2 cells. In addition to cytokine secretion, Th2 cell migration, adhesion, and homing are necessary

for disease initiation [162–171], and Th2 memory cells are important for maintaining disease [10]. Other lymphocytes including CD4+CD25+ T regulatory cells [96, 172, 173], CD8+ T cells [174–176], natural killer T cells [177, 178], and $\gamma\delta$ -T cells [179–183] may also be involved in disease pathogenesis.

Mouse models have confirmed the existence of a network of DC in the airway mucosa [184–190]. Intraepithelial airway DCs rapidly turn over during the inflammatory process, as a result of their recruitment, migration, and accumulation in the bronchial mucosa, which increases during the allergic response [191–193]. In addition, they play a role in *in vivo* Th2 cell priming and a critical role in secondary Th2 responses [14, 15, 39, 194–200]. In contrast to an apparent role for DC in allergic asthma, the function of macrophages remains unclear despite their obvious abundance within the lungs and inflammatory infiltrates [17, 201–206].

A large variety of inflammatory mediators, including chemokines, cytokines, mast cell-derived mediators and additional factors derived from cells from the respiratory epithelium, smooth muscle, and the nervous system play a role in allergic asthma. Recent studies show new factors that influence disease, such as the Tim-family genes [207]. The majority of experiments in mice has focused on pathogenesis and has demonstrated novel mechanisms that have yet to be proved in humans. These findings in mouse models have provided new insights into disease and pave the way for novel approaches for treatment.

Summary and Conclusion

Mouse models of asthma allow the elucidation of processes necessary for initial sensitization with allergen, early events in the lung associated with allergen response, and the determination of cellular and soluble mediators required for the initiation and perpetuation of allergic asthma. Insights from mouse models suggest that systemic immunization is necessary prior to the development of disease, that Th2 cytokines are important, that adjuvants facilitate these processes, and that the evolution from acute to ongoing inflammation involves a change in inflammatory cells and mediators. Importantly, insights derived from mouse models need to be verified in clinical disease. Nevertheless, these models have uncovered new mechanisms, created new paradigms, and lead to the discovery of novel potentially effective therapies. There are, however, notable discrepancies in certain aspects of allergic asthma between patients and mouse mod-

els that are difficult to reconcile. One example is the non-essential role for allergen-specific IgE in the mechanisms underlying AHR and inflammation in mice. Another is the transience of AHR in mice compared to humans. A third major difference appears to be the human thymic stromal lymphopoietin (TSLP) cytokine, which in atopic patients is known to influence DC-mediated allergic inflammation [208]. Despite these differences, questions about susceptibility, pathogenesis, airway remodeling, immunological memory, and treatment are being actively and successfully answered in mouse models. Furthermore, new mouse models of allergic asthma, such as the transgenic, T-bet transcription factor-deficient mice that develop spontaneous disease [88] or repeated challenge-induced chronic disease in A/J mice [12], are continuing to generate new advances in our understanding of allergic asthma. Do mouse models of allergic asthma mimic clinical disease? Yes.

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References

- WHO: WHO Fact Sheet Bronchial Asthma. Geneva, World Health Organization, 2000, No 206.
- Kung TT, Jones H, Adams GR, Umland SP, Kreutner W, Egan RW, Chapman RW, Watanick AS: Characterization of a murine model of allergic pulmonary inflammation. *Int Arch Allergy Immunol* 1994;105:83–90.
- Brusselle GG, Kips JC, Tavernier JH, van der Heyden JG, Cuvelier CA, Pauwels RA, Bluethmann H: Attenuation of allergic airway inflammation in IL-4-deficient mice. *Clin Exp Allergy* 1994;24:73–80.
- Gavett SH, Chen X, Finkelman F, Wills KM: Depletion of murine CD4⁺ T lymphocytes prevents antigen-induced airway hyperreactivity and pulmonary eosinophilia. *Am J Respir Cell Mol Biol* 1994;10:587–593.
- Lukacs NW, Strieter RM, Chensue SW, Kunkel SL: Interleukin-4-dependent pulmonary eosinophil infiltration in a murine model of asthma. *Am J Respir Cell Mol Biol* 1994;10:526–532.
- Busse WW, Lemanske RF Jr: Asthma. *N Engl J Med* 2001;344:350–362.
- Wills-Karp M, Santeliz J, Karp CL: The germless theory of allergic disease: Revisiting the hygiene hypothesis. *Nat Rev Immunol* 2001;1:69–75.
- Braun-Fahrlander C: Environmental exposure to endotoxin and other microbial products and the decreased risk of childhood atopy: Evaluating developments since April 2002. *Curr Opin Allergy Clin Immunol* 2003;3:325–329.
- Jungsuwadee P, Benkovsky M, Dekan G, Stingl G, Epstein MM: Recurrent aerosol allergen exposure suppresses inflammation in B cell deficient mice with established allergic asthma. *Clin Immunol*, in press.
- Mojtabavi N, Dekan G, Stingl G, Epstein MM: Long-lived Th2 memory in experimental allergic asthma. *J Immunol* 2002;169:4788–4796.
- Wills-Karp M, Luyimbazi J, Xu X, Schofield B, Neben TY, Karp CL, Donaldson DD: Interleukin-13: Central mediator of allergic asthma. *Science* 1998;282:2258–2261.
- Shinagawa K, Kojima M: Mouse model of airway remodeling – Strain differences. *Am J Respir Crit Care Med* 2003;168:959–967.
- Yu CK, Shieh CM, Lei HY: Repeated intratracheal inoculation of house dust mite (*Dermaphagoides farinae*) induces pulmonary eosinophilic inflammation and IgE antibody production in mice. *J Allergy Clin Immunol* 1999;104:228–236.
- Graffi SJ, Dekan G, Stingl G, Epstein MM: Systemic administration of antigen-pulsed dendritic cells induces experimental allergic asthma in mice upon aerosol antigen challenge. *Clin Immunol* 2002;103:176–184.
- Lambrech BN, Hoogsteden HC, Pauwels RA: Dendritic cells as regulators of the immune response to inhaled allergen: Recent findings in animal models of asthma. *Int Arch Allergy Immunol* 2001;124:432–446.
- Lambrech BN, Pauwels RA, Fazekas De St Groth B: Induction of rapid T cell activation, division, and recirculation by intratracheal injection of dendritic cells in a TCR transgenic model. *J Immunol* 2000;164:2937–2946.
- Janssen EM, Wauben MH, Nijkamp FP, van Eden W, van Oosterhout AJ: Immunomodulatory effects of antigen-pulsed macrophages in a murine model of allergic asthma. *Am J Respir Cell Mol Biol* 2002;27:257–264.
- Desiqueira A, Russo M, Steil AA, Facincone S, Mariano M, Jancar S: A new murine model of pulmonary eosinophilic hypersensitivity: Contribution to experimental asthma. *J Allergy Clin Immunol* 1997;100:383–388.
- Holt PG, Batty JE, Turner KJ: Inhibition of specific IgE responses in mice by pre-exposure to inhaled antigen. *Immunology* 1981;42:409–417.
