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## Are mouse models of allergic asthma useful for testing novel therapeutics?

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### Abstract

Experimental mouse allergic asthma is a reliable, clinically relevant facsimile of human disease. The focus here is to demonstrate that antigen-induced mouse allergic asthma is a useful model for testing novel therapeutics. Furthermore, it is especially crucial to treat mice during established disease, either during ongoing clinically manifest disease or to prevent disease relapses. In addition, our results indicate that lung Th2 resting memory cells are important targets for the treatment of allergic asthma.

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### Introduction

A central player in the mechanism underlying allergic asthma is the allergen-specific T-lymphocyte. These T cells respond to allergen and initiate a complex cascade of events involving other cells of the adaptive immune system, the innate immune system, and resident cells in the lungs. The result is reversible airway obstruction, caused by smooth muscle contraction, edema, mucus hypersecretion which leads to symptoms of shortness of breath, wheezing, and coughing. Although, plausible theoretical models of pathogenesis exist and generally effective drugs are available, allergic asthma remains a serious health problem with an escalating morbidity and mortality.

Several animal models have been developed to study disease pathogenesis and for drug discovery (Epstein, 2004a, b). They include mice, guinea pigs, rats, dogs, cats, monkeys, sheep, and horses. However, since the

first demonstration of mouse allergic asthma was reported in 1994, mice have become one of the most extensively studied model systems. Generally, disease is induced by systemic immunization and respiratory tract challenge with antigen (Ag). Remarkably, it is possible to establish disease with a large variety of Ags, routes of administration, adjuvants, dosing schedules, and mouse strains, leading to airway hyperresponsiveness (AHR), eosinophilic lung inflammation, mucus hypersecretion, and elevated IgE, that are characteristic of human disease. Additional approaches to generate disease include cellular transfers with activated Th2 cells or Ag-pulsed dendritic cells (DC) followed by respiratory tract challenge. The common feature of all of these models is respiratory tract Ag exposure in a recipient with Ag-specific T helper cells.

Another interesting mouse model takes advantage of the human-SCID (Severe combined immunodeficiency; T and B cell-deficient mice) chimera. In this model, PBMC from allergic patients are injected into SCID mice and then followed by respiratory tract challenge (with the allergen that the patient is allergic to). The

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result is pulmonary inflammation, AHR, and elevated IgE. The unique feature of this model is that the T cells and DC infiltrating the murine lung as well as the IgE are of human origin. The power of this model is that treatments target human cells. The drawback is that human cells may react differently when confronted with a foreign host.

In addition to Ag-induced asthma models, there are examples of spontaneous disease, which include naturally occurring *Ascaris suum*-allergic sheep, flea-allergic dogs, and t-bet deficient transgenic mice. The t-bet knockout mice develop asthma without allergen sensitization and challenge and may not represent allergic asthma but might provide models of intrinsic asthma.

## Pathobiology

My aim in here is to compare and contrast Ag-induced mouse allergic asthma with human disease and to demonstrate that this is a useful model for testing therapeutics. Although, mice develop characteristic features of allergic asthma closely resembling human disease, there are major differences between the two. These include: (1) transient AHR or lack of twitchy airways rather than persistent hyper-reactivity; (2) lack of a clear role for IgE and mast cells; (3) lack of a true sustained model of active, chronic disease inflammation; and (4) differences in lung anatomy and eosinophil function between mice and humans. Despite these differences these models mice develop features of clinical disease and are useful for testing novel therapeutic agents aimed at reducing lung inflammation, mucus hypersecretion, AHR, and IgE.

We have experience with several mouse models in our laboratory that are either generated by priming with soluble Ag, Ag precipitated in alum, Ag-pulsed DC, or with subcutaneous implantation of coagulated egg white, followed by aerosol Ag challenge (Table 1) (Graffi et al., 2002; Jungsuwadee et al., 2002; Mojtabavi et al., 2002). After immunization and aerosol challenge, we evaluate the mice for disease. To test for inflammation, we examine bronchoalveolar lavage fluid (BAL) and H&E stained lung sections. Mice are evaluated 48 h after the last aerosol challenge. Our main observations include: (1) in all models, there is always an increase in

eosinophils, lymphocytes, macrophages, and neutrophils in the airway; (2) histopathology evidence of airway inflammation resolves within 3 weeks; (3) BAL numbers cell counts correlate with the extent of lung parenchyma inflammation; and (4) Protocols in which ovalbumin (OVA) is the antigen and alum the adjuvant induce the highest eosinophilia.

Measurement of total cell counts in the BAL provides information on the severity of inflammation and numbers approximately 20,000 cells/ml of BAL in naive mice. Depending on the asthma induction protocol it rises to  $10^6$  cells/ml of BAL. BAL cell percentages vary in naive vs. diseased mice, from 0% to 80% eosinophils, 100% to 20% macrophages, <1% neutrophils, and 1% to 20% lymphocytes, and is dependent on the protocol used to induce disease. In the setting of allergic asthma, although the relative representation (%) of macrophages falls, the absolute number increases since BAL fluid cellularity increases.

Peribronchial and perivascular infiltrates are evident in all models. However, the cellular composition, severity of overall inflammation, and location of infiltrates is protocol-dependent. OVA/alum immunization induces diffuse infiltrates located near large airways and contain eosinophils > macrophages > lymphocytes > plasma cells > multinucleated giant cells > neutrophils. Soluble OVA (dissolved in PBS) immunization leads to focal, dense infiltrates in the lung periphery near small airways and consists of lymphocytes and macrophages > eosinophils > neutrophils > multinucleated giant cells. OVA-pulsed DC injection induces a diffuse, dense, infiltrate around large airways containing eosinophils, lymphocytes, macrophages > many multinucleated giant cells within infiltrates and large, activated macrophages within alveoli. These differences in the histopathology with different protocols are not well understood but might provide new insights into the heterogeneity of disease.

