No audible wheezing: nuggets and conundrums from mouse asthma models

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Mouse models of T helper type 2 (Th2) cell–biased pulmonary inflammation have elucidated mechanisms of sensitization, cell traffic, and induced airway hyperresponsiveness (AHR). Nonetheless, most mice lack intrinsic AHR, a central property of human asthma, and disparities persist regarding the contributions of eosinophils and mast cells and the sensitivity to induced AHR in the commonly used mouse strains. We suggest that these discordances, reflecting methodological and genetic differences, may be informative for understanding heterogeneity of human asthma.

Human bronchial asthma is heterogeneous in terms of severity, genetics, and in all likelihood pathophysiology. It is characterized physiologically by persistent AHR to pharmacologic bronchoconstrictors, and by variable, episodic intrathoracic airflow obstruction that is at least partly reversible with agonists of the β_2 adrenergic receptor. Asthma is also associated with Th2-like bronchial wall inflammation, regardless of whether individuals have allergen-specific IgE. Lymphocytes producing Th2-like cytokines (interleukin [IL] 4, 5, 9, and 13) (1), eosinophils, and mast cells (2) infiltrate the mucosal epithelium and submucosa. T cells in biopsies from the airways of subjects with asthma bear activation markers, whereas the eosinophils and mast cells show evidence of degranulation, suggesting concerted activation of these cell types. The epithelium may show goblet cell metaplasia or exfoliation. The infiltration of the airway smooth muscle with mast cells is a feature that distinguishes asthma from eosinophilic bronchitis (3), a syndrome in which mucosal inflammation is not accompanied by AHR or airflow obstruction. Airway "remodeling" associated with chronic asthmatic inflammation is characterized by hyperplasia of smooth muscle and mucous glands and accumulation of myofibroblasts and extracellular matrix in the subepithelial region. There is considerable evidence that AHR to spasmogens such as methacholine is an intrinsic, possibly inherited trait that is regulated separately from the inflammatory response, and precedes the development of clinical asthma in most individuals (4). An additional transient steroid-sensitive increment in AHR occurs following inhalation of allergen in atopic humans, indicating that allergic inflammation superimposes an inducible component of AHR onto an already hyperresponsive background (5).

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Lessons from mouse models: nuggets

Typical mouse models of allergen-mediated pulmonary inflammation involve intraperitoneal immunization with chicken egg ovalbumin (OVA) precipitated with aluminum hydroxide (alum), followed by repetitive challenge with OVA intratracheally, intranasally, or by aerosol. These conditions produce a robust eosinophilic inflammatory response that is typically distributed around bronchi and vascular structures, and AHR. These features are independent of IgE, B cells, or mast cells, but depend on $CD4^+$ T lymphocytes (6). Effector T lymphocytes are necessary and sufficient to provide the requisite Th2 cytokines that induce both histologic changes and induced AHR. The use of knockout mice and/or blockade with specific antibodies in wild-type mice revealed that IL-4 signaling through the IL-4 receptor α subunit (IL-4Rα), and subsequent STAT6-dependent transcriptional events are required for both the development of polarized OVA-specific Th2 cell populations and an IgE response from B cells (7, 8). IgE, but not Th2 cell polarization, can also be induced by STAT6 signaling initiated by IL-13 (9), which binds to the IL-4Rα/IL-13R1α heterodimer expressed by B cells and stromal cells, but not by T cells. Although dispensable for IgE generation, IL-13 is the major effector of airway mucosal pathology, targeting the epithelium for goblet cell metaplasia, epithelial cell–derived chemokine production, and AHR (10). The perivascular and peribronchial eosinophilia that is consistently observed in these models reflects the concerted actions of IL-5 and the chemokine eotaxin-1 (CCL13), the latter being a major product of IL-13–stimulated bronchial epithelial cells (11). Overexpression of IL-13 in the pulmonary epithelium also induces signature features of airway remodeling through activation of TGF-β1–matrix metalloprotease signaling (12). Thus, IL-4–dependent polarization of T cells provides the effector cytokines responsible for the core pathobiology of mouse models.