- Sedgwick JD, Holt PG: Down-regulation of immune responses to inhaled antigen: Studies on the mechanism of induced suppression. *Immunology* 1985;56:635–642.
- McMenamin C, Holt PG: The natural immune response to inhaled soluble protein antigens involves major histocompatibility complex (MHC) class I-restricted CD8⁺ T cell-mediated but MHC class II-restricted CD4⁺ T cell-dependent immune deviation resulting in selective suppression of immunoglobulin E production. *J Exp Med* 1993;178:889–899.
- Seymour BW, Gershwin LJ, Coffman RL: Aerosol-induced immunoglobulin (Ig)-E unresponsiveness to ovalbumin does not require CD8⁺ or T cell receptor (TCR)-gamma/delta⁺ T cells or interferon (IFN)-gamma in a murine model of allergen sensitization. *J Exp Med* 1998;187:721–731.
- Tsitoura DC, Yeung VP, DeKruyff RH, Umet-su DT: Critical role of B cells in the development of T cell tolerance to aeroallergens. *Int Immunol* 2002;14:659–667.
- Knott PG, Gater PR, Bertrand CP: Airway inflammation driven by antigen-specific resident lung CD4⁺ T cells in alphabeta-T cell receptor transgenic mice. *Am J Respir Crit Care Med* 2000;161:1340–1348.
- Wilder JA, Collie DD, Bice DE, Tesfaigzi Y, Lyons CR, Lipscomb MF: Ovalbumin aerosols induce airway hyperreactivity in naive DO11.10 T cell receptor transgenic mice without pulmonary eosinophilia or OVA-specific antibody. *J Leukoc Biol* 2001;69:538–547.
- Hogan SP, Koskinen A, Matthaai KI, Young IG, Foster PS: Interleukin-5-producing CD4⁺ T cells play a pivotal role in aeroallergen-induced eosinophilia, bronchial hyperreactivity, and lung damage in mice. *Am J Respir Crit Care Med* 1998;157:210–218.
- Kasai M, Kurasawa K, Nakajima H, Iwamoto I: T cell vaccination eliminates antigen-specific T cells and prevents antigen-induced eosinophil recruitment into the tissue. *Int Arch Allergy Immunol* 2001;125:59–66.
- Harris N, Campbell C, Le Gros G, Ronchese F: Blockade of CD28/B7 co-stimulation by mCTLA4-Hgammal1 inhibits antigen-induced lung eosinophilia but not Th2 cell development or recruitment in the lung. *Eur J Immunol* 1997;27:155–161.
- Lee SC, Jaffar ZH, Wan KS, Holgate ST, Roberts K: Regulation of pulmonary T cell responses to inhaled antigen: Role in Th1- and Th2-mediated inflammation. *J Immunol* 1999;162:6867–6879.
- Cohn L, Homer RJ, Marinov A, Rankin J, Bottomly K: Induction of airway mucus production by T helper 2 (Th2) cells: A critical role for interleukin 4 in cell recruitment but not mucus production. *J Exp Med* 1997;186:1737–1747.
- Henderson WR Jr, Chi EY, Maliszewski CR: Soluble IL-4 receptor inhibits airway inflammation following allergen challenge in a mouse model of asthma. *J Immunol* 2000;164:1086–1095.
- Jones HP, Hodge LM, Fujihashi K, Kiyono H, McGhee JR, Simecka JW: The pulmonary environment promotes Th2 cell responses after nasal-pulmonary immunization with antigen alone, but Th1 responses are induced during instances of intense immune stimulation. *J Immunol* 2001;167:4518–4526.
- Zhang Y, Lamm WJ, Albert RK, Chi EY, Henderson WR Jr, Lewis DB: Influence of the route of allergen administration and genetic background on the murine allergic pulmonary response. *Am J Respir Crit Care Med* 1997;155:661–669.
- Jungsuwadee P, Dekan G, Stingl G, Epstein MM: Recurrent aerosol antigen exposure induces distinct patterns of experimental allergic asthma in mice. *Clin Immunol* 2002;102:145–153.
- Gavett SH, O'Hearn DJ, Li X, Huang SK, Finkelman FD, Wills KM: Interleukin 12 inhibits antigen-induced airway hyperresponsiveness, inflammation, and Th2 cytokine expression in mice. *J Exp Med* 1995;182:1527–1536.
- Adel-Patient K, Nahori MA, Proust B, Lapa e Silva JR, Creminon C, Wal JM, Vargaftig BB: Elicitation of the allergic reaction in beta-lactoglobulin-sensitized Balb/c mice: Biochemical and clinical manifestations differ according to the structure of the allergen used for challenge. *Clin Exp Allergy* 2003;33:376–385.
- Jain VV, Businga TR, Kitagaki K, George CL, O'Shaughnessy PT, Kline JN: Mucosal immunotherapy with CpG oligodeoxynucleotides reverses a murine model of chronic asthma induced by repeated antigen exposure. *Am J Physiol Lung Cell Mol Physiol* 2003;285:L1137–L1146.
- Hoshino A, Tanaka Y, Akiba H, Asakura Y, Mita Y, Sakurai T, Takaoka A, Nakaike S, Ishii N, Sugamura K, Yagita H, Okumura K: Critical role for OX40 ligand in the development of pathogenic Th2 cells in a murine model of asthma. *Eur J Immunol* 2003;33:861–869.

- 39 Julia V, Hessel EM, Malherbe L, Glaichenhaus N, O'Garra A, Coffman RL: A restricted subset of dendritic cells captures airborne antigens and remains able to activate specific T cells long after antigen exposure. *Immunity* 2002; 16:271–283.
- 40 Constant SL, Lee KS, Bottomly K: Site of antigen delivery can influence T cell priming: Pulmonary environment promotes preferential Th2-type differentiation. *Eur J Immunol* 2000; 30:840–847.
- 41 Mehlhop PD, van de Rijn M, Goldberg AB, Brewer JP, Kurup VP, Martin TR, Oettgen HC: Allergen-induced bronchial hyperreactivity and eosinophilic inflammation occur in the absence of IgE in a mouse model of asthma. *Proc Natl Acad Sci USA* 1997;94:1344–1349.
- 42 Kim J, Merry AC, Nemzek JA, Bolgos GL, Siddiqui J, Remick DG: Eotaxin represents the principal eosinophil chemoattractant in a novel murine asthma model induced by house dust containing cockroach allergens. *J Immunol* 2001;167:2808–2815.
- 43 Batanero E, Barral P, Villalba M, Rodriguez R: Sensitization of mice with olive pollen allergen Ole e 1 induces a Th2 response. *Int Arch Allergy Immunol* 2002;127:269–275.
- 44 Yu C, Lee S, Wang J, Hsiue T, Lei H: Early-type hypersensitivity-associated airway inflammation and eosinophilia induced by *Dermaphagoides farinae* in sensitized mice. *J Immunol* 1996;156:1923–1930.
