

# Searching for an improved mouse model of allergic airway disease using dual allergen exposures

Irving C. Allen<sup>1</sup>

Asthma is a complex genetic disorder affected by a myriad of environmental stimuli, leading to suggestions that asthma is not a single disease but a syndrome of related diseases – a situation that complicates the development of relevant animal models (Busse and Lemanske, 2001; Drazen et al., 2000). Asthma is characterized by chronic airway inflammation, airway hyperresponsiveness (AHR) and reversible airflow obstruction. These three cardinal features distinguish asthma from other obstructive airway diseases, such as chronic obstructive airway disease and cystic fibrosis (Wardlaw et al., 2002). Chronic airway inflammation is mediated by eosinophils and typically involves the production of T-helper cell 2 (Th2)-associated cytokines, mucus hypersecretion, airway smooth muscle (ASM) hypertrophy, collagen deposition and mast cell degranulation (Wardlaw et al., 2002). In addition to airway inflammation, the presence of AHR is a common finding in asthmatic individuals and is characterized by an exaggerated response to a mild aerosol stimulus, such as methacholine (MCh). The degree of the resultant AHR correlates with asthma severity (Downie et al., 2007). Although still controversial, there is evidence that chronic exposure of ASM to inflammatory mediators affects smooth muscle physiology, thus contributing to its hyperresponsiveness. ASM constriction also contributes to the reversible airflow obstruction that sets asthma apart from other obstructive airway diseases (Wardlaw et al., 2002).

Animal models are vital to elucidating the pathophysiological mechanisms associated with human respiratory diseases and have been essential to the development of therapeutics. In particular, mouse models are excellent *in vivo* systems for studying sim-

ple physiological processes associated with respiratory disorders, owing to the ease with which the mouse genome can be manipulated and the availability of newly introduced techniques that allow accurate *in vivo* assessments of the physiological changes that mediate airflow obstruction and hyperresponsiveness. However, the complexity of the human airway and the structural, physiological, pharmacological and neuronal changes associated with AHR in humans challenge the development of relevant models (Canning, 2003; Joos, 2003). Only humans and a limited number of other mammalian species, such as the horse and cat, spontaneously develop asthma or asthma-like disease (Dye et al., 1996; Lavoie et al., 2001; Leguilette, 2003).

The majority of mouse studies use allergic lung disease as a surrogate model for asthma. Although this strategy significantly expanded our understanding of the mechanisms underlying airway diseases, these models do not fully recapitulate several distinct characteristics of asthma. The mouse allergic lung disease models are derived from short-term, acute exposures to simple protein antigens (e.g. ovalbumin), complex microorganisms (e.g. *Aspergillus*) or chemical compounds [e.g. toluene diisocyanate (TDI)]. Most of these models have been evaluated extensively and reproduce many of the features observed in human asthma including increases in antigen-specific IgE, increases in Th2-associated cytokines (IL-4, IL-5 and IL-13), eosinophilic lung inflammation, goblet cell metaplasia and AHR (Taube et al., 2004). The major differences that have been observed between current mouse models and human asthma include: acute peribronchiolar and perivascular inflammation in the lung parenchyma (rather than inflammation within the airway

wall in humans); a lack of activation, degranulation or intra-epithelial accumulation of eosinophils; significantly less plasma exudation in murine versus human airways; and a lack of structural changes in the airway other than goblet cell metaplasia (Kumar and Foster, 2002).

A new study by DiGiovanni et al. in a recent issue of *DMM* analyzes a novel mouse model of asthma based on concurrent dual allergen exposure (DiGiovanni et al., 2009). The rationale for using multiple allergens is based on the experience of human asthmatics who are frequently exposed to a wide spectrum of environmental stimuli, which may induce or exacerbate the respiratory symptoms associated with their disease. This situation is not recapitulated in the majority of existing mouse models, which are based on the sensitization and acute challenge of animals with single allergens. Thus, these researchers chronically exposed mice to ovalbumin (OVA) together with house dust mite (HDM) extract to determine how multiple allergens function synergistically, and to generate a more pertinent mouse model of asthma. They use the model to assess AHR and airway inflammation 24 hours following the final allergen challenge, and evaluate airway remodeling following a 4-week recovery phase.

It is well established that either OVA or HDM exposure can induce allergic airway disease in mice. The dual allergen exposure mice from the DiGiovanni et al. study recapitulate these findings and show increased granulocyte and lymphocyte counts in the bronchoalveolar lavage fluid (BALF), increased IL-4 production in splenocytes following allergen re-stimulation, increased mast cell numbers in the lung tissue (HDM exposure only) and increased airway responsiveness following challenges with the muscarinic acetylcholine receptor agonist MCh. In each case, concurrent exposure of both OVA and HDM only modestly increased airway inflammation and AHR above the levels observed for HDM alone. These findings suggest that HDM alone is sufficient for the inflammation and AHR in this model and does not show any additive effects with exposure to OVA.

In addition to assessing AHR and inflammation, the authors determine the influence of OVA and HDM on airway remodeling relative to one allergen alone. Their morphometric quantification of his-

<sup>1</sup>Lineberger Comprehensive Cancer Center, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA  
e-mail: coy\_allen@med.unc.edu

tology sections demonstrates a subtle increase in collagen deposition, smooth muscle contractile elements and goblet cells following the combination OVA and HDM challenge. Mice receiving both allergens show a slight increase in airway sensitivity to inflammatory mediators when compared with control animals exposed to one allergen, suggesting that the increased airway remodeling in this model may potentiate AHR during the recovery phase. Although this sustained AHR is an interesting observation, the combination model of OVA and HDM did not show significant changes in airway inflammation and constriction.

Although the authors observed both increases in markers associated with airway remodeling and sustained AHR throughout the recovery phase in mice exposed to the allergen combination, they point out that the combinatorial model did not result in a more robust induction of allergic airway disease compared with exposure to either allergen alone. They suggest that subtle variations of this model (i.e. choice of allergen, use of adjuvant during sensitization, duration of allergen exposure, length of recovery phase) may offer additional insights into the sustained AHR and may result in significantly different outcomes. A previous study, using a different HDM and OVA dual exposure model featuring re-stimulation with OVA following an 8-week recovery phase, successfully demonstrated that exposure to HDM extract establishes a local microenvironment in the lungs that ampli-

fies the immune response to otherwise innocuous doses of OVA (Fattouh et al., 2005). Although this model confirms the principles suggested by DiGiovanni et al. with respect to airway inflammation and hyperresponsiveness, the Fattouh et al. study does not directly address airway remodeling or sustained AHR throughout the recovery period. Thus, assuming OVA tolerance is not induced, it is interesting to speculate that these parameters would be enhanced by multiple exposures to OVA during the recovery period.

This model of dual allergen exposure addresses a variety of shortcomings associated with the traditional, single allergen mouse models of allergic airway disease. Future research might develop subtle variations of this model to determine how these variables affect airway pathophysiology and to establish a more robust mouse model for human asthma. Ultimately, these models may provide mechanistic insight into the host response when challenged with a variety of allergens that function either individually or synergistically to alter the lung microenvironment and influence airway disease.

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