Variables contributing to disparate experimental outcomes

Although allergen-induced models of pulmonary inflammation consistently elicit the features noted above in mice, discrepancies exist between models.

These discrepancies reflect several critical experimental variables noted below.

Strain. The most commonly used mouse strains in models of experimentally induced airway disease, BALB/c and C57BL/6, differ sharply in their propensity to Th2 versus Th1 cytokine production in response to certain infectious agents (13). In an analogous fashion, BALB/c mice develop a vigorous Th2 response following sensitization and challenge with OVA, characteristically involving more profound pulmonary eosinophilia, higher levels of allergen-specific IgE, and greater levels of induced AHR compared with C57BL/6 mice (8). BALB/c mice differ from C57BL/6 mice at genetic loci on chromosome 11 that control the robustness of IL-4 responses of T cells, a region syntenic with human chromosome 5q23-35 (14). This region contains the Th2 cytokine gene cluster, as well as a possible regulator of Th2 responses, T cell Ig domain, mucin domain-1, the mouse homologue of the hepatitis A receptor. Thus, the choice of strain may profoundly alter experimental outcomes in these lymphocyte–dependent models. The fact that most available knock-out strains of mice are created on a C57BL/6 genetic background has important implications for interpretations of allergic disease models where these mice are used. Furthermore, the backcrossing of C57BL/6 mice to the BALB/c background introduces the potential for confounding influences of retained C57BL/6 genes on chromosome 11 that may complicate comparisons with wild-type BALB/c mice, or even littermate controls.

As is the case with primary immune responses, innate tissue responses also differ amongst strains. Most mice are nonreactive or poorly reactive to pharmacologic bronchoconstrictors (15), and lack airway smooth muscle below the first few bronchial generations. However, A/J mice exhibit marked methacholine-induced bronchconstriction that is independent of allergen sensitization and challenge (referred to as naive AHR) (16). The naive AHR of the A/J strain is not associated with a definable abnormality in airway histology, and is not abrogated by crossing these mice with either *Rag2*-deficient mice (which lack mature B and T cells) or mice lacking IL-4Rα (17). The genotyping of phenotypically selected A/J mice that were bred over 8 generations onto the C57BL/6 background (which is hyporesponsive to methacholine) revealed that naive AHR is conferred by a major locus on A/J chromosome 2, and an interacting locus on chromosome 6 (18). It is noteworthy that chromosome 2 contains the mouse orthologue of a dysintergrin and metalloprotease 33 (*ADAM-33*), which was recently reported to be a gene with linkage to AHR in some humans studies. A/J mice also develop exuberant smooth muscle hyperplasia, eosinophilia, IL-13 production, and peribronchial fibrosis in response to repetitive intranasal administration of OVA in the absence of systemic sensitization, whereas BALB/c, C57BL/6, and C3H/Hej mice fail to respond to this protocol (19). Thus, existing mouse models can address the role of a given cell, mediator, or gene unequivocally on a single genetic background, but cannot address how broadly applicable a finding might be to an analogous experiment performed in a different strain. It is nonetheless tempting to speculate that naive AHR and a propensity for the development of exuberant remodeling in the A/J strain could be genetically linked in a manner directly relevant to human asthma.

Method of sensitization and nature of allergen. Toll-like receptors (TLRs) serve as innate receptors for pathogen-associated molecular patterns (PAMPs) and are a basis for the adjuvant effects of microbial constituents. Among other effects, PAMPs stimulate maturation of antigen presenting cells (APCs), and some can differentially potentiate subsequent Th1 or Th2 immune responses. The common experimental practice of inducing sensitization using intraperitoneal injections of alum-precipitated OVA bypasses this microbial influence. Resident APCs in the peritoneal cavity show signs of constitutive maturation, as evidenced by high levels of the costimulatory molecules CD40 and CD86, and MHC class II expression, even in mice that are deficient in TLR4, the receptor for lipopolysaccharide (LPS), or the adaptor molecule MyD88, which is used by most TLRs (20). In contrast, resident lung APCs do not constitutively express maturation markers, accounting for the fact that sensitization to OVA administered solely by intranasal administration requires the concomitant provision of LPS. Moreover, the dose of LPS used is critical: the immune response to the OVA is strongly Th2-like with sensitization in the context of low-dose LPS, but Th1-like with high-dose LPS. Both the Th2 and Th1 responses require TLR4 and MyD88 in this model (20). Thus, while intraperitoneal sensitization with alum-precipitated OVA reliably permits a robust Th2 response in the lung, it bypasses a range of signals from innate receptors that likely modify the nature of the immune and effector responses to allergens.