- 45 Bauer L, Bohle B, Jahn SB, Wiedermann U, Daser A, Renz H, Kraft D, Ebner C: Modulation of the allergic immune response in BALB/c mice by subcutaneous injection of high doses of the dominant T cell epitope from the major birch pollen allergen Bet v 1. *Clin Exp Immunol* 1997;107:536–541.
- 46 Justice JP, Borchers MT, Lee JJ, Rowan WH, Shibata Y, Van Scott MR: Ragweed-induced expression of GATA-3, IL-4, and IL-5 by eosinophils in the lungs of allergic C57BL/6J mice. *Am J Physiol Lung Cell Mol Physiol* 2002;282: L302–L309.
- 47 Cates EC, Gajewska BU, Goncharova S, Alvarez D, Fattouh R, Coyle AJ, Gutierrez-Ramos JC, Jordana M: Effect of GM-CSF on immune, inflammatory, and clinical responses to ragweed in a novel mouse model of mucosal sensitization. *J Allergy Clin Immunol* 2003;111: 1076–1086.
- 48 O'Brien R, Ooi MA, Clarke AH, Thomas WR: Immunologic responses following respiratory sensitization to house dust mite allergens in mice. *Immunol Cell Biol* 1996;74:174–179.
- 49 Cheng KC, Lee KM, Krug MS, Watanabe T, Suzuki M, Choe IS, Yoo TJ: House dust mite-induced sensitivity in mice. *J Allergy Clin Immunol* 1998;101:1.
- 50 Ichinose T, Takano H, Sadakane K, Yanagisawa R, Kawazato H, Sagai M, Shibamoto T: Differences in airway-inflammation development by house dust mite and diesel exhaust inhalation among mouse strains. *Toxicol Appl Pharmacol* 2003;187:29–37.
- 51 Ichinose T, Takano H, Miyabara Y, Yanagisawa R, Sagai M: Murine strain differences in allergic airway inflammation and immunoglobulin production by a combination of antigen and diesel exhaust particles. *Toxicology* 1997;122:183–192.
- 52 Sadakane K, Ichinose T, Takano H, Yanagisawa R, Sagai M, Yoshikawa T, Shibamoto T: Murine strain differences in airway inflammation induced by diesel exhaust particles and house dust mite allergen. *Int Arch Allergy Immunol* 2002;128:220–228.
- 53 Tategaki A, Kawamoto S, Okuda T, Aki T, Yasueda H, Suzuki O, Ono K, Shigeta S: A high-molecular-weight mite antigen (HM1) fraction aggravates airway hyperresponsiveness of allergic mice to house dusts and whole mite cultures. *Int Arch Allergy Immunol* 2002; 129:204–211.
- 54 Valenta R, Kraft D: From allergen structure to new forms of allergen-specific immunotherapy. *Curr Opin Immunol* 2002;14:718–727.
- 55 Alkan SS, Akdis AC, Feuerlein D, Gruninger M: Direct measurement of cytokines (IFN- γ , IL-4, -5, and -6) from organs after antigenic challenge. *Ann NY Acad Sci* 1996;796: 82–90.
- 56 Holt PG, Rose AH, Batty JE, Turner KJ: Induction of adjuvant-independent IgE responses in inbred mice: Primary, secondary, and persistent IgE responses to ovalbumin and ovomucoid. *Int Arch Allergy Appl Immunol* 1981;65:42–50.
- 57 Ormstad H, Groeng EC, Duffort O, Lovik M: The effect of endotoxin on the production of IgE, IgG1 and IgG2a antibodies against the cat allergen Fel d 1 in mice. *Toxicology* 2003;188: 309–318.
- 58 Eisenbarth SC, Piggott DA, Huleatt JW, Visintin I, Herrick CA, Bottomly K: Lipopolysaccharide-enhanced, toll-like receptor 4-dependent T helper cell type 2 responses to inhaled antigen. *J Exp Med* 2002;196:1645–1651.
- 59 Dabbagh K, Dahl ME, Stepick-Biek P, Lewis DB: Toll-like receptor 4 is required for optimal development of Th2 immune responses: Role of dendritic cells. *J Immunol* 2002;168:4524–4530.
- 60 Tulic MK, Holt PG, Sly PD: Modification of acute and late-phase allergic responses to ovalbumin with lipopolysaccharide. *Int Arch Allergy Immunol* 2002;129:119–128.
- 61 Watanabe J, Miyazaki Y, Zimmerman GA, Albertine KH, McIntyre TM: Endotoxin contamination of ovalbumin suppresses murine immunologic responses and development of airway hyper-reactivity. *J Biol Chem* 2003;278: 42361–42368.
- 62 Gerhold K, Bluemchen K, Franke A, Stock P, Hamelmann E: Exposure to endotoxin and allergen in early life and its effect on allergen sensitization in mice. *J Allergy Clin Immunol* 2003;112:389–396.
- 63 Rodriguez D, Keller AC, Faquim-Mauro EL, de Macedo MS, Cunha FQ, Lefort J, Vargaftig BB, Russo M: Bacterial lipopolysaccharide signaling through toll-like receptor 4 suppresses asthma-like responses via nitric oxide synthase 2 activity. *J Immunol* 2003;171:1001–1008.
- 64 Suzuki T, Kanoh T, Ishimori M, Ikeda S, Ohkuni H: Adjuvant activity of diesel exhaust particulates (DEP) in production of anti-IgE and anti-IgG1 antibodies to mite allergen in mice. *J Clin Lab Immunol* 1996;48:187–199.
- 65 Ormstad H: Suspended particulate matter in indoor air: Adjuvants and allergen carriers. *Toxicology* 2000;152:53–68.
- 66 Miyabara Y, Takano H, Ichinose T, Lim HB, Sagai M: Diesel exhaust enhances allergic airway inflammation and hyperresponsiveness in mice. *Am J Respir Crit Care Med* 1998;157: 1138–1144.
- 67 Seymour B, Pinkerton KE, Frieberthausen KE, Coffman RL, Gershwin LJ: Second-hand smoke is an adjuvant for T helper-2 responses in a murine model of allergy. *J Immunol* 1997; 159:6169–6175.
- 68 Barrett EG, Wilder JA, March TH, Espindola T, Bice DE: Cigarette smoke-induced airway hyperresponsiveness is not dependent on elevated immunoglobulin and eosinophilic inflammation in a mouse model of allergic airway disease. *Am J Respir Crit Care Med* 2002; 165:1410–1418.
- 69 Kheradmand F, Kiss A, Xu J, Lee SH, Kolattukudy PE, Corry DB: A protease-activated pathway underlying Th cell type 2 activation and allergic lung disease. *J Immunol* 2002;169: 5904–5911.
- 70 Garssen J, Vanbree L, Vandervliet H, Vanloveren H: Ozone induced impairment of pulmonary type iv hypersensitivity and airway hyperresponsiveness in mice. *Inhal Toxicol* 1997;9: 581–599.
- 71 Depuydt PO, Lambrecht BN, Joos GF, Pauwels RA: Effect of ozone exposure on allergic sensitization and airway inflammation induced by dendritic cells. *Clin Exp Allergy* 2002;32: 391–396.
