

EDITORIAL

Building a better mouse model: experimental models of chronic asthma

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Introduction

Allergic asthma is characterized by chronic mucosal inflammation mediated by CD4⁺ Th2 cells [1, 2]. Animal models of acute allergic airway disease have been used in order to elucidate the mechanisms leading to the development of inflammation. The acute airway inflammation induced in mice is characterized by accumulation of antigen-specific CD4⁺ effector cells and eosinophils surrounding the airways and blood vessels. However, the majority of these models involve systemic sensitization with relatively short-term administrations of high doses of aerosolized antigen, classically ovalbumin (OVA), and many do not show the typical chronic inflammatory and lung structural changes seen in most patients with asthma. For example, human asthma is not characterized by the degree of perivascular inflammation seen in the standard OVA-induced murine model; also, the degree of airway eosinophilia in human asthma is typically only 1–3% of bronchoalveolar lavage (BAL) cells, whereas the OVA murine model has 40–80% eosinophilia depending on the experimental model [3–5]. In addition, the induction of lung remodelling, including subepithelial fibrosis and goblet cell hyperplasia, is only mildly induced in the standard murine model of acute asthma. In recent years, several experimental protocols have been developed in an attempt to construct a model associated with airway inflammation and lung remodelling resembling human asthma.

Pulmonary over-expression of Th2-associated cytokines, including IL-4, -5, -9, -11, and -13, in transgenic mice has been used as an approach to model chronic asthma [6–10]. While these models provide valuable information regarding contributions of potential mediators to specific aspects of disease pathology, these models do not involve allergen challenge. Prolonged allergen exposure likely triggers a distinct array of immunological responses that result in chronic inflammation and impact airway integrity and function. Alternatively, experimental protocols involving repeated delivery or inhalation exposure of antigens to sensitized animals over the course of weeks and months have been developed.

Chronic inflammation

Allergic asthma is characterized by chronic lung inflammation and repeated inflammatory exacerbations. The composi-

tion and localization of inflammation in acute models of experimental allergic asthma differ from that observed in individuals with asthma. Acute exposure to antigen usually results in parenchymal, perivascular, and peribronchial inflammation, composed primarily of eosinophils. Human asthma is associated with persistent bronchial wall inflammation, including infiltration of Th2-cytokine producing lymphocytes, eosinophils, and mast cells into the submucosa and mucosal epithelium [11–13]. In the current issue of *Clinical and Experimental Allergy*, Wegmann et al. repetitively exposed sensitized animals (two times a week for 12 weeks) to low-dose OVA (1% wt/vol in phosphate buffered saline) in order to develop inflammation in both the proximal and distal airways [14]. In chronically exposed animals, lymphocyte accumulation predominated in the proximal airways, but both eosinophils and lymphocytes were detected in distal airways. Similar inflammatory infiltrate has been observed in other models of prolonged antigen exposure [15, 16]. In contrast, a severe and persistent airway eosinophilia was observed following chronic exposure to house dust mite (HDM) extract [17]. Interestingly, Wegmann et al. report the persistence of significant inflammation 6 weeks after the final allergen exposure. McMillan and Lloyd [15] have also shown sustained mononuclear and eosinophil inflammation following prolonged OVA (5%) challenges. In contrast, intraepithelial eosinophil infiltration and mononuclear accumulation in the airway wall returned to baseline within 2 weeks after cessation of antigenic challenge in a similar model of chronic low-dose OVA (2.5%) or HDM exposure [16, 17]. It remains to be determined whether persistent or chronic pulmonary inflammation such as seen in human asthma is dependent upon continued allergen exposure.

Lung remodelling

Airway remodelling, an important contributor to reduced lung function in asthmatics, has been proposed to result from repeated cycles of airway injury induced by inflammatory responses followed by processes inherent in the lung to repair the damaged airway [18, 19]. Airway remodelling associated with chronic asthmatic inflammation is characterized by hyperplasia of smooth muscle and mucus-producing cells and accumulation of myofibroblasts and extracellular matrix (ECM) in the subepithelial region [20]. Defining the mechanisms of asthmatic lung remodelling can have critical clinical consequences as tissue remodelling and fibrosis are important pathological features of lung disease, and do not appear to be clearly reversed by existing therapy [21–23]. Murine models of chronic asthma have utilized repeated antigen exposure to

