

## Review

# What have transgenic and knockout animals taught us about respiratory disease?

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## Abstract

Over the past decade there has been a significant shift to the use of murine models for investigations into the molecular basis of respiratory diseases, including asthma and chronic obstructive pulmonary disease. These models offer the exciting prospect of dissecting the complex interaction between cytokines, chemokines and growth related peptides in disease pathogenesis. Furthermore, the receptors and the intracellular signalling pathways that are subsequently activated are amenable for study because of the availability of monoclonal antibodies and techniques for targeted gene disruption and gene incorporation for individual mediators, receptors and proteins. However, it is clear that extrapolation from these models to the human condition is not straightforward, as reflected by some recent clinical disappointments. This is not necessarily a problem with the use of mice itself, but results from our continued ignorance of the disease process and how to improve the modelling of complex interactions between different inflammatory mediators that underlie clinical pathology. This review highlights some of the strengths and weaknesses of murine models of respiratory disease.

**Keywords:** asthma, chemokines, cytokines, inflammation, murine

## Introduction

The incidence of respiratory diseases such as asthma and chronic obstructive pulmonary disease (COPD) continue to increase despite the availability of current methods of treatment and there is therefore a need to improve our understanding of the pathophysiology of these diseases to permit the development of novel therapeutic agents. Although the exact causes of asthma and COPD are not completely understood, it is clear that both diseases are characterized by inflammation of the airways and a decline in respiratory function. In asthma, several inflammatory cell types are thought to contribute toward the pathogenesis of this disease, including eosinophils [1\*] and CD4<sup>+</sup> T lym-

phocytes [2\*], whereas it is thought that CD8<sup>+</sup> lymphocytes [3\*] and neutrophils [4\*] are important in COPD. Another important feature of these diseases is the presence of airway wall remodelling. There is evidence of hyperplasia/hypertrophy of airway smooth muscle, increased collagen deposition beneath the basement membrane, increased production of mucus, angiogenesis and alterations in the extracellular matrix in asthma [5\*]. In COPD, there is evidence of mucous gland hyperplasia, increased hypertrophy of bronchiolar smooth muscle, fibrosis of the small airways and, in emphysema, destruction of alveolar tissue [6\*]. On the basis of the findings obtained from autopsy, the analysis of biological fluids

and, more recently, biopsies from individuals with respiratory disease, a variety of animal models have been used to study many of the characteristic features of these diseases. For example, in asthma research, there are models of airway inflammation that have been developed in sheep, dogs, cats, rabbits, rats, guinea-pigs and primates. In general, these models are useful; moreover, there are known instances of natural sensitivity to environmental allergens in sheep, dogs and primates. Furthermore, their large size means that repeated measurements can be made quite easily within the same animal.

The mainstay of treatment for asthma includes bronchodilators such as  $\beta_2$ -adrenoceptor agonists and glucocorticosteroids; for COPD, ipratropium bromide and  $\beta_2$ -adrenoceptor agonists are used. With the aid of animal models, a new class of anti-asthma drug (the leukotriene antagonists) has been introduced clinically [7] and clinical trials are in progress with another drug class, the phosphodiesterase 4 (PDE4) inhibitors [8]. Although the introduction of one new drug after 30 years for the treatment of asthma seems disappointing, it is worth remembering that our understanding of the disease process has altered from a simple model of controlling bronchoconstriction to attempts at modulating the inflammatory response and the remodelling of the structural airway. Furthermore, animal models have been useful in the development of better bronchodilator drugs such as long-acting  $\beta_2$ -adrenoceptor agonists, including salmeterol and formoterol, better glucocorticosteroids (for example fluticasone) and in the development of leukotriene antagonists. Despite the criticisms and imperfections of animal models in general, they still offer us a useful tool in the study of respiratory airway disease.