- 72 Goldsmith CAW, Ning Y, Qin G, Imrich A, Lawrence J, Murthy GKG, Catalano PJ, Kobzik L: Combined air pollution particle and ozone exposure increases airway responsiveness in mice. *Inhal Toxicol* 2002;14:325–347.
- 73 Hayashi T, Adachi Y, Hasegawa K, Morimoto M: Less sensitivity for late airway inflammation in males than females in BALB/c mice. *Scand J Immunol* 2003;57:562–567.
- 74 Russo M, Jancar S, Pereira de Siqueira AL, Mengel J, Gomes E, Ficker SM, Caetano de Faria AM: Prevention of lung eosinophilic inflammation by oral tolerance. *Immunol Lett* 1998;61:15–23.
- 75 Russo M, Nahori MA, Lefort J, Gomes E, de Castro Keller A, Rodriguez D, Ribeiro OG, Adriouch S, Gallois V, de Faria AM, Vargaftig BB: Suppression of asthma-like responses in different mouse strains by oral tolerance. *Am J Respir Cell Mol Biol* 2001;24:518–526.
- 76 Wiedermann U, Jahn SB, Fritsch R, Bauer L, Renz H, Kraft D, Ebner C: Effects of adjuvants on the immune response to allergens in a murine model of allergen inhalation: Cholera toxin induces a Th1-like response to Bet v 1, the major birch pollen allergen. *Clin Exp Immunol* 1998;111:144–151.

- 77 Lima C, Perini A, Garcia ML, Martins MA, Teixeira MM, Macedo MS: Eosinophilic inflammation and airway hyper-responsiveness are profoundly inhibited by a helminth (*Ascaris suum*) extract in a murine model of asthma. *Clin Exp Allergy* 2002;32:1659–1666.
- 78 Chu HW, Honour JM, Rawlinson CA, Harbeck RJ, Martin RJ: Effects of respiratory *Mycoplasma pneumoniae* infection on allergen-induced bronchial hyperresponsiveness and lung inflammation in mice. *Infect Immun* 2003;71:1520–1526.
- 79 Kips JC, Brusselle GG, Joos GF, Peleman RA, Devos RR, Tavernier JH, Pauwels RA: Importance of interleukin-4 and interleukin-12 in allergen-induced airway changes in mice. *Int Arch Allergy Immunol* 1995;107:115–118.
- 80 Hofstra CL, VanArk I, Hofman G, Kool M, Nijkamp FP, VanOosterhout A: Prevention of Th2-like cell responses by coadministration of IL-12 and IL-18 is associated with inhibition of antigen-induced airway hyperresponsiveness, eosinophilia, and serum IgE levels. *J Immunol* 1998;161:5054–5060.
- 81 Kuribayashi K, Kodama T, Okamura H, Sugita M, Matsuyama T: Effects of post-inhalation treatment with interleukin-12 on airway hyper-reactivity, eosinophilia and interleukin-18 receptor expression in a mouse model of asthma. *Clin Exp Allergy* 2002;32:641–649.
- 82 Hayashi T, Maeda K, Hasegawa K, Nakai S, Hamachi T, Iwata H: Systemic administration of interferon-gamma-expressing plasmid reduces late allergic bronchitis in a mouse model of asthma. *Int J Exp Pathol* 2002;83:81–86.
- 83 Leonard P, Sur S: Interleukin-12: Potential role in asthma therapy. *BioDrugs* 2003;17:1–7.
- 84 Rook GA, Martinelli R, Brunet LR: Innate immune responses to mycobacteria and the downregulation of atopic responses. *Curr Opin Allergy Clin Immunol* 2003;3:337–342.
- 85 Erb KJ, Holloway JW, Sobeck A, Moll H, Le Gros G: Infection of mice with *Mycobacterium bovis*-*Bacillus Calmette-Guérin* (BCG) suppresses allergen-induced airway eosinophilia. *J Exp Med* 1998;187:561–569.
- 86 Hussain I, Kline JN: CpG oligodeoxynucleotides in asthma. *Curr Opin Investig Drugs* 2001;2:914–918.
- 87 Jahn-Schmid B, Wiedermann U, Bohle B, Repa A, Kraft D, Ebner C: Oligodeoxynucleotides containing CpG motifs modulate the allergic TH2 response of BALB/c mice to Bet v 1, the major birch pollen allergen. *J Allergy Clin Immunol* 1999;104:1015–1023.
- 88 Finotto S, Neurath M, Glickman J, Qin S, Lehr H, Green F, Ackerman K, Haley K, Galle P, Szabo S, Drazen J, DeSanctis G, Glimcher L: Development of spontaneous airway changes consistent with human asthma in mice lacking T-bet. *Science* 2002;295:336–338.
- 89 Cross ML, Gill HS: Can immunoregulatory lactic acid bacteria be used as dietary supplements to limit allergies? *Int Arch Allergy Immunol* 2001;125:112–119.
- 90 Hansen G, Berry G, DeKruyff RH, Umetsu DT: Allergen-specific Th1 cells fail to counterbalance Th2 cell-induced airway hyper-reactivity but cause severe airway inflammation. *J Clin Invest* 1999;103:175–183.
- 91 Li L, Xia Y, Nguyen A, Feng L, Lo D: Th2-induced eotaxin expression and eosinophilia coexist with Th1 responses at the effector stage of lung inflammation. *J Immunol* 1998;161:3128–3135.
- 92 Randolph DA, Stephens R, Carruthers CJ, Chaplin DD: Cooperation between Th1 and Th2 cells in a murine model of eosinophilic airway inflammation. *J Clin Invest* 1999;104:1021–1029.
- 93 Cohn L, Homer RJ, MacLeod H, Mohrs M, Brombacher F, Bottomly K: Th2-induced airway mucus production is dependent on IL-4 α , but not on eosinophils. *J Immunol* 1999;162:6178–6183.
- 94 Ferreira MB, da Silva SL, Carlos AG: Atopy and helminths. *Allergy Immunol* 2002;34:10–12.
- 95 Akbari O, DeKruyff RH, Umetsu DT: Pulmonary dendritic cells producing IL-10 mediate tolerance induced by respiratory exposure to antigen. *Nat Immunol* 2001;2:725–731.
- 96 Akbari O, Freeman GJ, Meyer EH, Greenfield EA, Chang TT, Sharpe AH, Berry G, DeKruyff RH, Umetsu DT: Antigen-specific regulatory T cells develop via the ICOS-ICOS-ligand pathway and inhibit allergen-induced airway hyperreactivity. *Nat Med* 2002;8:1024–1032.
- 97 Untersmayr E, Scholl I, Swoboda I, Beil WJ, Forster-Waldl E, Walter F, Riemer A, Kraml G, Kinaciyan T, Spitzauer S, Boltz-Nitulescu G, Scheiner O, Jensen-Jarolim E: Antacid medication inhibits digestion of dietary proteins and causes food allergy: A fish allergy model in BALB/c mice. *J Allergy Clin Immunol* 2003;112:616–623.
- 98 Herz U, Braun A, Ruckert R, Renz H: Various immunological phenotypes are associated with increased airway responsiveness. *Clin Exp Allergy* 1998;28:625–634.