In contrast to the weak sensitizing properties of OVA in the lung, protease-containing allergens (derived from cockroach, dust mites, and fungi) efficiently sensitize mice through intranasal application without the requirement for systemic immunization or adjuvant (21). Such "natural allergens," which also elicit sensitization in human subjects, may function by directly activating cells of the innate immune system (APCs and mast cells) (21, 22), thus fulfilling the functions of both adjuvants and antigens. Protocols using protease allergens may thus induce certain functions of the innate immune system that modify the nature of the subsequent immune response, and that may be missing in protocols that use OVA as an antigen.

Conundrums: contributions of eosinophils and mast cells

Eosinophils and mast cells are main effector cells of Th2-polarized immune defenses of the mouse against helminthic parasites such as *Trichinella spiralis*. Normal mouse lung contains no eosin-

ophils and sparse numbers of mast cells, which are confined to the paratracheal tissues. In OVA-induced pulmonary inflammation, sharp increases in tissue eosinophils reflect their recruitment as mature cells from peripheral blood, while a modest reactive intraepithelial mast cell hyperplasia (23) is attributable to the comitogenic effects of Th2 cell cytokines that synergize with the homeostatic mast cell growth factor, stem cell factor. The specific contribution of each cell type to the pathophysiology of OVA-induced models remains a subject of considerable debate. Mice lacking IL-5 (24) or IL-5 and eotaxin-1 (25) exhibit attenuated OVA-induced pulmonary eosinophil recruitment and impaired induction of AHR (24, 25), goblet cell metaplasia (24), and production of IL-13 by $CD4^+$ T cells ex vivo (25). In contrast, a strain lacking CCR3, an essential chemokine receptor for eosinophil recruitment, showed no pulmonary eosinophil recruitment, but unimpaired induction of AHR and goblet cell metaplasia (26). Conflicting data were obtained in studies where depletion of eosinophils was accomplished by blocking antibodies to IL-5 (8, 27). Studies reporting a critical role for eosinophils in AHR have often used the comparatively weakly Th2-responding C57BL/6 mouse strain, or backcrosses to BALB/c from C57BL/6 (25), whereas several studies failing to show such requirements have been performed in strains with a BALB/c background (8, 26). Recently, experiments conducted on C57BL/6 mice deficient in eosinophils due to expression of diphtheria toxin A under control of an eosinophil-specific promoter revealed that induced AHR and goblet cell metaplasia required eosinophils (28). By contrast, BALB/c mice made deficient in eosinophils by deletion of a high-affinity GATA binding site in the GATA-1 promoter showed no requirement for eosinophils in either AHR or goblet cell metaplasia (29). Interpretation of the role for eosinophils in mouse AHR is further complicated by the finding that very high levels of bronchial eosinophilia (driven by an IL-5 transgene) protected C3H/Hej mice against OVA-induced AHR, reflecting the provision of TGF-β1 (30), a cytokine that can down-modulate Th2 responses. A critical observation in this study was that reduction of eosinophil numbers with an anti-IL-5 antibody amplified OVA-induced AHR, suggesting that the dose–response curve relating eosinophils to AHR in mice may be bell-shaped. The

Table I. Pathologic features of mouse models of allergen-induced pulmonary disease and human asthma