### **Murine models of airway inflammation**

The use of mice as models of human respiratory diseases began to emerge in the early 1990s, and there were more than 500 publications in the latter half of that decade. The principal reason for using mice is that it enables investigators to study the role of the immune system in respiratory disease. Indeed, considerable attention is now focused on understanding the role of cytokines, chemokines and growth related peptides in asthma because these substances are often detected in bronchial tissue and have a wide variety of pharmacological and immunological activities [9]. The mouse model is amenable for study because of the existence of monoclonal antibodies specific for murine proteins, and the availability of knockout and transgenic mice. It is these latter two aspects that make the use of the mouse a powerful biological tool in the study of inflammatory disease because this technology is not currently available in other species. Furthermore, the lack of selective non-peptide antagonists to many of the cytokine, chemokine and growth factor receptors makes these models highly attractive.

As with other animal models, murine models of allergic inflammation show many of the characteristic features of the clinical disease. Thus, allergic mice undergo early and late 'asthmatic' responses [10] and demonstrate serum-specific IgE, recruitment of lymphocytes, eosinophils and bronchial hyper-responsiveness to human relevant antigens such as Der p1 [11]; murine models can also be used to study various aspects of airway remodelling after protocols involving chronic challenge with antigens [12]. Moreover, many of the cytokines, chemokines, growth related peptides and their receptors that are expressed in human respiratory disease are also found in these allergic models of inflammation.

Another important aspect of any 'asthma' model is whether some of the characteristic features of the disease are attenuated by drug treatment. Thus,  $\beta_2$ -adrenoceptor agonists provide effective bronchoprotection against antigen challenge, and glucocorticosteroids attenuate the development of the late asthmatic response [10]. Similarly, glucocorticosteroids are effective at inhibiting the recruitment of eosinophils and bronchial hyper-responsiveness after chronic challenge with antigens [13]. There is currently enormous interest in the possible anti-inflammatory activity of PDE4 inhibitors for the treatment of asthma and COPD [8] and it is of interest that the PDE4 inhibitor, rolipram, attenuated eosinophilia and bronchial hyper-responsiveness in a murine model of allergic inflammation [14]. As with other models, murine models of respiratory disease can be modulated by current therapeutic modalities and therefore offer the possibility of testing potentially novel anti-inflammatory agents. It is also clear that many false positives will be found that will fail the clinical test and will therefore require an adjustment to our current concepts of disease pathophysiology. This iterative process between biological modelling and clinical evaluation is not unique to human respiratory disease but is also a feature of other human diseases, including cardiovascular disease and cancer.

### **What we have learned from these models**

It is immediately obvious that our understanding of the role of the immune system in the initiation and propagation of the inflammatory response in the airways has increased enormously. Furthermore, the complex pathways that are being realized have offered an array of potential target sites for the development of novel therapeutic strategies for the control of inflammatory disease. The current model of airway inflammation in the mouse is one driven by T helper type 2 (Th2) lymphocytes secondary to antigen presentation from dendritic cells [2]. Antigen-specific Th2 cell clones generate a range of cytokines, including interleukin (IL)-4, IL-5, IL-9 and IL-13, which are important for the regulation of a range of inflammatory cells, including B cells, eosinophils, epithelial cells and fibroblasts. Both IL-4 and IL-13 are important in the

isotype switching of B cells to the IgE-secreting phenotype and have also been implicated in the recruitment of eosinophils to the airways. Furthermore, cytokines such as IL-4 and IL-9 can induce eosinophil recruitment by the stimulation of chemokine production from airway epithelium and fibroblasts, whereas IL-5 is an important cytokine for the development, recruitment and maintenance of eosinophils within the airways. Cytokines are also implicated in airway remodelling associated with asthma, because several studies have shown the ability of IL-6, IL-9 and IL-11 to promote fibroblast proliferation and subepithelial fibrosis. The interaction between the immune system and resident cells such as the epithelium and fibroblasts highlights multiple interactions and interconnected networks that are thought to be important in the propagation of the inflammatory response and airway wall remodelling [2,15,16].