- 99 Whitehead GS, Walker JK, Berman KG, Foster WM, Schwartz DA: Allergen-induced airway disease is mouse strain dependent. *Am J Physiol Lung Cell Mol Physiol* 2003;285:L32–L42.
- 100 De Sanctis G, Merchant M, Beier DR, Dredge RD, Grobholz JK, Martin TR, Lander ES, Drazen JM: Quantitative locus analysis of airway hyperresponsiveness in A/J and C57BL/6J mice. *Nat Genet* 1995;11:150–154.
- 101 Kannan MS, Deshpande DA: Allergic asthma in mice: What determines the phenotype? *Am J Physiol Lung Cell Mol Physiol* 2003;285:L29–L31.
- 102 Takeda K, Haczku A, Lee JJ, Irvin CG, Gelfand EW: Strain dependence of airway hyperresponsiveness reflects differences in eosinophil localization in the lung. *Am J Physiol Lung Cell Mol Physiol* 2001;281:L394–L402.
- 103 Brewer JP, Kisselgof AB, Martin TR: Genetic variability in pulmonary physiological, cellular, and antibody responses to antigen in mice. *Am J Respir Crit Care Med* 1999;160:1150–1156.
- 104 Roche WR: Inflammatory and structural changes in the small airways in bronchial asthma. *Am J Respir Crit Care Med* 1998;157:191S–194S.
- 105 Drazen JM, Finn PW, De SG: Mouse models of airway responsiveness: Physiological basis of observed outcomes and analysis of selected examples using these outcome indicators. *Annu Rev Physiol* 1999;61:593–625.
- 106 Hamelmann E, Schwarze J, Takeda K, Oshiba A, Larsen GL, Irvin CG, Gelfand EW: Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmography. *Am J Respir Crit Care Med* 1997;156:766–775.
- 107 Lundblad LK, Irvin CG, Adler A, Bates JH: A reevaluation of the validity of unrestrained plethysmography in mice. *J Appl Physiol* 2002;93:1198–1207.
- 108 Hantos Z, Brusasco V: Assessment of respiratory mechanics in small animals: The simpler the better? *J Appl Physiol* 2002;93:1196–1197.
- 109 Mitzner W, Tankersley C: Interpreting Penh in mice. *J Appl Physiol* 2003;94:828–831.
- 110 Irvin CG, Bates JH: Measuring the lung function in the mouse: The challenge of size. *Respir Res* 2003;4:4.
- 111 Dohi M, Tsukamoto S, Nagahori T, Shinagawa K, Saitoh K, Tanaka Y, Kobayashi S, Tanaka R, To Y, Yamamoto K: Noninvasive system for evaluating the allergen-specific airway response in a murine model of asthma. *Lab Invest* 1999;79:1559–1571.
- 112 Corry DB, Grunig G, Hadeiba H, Kurup VP, Warnock ML, Sheppard D, Rennick DM, Locksley RM: Requirements for allergen-induced airway hyperreactivity in T and B cell-deficient mice. *Mol Med* 1998;4:344–355.
- 113 Townley RG, Horiba M: Airway hyperresponsiveness: A story of mice and men and cytokines. *Clin Rev Allergy Immunol* 2003;24:85–110.
- 114 De Sanctis GT, Itoh A, Green FH, Qin S, Kimura T, Grobholz JK, Martin TR, Maki T, Drazen JM: T-lymphocytes regulate genetically determined airway hyperresponsiveness in mice. *Nat Med* 1997;3:460–462.
- 115 Hadeiba H, Corry DB, Locksley RM: Baseline airway hyperreactivity in A/J mice is not mediated by cells of the adaptive immune system. *J Immunol* 2000;164:4933–4940.
- 116 De Sanctis GT, Singer JB, Jiao A, Yandava CN, Lee YH, Haynes TC, Lander ES, Beier DR, Drazen JM: Quantitative trait locus mapping of airway responsiveness to chromosomes 6 and 7 in inbred mice. *Am J Physiol* 1999;277:L1118–L1123.
- 117 Renz H, Smith HR, Henson JE, Ray BS, Irvin CG, Gelfand EW: Aerosolized antigen exposure without adjuvant causes increased IgE production and increased airway responsiveness in the mouse. *J Allergy Clin Immunol* 1992;89:1127–1138.

- 118 Cohn L, Whittaker L, Niu N, Homer RJ: Cytokine regulation of mucus production in a model of allergic asthma. *Novartis Found Symp* 2002;248:201–213.
- 119 Zhu Z, Homer RJ, Wang Z, Chen Q, Geba GP, Wang J, Zhang Y, Elias JA: Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eosinophil production. *J Clin Invest* 1999;103:779–788.
- 120 Taube C, Duez C, Cui ZH, Takeda K, Rha YH, Park JW, Balhorn A, Donaldson DD, Dakhama A, Gelfand EW: The role of IL-13 in established allergic airway disease. *J Immunol* 2002;169:6482–6489.
- 121 Kibe A, Inoue H, Fukuyama S, Machida K, Matsumoto K, Koto H, Ikegami T, Aizawa H, Hara N: Differential regulation by glucocorticoid of interleukin-13-induced eosinophilia, hyperresponsiveness, and goblet cell hyperplasia in mouse airways. *Am J Respir Crit Care Med* 2003;167:50–56.
- 122 Herz U, Lupp U, Daser A, Gelfand EW, Renz H: Murine animal models to study the central role of T cells in immediate-type hypersensitivity responses. *Adv Exp Med Biol* 1996;409:25–32.
- 123 Takeda K, Hamelmann E, Joetham A, Shultz LD, Larsen GL, Irvin CG, Gelfand EW: Development of eosinophilic airway inflammation and airway hyperresponsiveness in mast cell-deficient mice. *J Exp Med* 1997;186:449–454.
- 124 Kung T, Stelts D, Zurcher J, Jones H, Umland S, Kreutner W, Egan R, Chapman R: Mast cells modulate allergic pulmonary eosinophilia in mice. *Am J Respir Cell Mol Biol* 1995;12:404–409.
- 125 MacLean JA, Sauty A, Luster AD, Drazen JM, De Sanctis GT: Antigen-induced airway hyperresponsiveness, pulmonary eosinophilia, and chemokine expression in B cell-deficient mice. *Am J Respir Cell Mol Biol* 1999;20:379–387.
- 126 Korsgren M, Erjefalt JS, Korsgren O, Sundler F, Persson CG: Allergic eosinophil-rich inflammation develops in lungs and airways of B cell-deficient mice. *J Exp Med* 1997;185:885–892.
- 127 Hamelmann E, Vella AT, Oshiba A, Kappler JW, Marrack P, Gelfand EW: Allergic airway sensitization induces T cell activation but not airway hyperresponsiveness in B cell-deficient mice. *Proc Natl Acad Sci USA* 1997;94:1350–1355.
- 128 Busse WW: Anti-immunoglobulin E (omalizumab) therapy in allergic asthma. *Am J Respir Crit Care Med* 2001;164:S12–S17.