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induce changes in the airway wall that mimic many features of human asthma [24]. Excess mucus secretion from hyperplastic goblet cells is a characteristic feature of the asthmatic airway [25]. In chronic experimental asthma, goblet cell hyperplasia is a consistent finding following prolonged antigen challenge. Another prominent feature of airway remodelling observed in asthma patients is an increase in matrix deposition in the subepithelial region of the bronchioles [26–29]. Repeat antigen challenge of sensitized animals over the course of 8–12 weeks leads to dense collagen deposition around airways [24, 30]. Total collagen content of the lungs has also been reported to increase in chronic experimental asthma [15, 31]. Importantly, the subepithelial fibrotic zone was not resolved or diminished weeks after cessation of allergen challenge [15–17]. An increase in airway smooth muscle mass is another characteristic feature of human chronic asthma [32]. Chronic allergen challenge in experimental models, including the model described by Wegmann and colleagues results in smooth muscle proliferation and an increase in the thickness of the smooth muscle layer [14, 15, 17, 31, 33]. Interestingly, the number of proliferating smooth muscle cells has been shown to continue to increase in the absence of further allergen challenge [15]. Collectively, these studies demonstrate that chronic exposure to allergens elicits persistent lung structural changes that results in an increase in airway wall thickening and draw attention to the need to develop new therapeutic approaches to reverse and prevent airway structural changes.

Recently there has been a lot of attention on the role of eosinophils in the pathophysiology of airway remodelling [34]. Clinical investigations have demonstrated that thickening of the subepithelial basement membrane was associated with increases in bronchial mucosal eosinophils in severe asthmatics [35]. Furthermore, tissue eosinophilia and eosinophil degranulation have been associated with several fibrotic syndromes [36, 37]. Eosinophils are a source of several molecules implicated in tissue remodelling processes such as transforming growth factor- β_1 (TGF- β_1), vascular endothelial growth factor (VEGF), matrix metalloproteinase-9 (MMP-9), tissue inhibitor of metalloproteinase-1, and IL-13 [34]. TGF- β_1 is a potent regulator of fibroblast/myofibroblast function in particular the regulation of expression of several ECM proteins including collagens, proteoglycans, and tenascin. Elevated levels of TGF- β_1^+ eosinophils are present in the asthmatic airways compared to normal individuals [29]. Reduction of airway eosinophilia by anti-IL-5 therapy significantly reduced TGF- β_1^+ eosinophils in airway tissue and TGF- β_1 levels in BAL fluid (BALF) of asthmatics. The reduction in eosinophil numbers and TGF- β_1 levels was associated with reduced expression of ECM proteins in the bronchial mucosa reticular basement membrane of asthmatics [29]. Wegmann and colleagues demonstrated increased levels of TGF- β_1 in BALF during the chronic phase and notably this increase was associated with elevated levels of eosinophils in the distal airways. It remains to be determined whether these eosinophils express TGF- β_1 . Employing a similar model of chronic experimental asthma, Kumar and colleagues have demonstrated a role for IL-13 in airway remodelling [38]. Interestingly, IL-13 promotes fibroblast activity and remodelling events through the up-regulation of epithelial cell and fibroblast-derived TGF- β_1 [39].

Airway hyper-reactivity

Acute models of allergic asthma have demonstrated the importance of Th2 cytokines in the pathogenesis of allergen-induced airway hyper-responsiveness (AHR) [40–43]. Although these studies and others have increased our understanding of the mechanisms underlying transient responses to allergens, they do not necessarily provide an experimental model of the sustained AHR present in asthma. As such, chronic models of experimental asthma associated with sustained AHR are needed. Leigh et al. and others have demonstrated sustained AHR persisting 5–8 weeks following chronic allergen exposure that was associated with the presence of airway wall remodelling [17, 30, 33]. These studies are in contrast to other studies, including Wegmann et al., that demonstrate progressive AHR with chronic allergen exposure, but in the absence of continued allergen challenge, reactivity to cholinergic stimulation returns to baseline [14, 15]. The conflicting results may be related to the marked differences in antigen delivery (intranasal bolus vs. aerosolized antigen) utilized in the studies.

In summary, acute models of allergic asthma have provided a wealth of data regarding the basic mechanisms of allergic inflammation and the underlying immunologic responses. However, the value of short-term models is limited as these models fail to reproduce a number of characteristics of human asthma in regard to inflammatory infiltrate, lung remodelling and sustained AHR. Experimental protocols that utilize prolonged antigen exposure have enriched the standard animal model to more typify the morphological and functional changes evident in human disease. These models have considerable potential for the investigation of pathogenic mechanisms and potential treatments of chronic human asthma. Likely, future models involving even lower doses of antigen and extended chronicity will be enlightening; perhaps transgenic approaches involving over-expression of inducible allergen transgenes may be informative.

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