However, it is also clear that these models have given us conflicting information about the critical importance of single proteins to the inflammatory response; this is more a reflection of the exuberance of investigators in pursuing the 'holy grail' of inflammation, namely a 'single mediator' hypothesis. As an example, the role of eosinophils in the pathophysiology of asthma is central to our thinking on this disease [1]; although there are numerous reports supporting this view, this is not a universal finding [17\*\*]. This is a picture that is mirrored in murine models, in which there are numerous reports supporting a role for IL-5 and eosinophils in the 'asthma' response [10,18] but under different experimental conditions bronchial hyper-responsiveness is not dependent on the eosinophil [11,18]. It is clear that the lack of a unified hypothesis for the role of various inflammatory substances and cells in the allergic response is a consequence of the sheer complexity of a process that is not completely understood and is unlikely to be described by a linear function but one that is highly complex [19\*,20\*].

Another important characteristic feature of asthma is bronchial hyper-responsiveness that is a major determinant of the irritability of the airways to environmental stimuli such as cold air, exercise, distilled water, pollutants and allergens. It is clear that strategies designed to suppress bronchial hyper-responsiveness will have a beneficial therapeutic outcome [21]; understanding the mechanisms that lead to this phenomenon is therefore of considerable importance. There are challenges to the measurement of respiratory mechanics in the mouse, and current methods are available that permit the determination of airway responsiveness to either serotonin or methacholine in this species [22]. The change in responsiveness typically observed in such experiments is approx. 2–5-fold, which is of a similar magnitude to that normally seen after an exacerbation of asthma. Before antigen challenge the baseline responsiveness to spasmogens is no different

from that in controls; in this respect, mice, like other animal models, differ from humans; they are therefore models of asthma exacerbation to allergens. However, there are examples of mice with a genetic predisposition to increased airway sensitivity to spasmogens in comparison with other strains [22], or altered responsiveness after the incorporation of transgenes (such as IL-6, IL-9 and IL-11) [15]. Superimposing the allergic response determined by Th2 cells in these models could be used to study the interaction between allergic inflammation and structural cells in the overall disease process [23].

Thus, it is clear from current models that specific cytokines and chemokines can be targeted to modulate airway inflammation. However, there are a number of conflicting reports citing the importance of several of these cytokines to this response. In some cases, gene knockout strategies often reveal alternative pathways that are 'recessive' under physiological conditions but become important when a 'dominant' pathway is removed. Alternatively, dependence on a particular pathway might be under the control of genetic differences between species, which begs the question as to which model is a better approximation of the clinical condition [18]. If we bear these caveats in mind, important information on the roles of individual cytokines and chemokines, or groups of these substances, in the inflammatory response can still be obtained and novel anti-inflammatory drugs can be developed and ultimately tested in the clinic, which is the key test of any drug under investigation.

### Potential novel therapeutic agents

Mice have long been the domain of immunologists and it is only recently that pharmacologists have discovered the possibilities that are being offered in understanding the role of the immune system in the context of human airway disease. The ultimate test of whether murine models can teach us anything about respiratory disease will be in the development of novel anti-inflammatory agents. Several novel therapeutic agents have been tested in the clinic. Thus, rhuMab 25, monoclonal antibody against human IgE [24] and IL-4R [25] have had modest clinical effects, whereas anti-IL5 antibody [26] and IL-12 [27] treatment did not seem to modulate the late asthmatic response or bronchial hyper-responsiveness. It is clear that these studies rely on the 'single mediator' hypothesis, and strategies designed to suppress inflammatory cell function will prove more successful. To this end, drug companies are attempting to define novel targets involved in the intracellular signalling pathways used by cytokines and chemokines that can be readily tested in murine models. Moreover, strategies attempting to suppress Th2 lymphocyte function by upregulating Th1 lymphocyte activity, or downregulating antigen presentation processes, offer an exciting new area of research that can be readily tested in murine models [28].

## Conclusion

Murine models of respiratory disease have taught us much about the role of the immune system in these diseases and the complexity of airway inflammation. We have gone through the first phase of the use of these models, investigating the effect of removing or adding single mediator genes on the inflammatory response. It is the next step, understanding the integration of different signals and their pathways to the overall inflammatory response, that will bring us closer to defining novel therapeutic pathways in respiratory disease.

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