There is some controversy about the best technique to measure AHR in mice. We observed that methacholine-induced AHR measured 24 h after the last aerosol challenge was only observed in sensitized mice measured by Penh using the Buxco Electronics Inc. non-invasive plethysmography equipment. All mice previously immunized and challenged with respiratory Ag show AHR and can be elicited with methacholine for approximately 10 days after the last aerosol challenge.

**Table 1.** Protocols for inducing allergic asthma in C57BL/6 and BALB/c mice

| Immunization                                                                                 | Aerosol challenge                          |
|----------------------------------------------------------------------------------------------|--------------------------------------------|
| OVA 25 µg/alum i.p. days 0 & 5                                                               | 1% OVA, 60 min twice daily on days 12 & 13 |
| OVA 10 µg in PBS i.p. days 0 & 21                                                            | 1% OVA, 60 min twice daily on days 28 & 29 |
| OVA-pulsed DC $2 \times 10^5$ i.p. day 0                                                     | 1% OVA, 60 min twice daily on days 7 & 8   |
| Egg white implants ( $2 \times 2$ mm <sup>2</sup> ) coagulated egg white, subcutaneous day 0 | 1% OVA, 60 min twice daily on days 10 & 11 |

Periodic Acid Schiff-stained lung section are used to evaluate mucus secretion. Naive mice have low goblet cell production of mucus. However, mucus is dramatically increased during asthma. Mucus is present in the large and/or small airways and within airway lumen and is associated with goblet cell hyperplasia. Mucus secretion decreases to baseline within 30 days.

OVA-specific Ab is usually detectable in serum and in BAL fluid. IgG1 is always 1–2 magnitudes higher than IgE and serum levels exceed those found in BAL fluid. OVA-specific IgE but not IgG1 decreases with time and neither isotype is necessary for the generation of allergic asthma in mice. An example of allergic asthma that does not seem to require Ag-specific IgE is that of egg white implant immunized mice. Unlike other OVA-mediated models, these mice do not show an increase in OVA-specific IgE in serum or in BAL fluid.

## Treatment

Treatment experiments allow both the evaluation of drug efficacy and serve as a useful probe for pathogenesis. Although disease prevention is often studied, our approach is focused on preventing disease relapses in mice with allergic asthma, inhibiting overt (clinically manifest) disease, and ultimately preventing disease progression (Jungsuwadee et al., 2002). We observed that mice with disease relapse (induced during recovery) and in mice recurrently aerosol-Ag challenged for the induction of overt disease had increased IgE and IgG1, AHR, and mucus secretion, compared to acute onset disease. Both phases of disease (relapse and overt disease) lead to a change in quality of inflammatory infiltrates in the lung parenchyma characterized by fewer eosinophils, more plasma cells, macrophages, and lymphocytes compared to acute onset disease. Thus, suggesting that it is important to evaluate treatment modalities during established disease. In a treatment study using corticosteroids as a reference standard to prevent relapse and to inhibit active ongoing disease, we found that inhaled dexamethasone differentially inhibited airway inflammation compared to parenchymal inflammation and affected relapse and overt disease differently (Jungsuwadee et al., 2004a). These findings demonstrate the importance of evaluating both airway and parenchymal inflammation in the context of clinically relevant phases of disease. We chose to measure drug efficacy in overt disease 3 or 4 weeks after priming and weekly aerosol-Ag challenges. The reason is that after 6 weeks of challenges, inflammation resolves (Jungsuwadee et al., 2004b). This is a major difficulty in studying chronic inflammation in mouse models.

In a parallel project, we are exploring the role of long-lived memory Th2 cells in the lungs of mice recovered from allergic asthma and attempting to target them for the treatment of disease. Using the soluble OVA immunization (without alum) protocol, we found that lung lesions resemble human infiltrates and upon aerosol Ag rechallenge 800 days after initial priming; mice develop eosinophilic lung inflammation, mucus hypersecretion, AHR, IgG1, and IgE. The recovery phase is characterized by an absence of BAL inflammation, mucus secretion, AHR, and IgE. However, the mice have persistent inflammatory lung infiltrates and elevated levels of IgG1 up until 800 days. The lung infiltrates contain resting memory lung CD4+ T cells, which respond in vitro to Ag and rapidly upon aerosol Ag upregulate lung Th2 cytokine RNA in vivo. Our aim with treatment is to inhibit activation and cytokine production by resting lung Th2 memory cells. To generate a reference standard for novel treatments, we treat fully recovered mice with intranasal dexamethasone, which results in a significant reduction in BAL inflammation, eosinophilia, and mucus hypersecretion upon respiratory tract re-challenge. Currently, we are studying the effectiveness of T lymphocyte specific inhibitors.

## Summary

Mouse allergic asthma models mimic clinically important human disease, albeit with several notable caveats. It is important to focus on the treatment of clinically relevant phases of the disease process, i.e., flares of symptoms and inflammation. Histopathologic and functional assessments of infiltrating inflammatory cells in relapses of allergic asthma suggest that lung Th2 resting memory cells are crucial targets for the treatment of disease.

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