- 129 Hsu C, MacGlashan DJ: IgE antibody up-regulates high affinity IgE binding on murine bone marrow-derived mast cells. *Immunol Lett* 1996;52:129–134.
- 130 Nagai H, Yamaguchi S, Tanaka H: The role of interleukin-5 (IL-5) in allergic airway hyperresponsiveness in mice. *Ann NY Acad Sci* 1996;796:91–96.
- 131 Kobayashi T, Miura T, Haba T, Sato M, Serizawa I, Nagai H, Ishizaka K: An essential role of mast cells in the development of airway hyperresponsiveness in a murine asthma model. *J Immunol* 2000;164:3855–3861.
- 132 Yiamouyiannis CA, Schramm CM, Puddington L, Stengel P, Baradaran-Hossein E, Wolyniec WW, Whiteley HE, Thrall RS: Shifts in lung lymphocyte profiles correlate with the sequential development of acute allergic and chronic tolerant stages in a murine asthma model. *Am J Pathol* 1999;154:1911–1921.
- 133 Sakai K, Yokoyama A, Kohno N, Hamada H, Hiwada K: Prolonged antigen exposure ameliorates airway inflammation but not remodeling in a mouse model of bronchial asthma. *Int Arch Allergy Immunol* 2001;126:126–134.
- 134 Swirski FK, Sajic D, Robbins CS, Gajewska BU, Jordana M, Stampfli MR: Chronic exposure to innocuous antigen in sensitized mice leads to suppressed airway eosinophilia that is reversed by granulocyte macrophage colony-stimulating factor. *J Immunol* 2002;169:3499–3506.
- 135 Hogaboam CM, Blease K, Mehrad B, Steinhilber ML, Standiford TJ, Kunkel SL, Lukacs NW: Chronic airway hyperactivity, goblet cell hyperplasia, and peribronchial fibrosis during allergic airway disease induced by *Aspergillus fumigatus*. *Am J Pathol* 2000;156:723–732.
- 136 Temelkovski J, Hogan SP, Shepherd DP, Foster PS, Kumar RK: An improved murine model of asthma: Selective airway inflammation, epithelial lesions and increased methacholine responsiveness following chronic exposure to aerosolised allergen. *Thorax* 1998;53:849–856.
- 137 Trifilieff A, El-Hashim A, Bertrand C: Time course of inflammatory and remodeling events in a murine model of asthma: Effect of steroid treatment. *Am J Physiol Lung Cell Mol Physiol* 2000;279:L1120–L1128.
- 138 Kumar RK, Foster PS: Modeling allergic asthma in mice: Pitfalls and opportunities. *Am J Respir Cell Mol Biol* 2002;27:267–272.
- 139 Komai M, Tanaka H, Masuda T, Nagao K, Ishizaki M, Sawada M, Nagai H: Role of Th2 responses in the development of allergen-induced airway remodelling in a murine model of allergic asthma. *Br J Pharmacol* 2003;138:912–920.
- 140 Holgate ST, Peters-Golden M, Panettieri RA, Henderson WR Jr: Roles of cysteinyl leukotrienes in airway inflammation, smooth muscle function, and remodeling. *J Allergy Clin Immunol* 2003;111:S18–S34.
- 141 Bates CA, Silkoff PE: Exhaled nitric oxide in asthma: From bench to bedside. *J Allergy Clin Immunol* 2003;111:256–262.
- 142 De Bie JJ, Hessel EM, Van Ark I, Van Esch B, Hofman G, Nijkamp FP, Van Oosterhout AJ: Effect of dexamethasone and endogenous corticosterone on airway hyperresponsiveness and eosinophilia in the mouse. *Br J Pharmacol* 1996;119:1484–1490.
- 143 Blyth DI, Wharton TF, Pedrick MS, Savage TJ, Sanjar S: Airway subepithelial fibrosis in a murine model of atopic asthma: Suppression by dexamethasone or anti-interleukin-5 antibody. *Am J Respir Cell Mol Biol* 2000;23:241–246.
- 144 Vanacker NJ, Palmans E, Kips JC, Pauwels RA: Fluticasone inhibits but does not reverse allergen-induced structural airway changes. *Am J Respir Crit Care Med* 2001;163:674–679.
- 145 Jeffery P, Godfrey RW, Ädelroth E, Nelson F, Rogers A, Johansson S-Å: Effects of treatment on airway inflammation and thickening of basement membrane reticular collagen in asthma: A quantitative light and electron microscopic study. *Am Rev Respir Dis* 1992;145:890–899.
- 146 Mathur M, Herrmann K, Li X, Qin Y, Weinstein J, Elliott D, Monahan J, Padrid P: TRFK-5 reverses established airway eosinophilia but not established hyperresponsiveness in a murine model of chronic asthma. *Am J Respir Crit Care Med* 1999;159:580–587.
- 147 Jungsuwadee P, Dekan G, Stingl G, Epstein MM: Inhaled dexamethasone differentially attenuates disease relapse and established allergic asthma in mice. *Int Arch Allergy Immunol*, in press.
- 148 Garlisi CG, Falcone A, Kung TT, Stelts D, Pennline KJ, Beavis AJ, Smith SR, Egan RW, Umland SP: T cells are necessary for Th2 cytokine production and eosinophil accumulation in airways of antigen-challenged allergic mice. *Clin Immunol Immunopathol* 1995;75:75–83.
- 149 Foster PS, Martinez-Moczygemba M, Huston DP, Corry DB: Interleukins-4, -5, and -13: Emerging therapeutic targets in allergic disease. *Pharmacol Ther* 2002;94:253–264.
- 150 Wills-Karp M, Chiaramonte M: Interleukin-13 in asthma. *Curr Opin Pulm Med* 2003;9:21–27.
- 151 Shimoda K, van Deursen J, Sangster MY, Sarawar SR, Carson RT, Tripp RA, Chu C, Quelle FW, Nosaka T, Vignali DA, Doherty PC, Grosveld G, Paul WE, Ihle JN: Lack of IL-4-induced Th2 response and IgE class switching in mice with disrupted Stat6 gene. *Nature* 1996;380:630–633.
- 152 Kuperman D, Schofield B, Wills-Karp M, Grusby MJ: Signal transducer and activator of transcription factor 6 (Stat6)-deficient mice are protected from antigen-induced airway hyperresponsiveness and mucus production. *J Exp Med* 1998;187:939–948.
- 153 Miyata S, Matsuyama T, Kodama T, Nishio Y, Kuribayashi K, Takeda K, Akira S, Sugita M: STAT6 deficiency in a mouse model of allergen-induced airways inflammation abolishes eosinophilia but induces infiltration of CD8(+) T cells. *Clin Exp Allergy* 1999;29:114–123.

- 154 Foster PS, Webb DC, Yang M, Herbert C, Kumar RK: Dissociation of T helper type 2 cytokine-dependent airway lesions from signal transducer and activator of transcription 6 signalling in experimental chronic asthma. *Clin Exp Allergy* 2003;33:688–695.