	Demonstrated in mouse only	Demonstrated in human only	Demonstrated in both
Immune response			
Th2 cell cytokines (IL-4, IL-5, IL-9, IL-13)			+
IgE			+
IgG_1	+		
Smooth muscle response			
Intrinsic AHR		+ (except A/J mouse strain)	
Smooth muscle beyond large airways		+	
Allergen-induced AHR			+
Hypertrophy or hyperplasia			+
Epithelium			
Goblet cell metaplasia or mucous plugging			+
Exfoliation		+	
Subepithelial fibrosis			+
Eosinophils			
Peribronchial			+
Intraepithelial		+	
Degranulation		+	
Importance for induced AHR	+ (mostly in C57BL/6)		
Importance for remodeling			+
Mast cells			
Smooth muscle		+	
Intraepithelial			+
Degranulation			+
Involvement in exacerbations		+	

inconsistencies regarding the role of eosinophils in the short-term models of airway disease are contrasted by the relatively consistent demonstration that eosinophils are required for remodeling in models using a longer period of allergen challenge (29, 31), possibly through pathways involving TGF-β1 (31) and/or cysteinyl leukotrienes (32).

Mast cells generate and release numerous mediators (leukotrienes, prostaglandin D_2, histamine, proteases, and cytokines) that potently modulate smooth muscle and/or Th2 responses, and contribute substantially to asthmatic early and late-phase responses to allergen challenge in humans. The contribution of mast cells to the induction of AHR, eosinophilia, or both in mice depends on the strength of OVA sensitization and challenge protocols. Intraperitoneal sensitization to OVA without alum, sensitization with OVA/alum followed by a minimal number of airway challenges, or weak sensitization induced only by successive intranasal challenge with OVA (33) each reveal substantial contributions from mast cells to the effector phase. Although strong systemic sensitization or higher-dose airway challenges override the window for detecting a mast cell contribution, there is direct evidence that IgE-dependent mast cell activation induces AHR in naive mice without OVA-induced pulmonary inflammation (34), and potentiates OVA-induced AHR in actively sensitized mice (35). Furthermore, both C57BL/6 (35) and BALB/c mice (34) lacking FcεRI show deficits in AHR in weak sensitization and challenge protocols, reflecting amplification by mast cells of afferent and/or efferent events. Mast cell infiltration of airway smooth muscle, a major correlate of human AHR and asthma in humans (3), has not yet been modeled in mice.

Implications of mouse models for the pathophysiology of asthma

No truly authentic "asthmatic mouse" exists, and the available models used currently cannot dissect the complex mechanisms of spontaneous and variable airflow obstruction, disease exacerbations, or naive AHR. However, the allergen-induced models have proven invaluable for dissection of afferent innate and adaptive mechanisms leading to sensitization, and efferent mechanisms for effector cell and end-organ responses (Table I). Extrapolation of these mechanisms to the pathophysiology of human disease, and to the development of potential pharmacotherapeutic targets, requires caution as the models introduce the potential bias of genetic purity that may not translate to human populations. Furthermore, such extrapolation must account for distinctions between mediators, cells, and receptors that are crucial for sensitization from those that amplify effector responses or contribute to their resolution or perpetuation. The failure of a soluble recombinant form of the IL-4 receptor extracellular domain administered by inhalation to substantially improve asthma control (36) may relate to the central involvement of IL-4 in inductive events, but not effector events, especially induced AHR as shown in mice. Although monoclonal anti–IL-5 antibodies blunt peripheral blood and sputum eosinophil counts in human asthmatics, they do not alter impaired airflow or AHR, a finding some mouse strains. The additional finding that prolonged (3 month) administration of anti–IL-5 to a small group of humans with asthma decreased several indices of remodeling (36) would seem to reflect a broadly applicable, central role for eosinophils in a process that can readily be reproduced in mice. The fact that nonanaphylactogenic humanized monoclonal antibodies to the FcεRI binding site of human IgE improve asthma control in atopic subjects (36) suggests that further manipulations of mast cell–dependent models may prove valuable for determining mechanisms of disease exacerbations. The evidence implicating IL-13 as a central effector of both induced AHR and remodeling responses in the mouse awaits validation using pharmacologic antagonists in human asthma. Finally, the accumulating body of evidence suggests that the A/J mouse provides an opportunity to study the influence of major genes, localized but yet to be identified, on constitutive AHR and susceptibility to chronic Th2 cell–like bronchial inflammation with remodeling.

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