- 155 Das J, Chen CH, Yang L, Cohn L, Ray P, Ray A: A critical role for NF-kappa B in GATA3 expression and TH2 differentiation in allergic airway inflammation. *Nat Immunol* 2001;2:45–50.
- 156 Zhang DH, Yang L, Cohn L, Parkyn L, Homer R, Ray P, Ray A: Inhibition of allergic inflammation in a murine model of asthma by expression of a dominant-negative mutant of GATA-3. *Immunity* 1999;11:473–482.
- 157 Ray A, Cohn L: Th2 cells and GATA-3 in asthma: New insights into the regulation of airway inflammation. *J Clin Invest* 1999;104:985–993.
- 158 Robinson DS, Hamid Q, Ying S, Tsicopoulos A, Barkans J, Bentley AM, Corrigan C, Durham SR, Kay AB: Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med* 1992;326:298–304.
- 159 Li XM, Schofield BH, Wang QF, Kim KH, Huang SK: Induction of pulmonary allergic responses by antigen-specific Th2 cells. *J Immunol* 1998;160:1378–1384.
- 160 Coyle AJ, Tsuyuki S, Bertrand C, Huang S, Aguet M, Alkan SS, Anderson GP: Mice lacking the IFN-gamma receptor have impaired ability to resolve a lung eosinophilic inflammatory response associated with a prolonged capacity of T cells to exhibit a Th2 cytokine profile. *J Immunol* 1996;156:2680–2685.
- 161 Wilson RA, Coulson PS, Betts C, Dowling MA, Smythies LE: Impaired immunity and altered pulmonary responses in mice with a disrupted interferon-gamma receptor gene exposed to the irradiated *Schistosoma mansoni* vaccine. *Immunology* 1996;87:275–282.
- 162 Henderson WR, Chi EY, Albert RK, Chu SJ, Lamm W, Rochon Y, Jonas M, Christie PE, Harlan JM: Blockade of CD49d (alpha4) integrin on intrapulmonary but not circulating leukocytes inhibits airway inflammation and hyperresponsiveness in a mouse model of asthma. *J Clin Invest* 1997;100:3083–3092.
- 163 Lobb RR, Abraham WM, Burkly LC, Gill A, Ma W, Knight JA, Leone DR, Antognetti G, Pepinsky RB: Pathophysiologic role of alpha 4 integrins in the lung. *Ann NY Acad Sci* 1996;796:113–123.
- 164 Iwamoto I, Nakao A: Induction of Th2 cell tolerance to a soluble antigen by blockade of the LFA-1-dependent pathway prevents allergic inflammation. *Immunol Res* 1995;14:263–270.
- 165 Buckley TL, Bloemen PG, Henricks PA, van den Tweel MC, Redegeld FA, Koster AS, Nijkamp FP: LFA-1 and ICAM-1 are crucial for the induction of hyperactivity in the mouse airways. *Ann NY Acad Sci* 1996;796:149–161.
- 166 Lukacs NW, Strieter RM, Chensue SW, Kunkel SL: Activation and regulation of chemokines in allergic airway inflammation. *J Leukoc Biol* 1996;59:13–17.
- 167 Wolyniec WW, De Sanctis GT, Nabozny G, Torcellini C, Haynes N, Joetham A, Gelfand EW, Drazen JM, Noonan TC: Reduction of antigen-induced airway hyperactivity and eosinophilia in ICAM-1-deficient mice. *Am J Respir Cell Mol Biol* 1998;18:777–785.
- 168 Borchers MT, Crosby J, Farmer S, Sypek J, Ansay T, Lee NA, Lee JJ: Blockade of CD49d inhibits allergic airway pathologies independent of effects on leukocyte recruitment. *Am J Physiol Lung Cell Mol Physiol* 2001;280:L813–L821.
- 169 Keramidaris E, Merson TD, Steeber DA, Tedder TF, Tang ML: L-selectin and intercellular adhesion molecule 1 mediate lymphocyte migration to the inflamed airway/lung during an allergic inflammatory response in an animal model of asthma. *J Allergy Clin Immunol* 2001;107:734–738.
- 170 Lee KS, Jin SM, Kim HJ, Lee YC: Matrix metalloproteinase inhibitor regulates inflammatory cell migration by reducing ICAM-1 and VCAM-1 expression in a murine model of toluene diisocyanate-induced asthma. *J Allergy Clin Immunol* 2003;111:1278–1284.
- 171 Sheppard D: Functions of pulmonary epithelial integrins: From development to disease. *Physiol Rev* 2003;83:673–686.
- 172 Umetsu DT, Akbari O, Dekruyff RH: Regulatory T cells control the development of allergic disease and asthma. *J Allergy Clin Immunol* 2003;112:480–487.
- 173 Yssel H, Lecart S, Pene J: Regulatory T cells and allergic asthma. *Microbes Infect* 2001;3:899–904.
- 174 Erb KJ, Le Gros G: The role of Th2 type CD4+ T cells and Th2 type CD8+ T cells in asthma. *Immunol Cell Biol* 1996;74:206–208.
- 175 Hamelmann E, Oshiba A, Paluh J, Bradley K, Loader J, Potter TA, Larsen GL, Gelfand EW: Requirement for CD8+ T cells in the development of airway hyperresponsiveness in a murine model of airway sensitization. *J Exp Med* 1996;183:1719–1729.
- 176 Allakhverdi Z, Lamkhioued B, Olivenstein R, Hamid Q, Renzi PM: CD8 depletion-induced late airway response is characterized by eosinophilia, increased eotaxin, and decreased IFN-gamma expression in rats. *Am J Respir Crit Care Med* 2000;162:1123–1131.
- 177 Lisbonne M, Diem S, De Castro Keller A, Lefort J, Araujo LM, Hachem P, Fournieu JM, Sidobre S, Kronenberg M, Taniguchi M, Van Endert P, Dy M, Askenase P, Russo M, Vargaftig BB, Herbelin A, Leite-De-Moraes MC: Cutting edge: Invariant Valpha14 NKT cells are required for allergen-induced airway inflammation and hyperactivity in an experimental asthma model. *J Immunol* 2003;171:1637–1641.
- 178 Akbari O, Stock P, Meyer E, Kronenberg M, Sidobre S, Nakayama T, Taniguchi M, Grusby MJ, DeKruyff RH, Umetsu DT: Essential role of NKT cells producing IL-4 and IL-13 in the development of allergen-induced airway hyperactivity. *Nat Med* 2003;9:582–588.
- 179 McMenamin C, Pimm C, McKersey M, Holt PG: Regulation of IgE responses to inhaled antigen in mice by antigen-specific gamma delta T cells. *Science* 1994;265:1869–1871.
- 180 Zuany-Amorim C, Ruffie C, Haile S, Vargaftig BB, Pereira P, Pretolani M: Requirement for gammadelta T cells in allergic airway inflammation. *Science* 1998;280:1265–1267.
- 181 Schramm CM, Puddington L, Yiamouyianis CA, Lingenheld EG, Whiteley HE, Wolyniec WW, Noonan TC, Thrall RS: Proinflammatory roles of T-cell receptor (TCR)gammadelta and TCRalphabeta lymphocytes in a murine model of asthma. *Am J Respir Cell Mol Biol* 2000;22:218–225.
- 182 Cui ZH, Joetham A, Aydtug MK, Hahn YS, Born WK, Gelfand EW: Reversal of allergic airway hyperactivity after long-term allergen challenge depends on gamma delta T cells. *Am J Respir Crit Care Med*, in press.
- 183 Hahn YS, Taube C, Jin N, Takeda K, Park JW, Wands JM, Aydtug MK, Roark CL, Lahn M, O'Brien RL, Gelfand EW, Born WK: V gamma4+ gamma delta T cells regulate airway hyperactivity to methacholine in ovalbumin-sensitized and challenged mice. *J Immunol* 2003;171:3170–3178.
- 184 Schon HM, Oliver J, McMenamin PG, Holt PG: Studies on the density, distribution, and surface phenotype of intraepithelial class II major histocompatibility complex antigen (Ia)-bearing dendritic cells (DC) in the conducting airways. *J Exp Med* 1991;173:1345–1356.
- 185 Holt PG, Stumbles PA: Regulation of immunologic homeostasis in peripheral tissues by dendritic cells: The respiratory tract as a paradigm. *J Allergy Clin Immunol* 2000;105:421–429.
- 186 Holt PG, Stumbles PA: Characterization of dendritic cell populations in the respiratory tract. *J Aerosol Med* 2000;13:361–367.
- 187 McWilliam AS, Nelson DJ, Holt PG: The biology of airway dendritic cells. *Immunol Cell Biol* 1995;73:405–413.
- 188 Vermaelen KY, Pauwels RA: Accelerated airway dendritic cell maturation, trafficking and elimination in a mouse model of asthma. *Am J Respir Cell Mol Biol* 2003;29:405–409.
- 189 Cheng X, Wang C, Qian G, Zhu B: CD80, but not CD86 were up-regulated on the spleen-derived dendritic cells from OVA-sensitized and challenged BALB/c mice. *Immunol Lett* 2003;89:31–38.
- 190 Upham JW, Stumbles PA: Why are dendritic cells important in allergic diseases of the respiratory tract? *Pharmacol Ther* 2003;100:75–87.
- 191 Stumbles PA, Strickland DH, Pimm CL, Proksch SF, Marsh AM, McWilliam AS, Bosco A, Tobagus I, Thomas JA, Napoli S, Proudfoot AE, Wells TN, Holt PG: Regulation of dendritic cell recruitment into resting and inflamed airway epithelium: Use of alternative chemokine receptors as a function of inducing stimulus. *J Immunol* 2001;167:228–234.

- 192 Huh JC, Strickland DH, Jahnsen FL, Turner DJ, Thomas JA, Napoli S, Tobagus I, Stumbles PA, Sly PD, Holt PG: Bidirectional interactions between antigen-bearing respiratory tract dendritic cells (DCs) and T cells precede the late phase reaction in experimental asthma: DC activation occurs in the airway mucosa but not in the lung parenchyma. *J Exp Med* 2003;198:19–30.
- 193 Stumbles PA, Upham JW, Holt PG: Airway dendritic cells: Co-ordinators of immunological homeostasis and immunity in the respiratory tract. *APMIS* 2003;111:741–755.
- 194 Lambrecht BN, Salomon B, Klatzmann D, Pauwels RA: Dendritic cells are required for the development of chronic eosinophilic airway inflammation in response to inhaled antigen in sensitized mice. *J Immunol* 1998;160:4090–4097.
- 195 Lambrecht BN, De Veerman M, Coyle AJ, Gutierrez Ramos JC, Thielemans K, Pauwels RA: Myeloid dendritic cells induce Th2 responses to inhaled antigen, leading to eosinophilic airway inflammation. *J Clin Invest* 2000;106:551–559.
- 196 Schipf A, Heilmann A, Boue L, Mossmann H, Brouck T, Rocken M: Th2 cells shape the differentiation of developing T cell responses during interactions with dendritic cells in vivo. *Eur J Immunol* 2003;33:1697–1706.
- 197 Stumbles PA, Thomas JA, Pimm CL, Lee PT, Venaille TJ, Proksch S, Holt PG: Resting respiratory tract dendritic cells preferentially stimulate T helper cell type 2 (Th2) responses and require obligatory cytokine signals for induction of Th1 immunity. *J Exp Med* 1998;188:2019–2031.
- 198 Stumbles PA: Regulation of T helper cell differentiation by respiratory tract dendritic cells. *Immunol Cell Biol* 1999;77:428–433.
- 199 van Rijt LS, Lambrecht BN: Role of dendritic cells and Th2 lymphocytes in asthma: Lessons from eosinophilic airway inflammation in the mouse. *Microsc Res Tech* 2001;53:256–272.
- 200 Holt PG, Haining S, Nelson DJ, Sedgwick JD: Origin and steady-state turnover of class II MHC-bearing dendritic cells in the epithelium of the conducting airways. *J Immunol* 1994;153:256–261.
- 201 Tang C, Inman MD, van Rooijen N, Yang P, Shen H, Matsumoto K, O'Byrne PM: Th type 1-stimulating activity of lung macrophages inhibits Th2-mediated allergic airway inflammation by an IFN-gamma-dependent mechanism. *J Immunol* 2001;166:1471–1481.
- 202 Pynaert G, Rottiers P, Haegeman A, Sehra S, Korf J, Grooten J: Antigen presentation by local macrophages promotes nonallergic airway responses in sensitized mice. *Am J Respir Cell Mol Biol* 2003;29:634–641.
- 203 Damiani G: The role of macrophages and lymphocytes in bronchial asthma: Perspectives for new research models. *Eur Respir J Suppl* 1989;6:469s–472s.
- 204 Holt PG, Degebrodt A, O'Leary C, Krska K, Plozza T: T cell activation by antigen-presenting cells from lung tissue digests: Suppression by endogenous macrophages. *Clin Exp Immunol* 1985;62:586–593.
- 205 Thepen T, McMenamin C, Oliver J, Kraal G, Holt PG: Regulation of immune response to inhaled antigen by alveolar macrophages: Differential effects of in vivo alveolar macrophage elimination on the induction of tolerance vs. immunity. *Eur J Immunol* 1991;21:2845–2850.
- 206 Bilyk N, Holt PG: Cytokine modulation of the immunosuppressive phenotype of pulmonary alveolar macrophage populations. *Immunology* 1995;86:231–237.
- 207 Kuchroo VK, Umetsu DT, DeKruyff RH, Freeman GJ: The TIM gene family: Emerging roles in immunity and disease. *Nat Rev Immunol* 2003;3:454–462.
- 208 Soumelis V, Reche PA, Kanzler H, Yuan W, Edward G, Homey B, Gilliet M, Ho S, Antonenko S, Lauerma A, Smith K, Gorman D, Zurawski S, Abrams J, Menon S, McClanahan T, de Waal-Malefyt Rd R, Bazan F, Kastelein RA, Liu YJ: Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat Immunol* 2002;3:673–680.