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The use of knockouts to study determinants of airway hyperresponsiveness

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Nonspecific bronchial hyperresponsiveness (BHR) describes the characteristic altered airway behavior observed in asthma; namely, the propensity to develop bronchoconstriction in response to a wide variety of physical, chemical, pharmacologic, and physiologic stimuli at concentrations or levels of intensity that do not induce any change in airway caliber in healthy subjects (1). The two main determinants of BHR are increases, respectively, in the reactivity and the sensitivity of the airways (2, 3). The hypersensitivity is characterized by a leftward shift of the dose-response curve to the bronchoconstrictor effect of stimuli such as histamine or methacholine. The hyperreactivity is reflected by a steeper slope of the dose-response curve and by an increased degree of maximal airway narrowing. On the basis of mathematic models, increased sensitivity has been attributed predominantly to thickening of the airway wall, due to the presence of an inflammatory process in the airway mucosa (4, 5). Increased airway reactivity has been largely explained by the presence of more chronic, structural changes in asthmatic airways, such as thickening of the smooth-muscle layer and changes in the adventitial structure, leading to a loss of elastic parenchymal recoil on contracting airway smooth muscle (6, 7).

The exact functional contribution of the various inflammatory and structural cells, mediators, cytokines, and growth factors present in asthmatic airways to both determinants of BHR remains to be fully established. To date, for obvious ethical reasons, studies in man have been restricted to the combination of morphologic assessment and *in vitro* experiments. In addition to these data, a more functional evaluation in an *in vivo* animal model may be of interest. In asthma research, this has resulted in the development of a multitude of animal models that have examined the effect of exogenous administration of a given

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cell or mediator on airway structure and function. This is complemented by studies examining the effect of neutralizing specific cells or antagonizing endogenously released mediators in models of allergic inflammation. Gene knockout technology seems particularly suitable in this latter approach, as this avoids the drawbacks associated with blocking antibodies such as the potential development of neutralizing antibodies upon repeated administration or an incomplete blockade by the antibodies due to problems of insufficient dosing or penetration into the tissue. Obviously, the closer the model in wild-type (WT) animals mimicks the full asthma phenotype, the more relevant the information derived from gene knockout models will be.

However, a few caveats need to be borne in mind in this respect. First of all, no “gold standard” lung-function index has been invariably accepted in mice (8). The indices used include lung resistance based on the principles of Amdur & Mead, overflow of insufflation pressure as described by Konzett & Rössler and sometimes expressed as the airway pressure over time index (APTI), or the enhanced pause (penH) value (9–12). These various indices reflect to a variable degree changes in airway sensitivity and reactivity. In the vast majority of murine models, these two components of BHR, as in man, are not specifically examined.

Secondly, the overall increase in airway responsiveness obtained in murine models is generally smaller than the difference in airway responsiveness that exists between healthy and asthmatic subjects. This probably relates, at least in part, to the differences observed at a morphologic level. In most of the murine models of allergen exposure, only acute inflammatory changes are induced, without any concomitant structural change that might affect responsiveness to a larger degree. The distribution of the inflammation is also different from human asthma. In animal models, the inflammatory changes not only concentrate in or around the airways, but also frequently include parenchymal, mainly perivascular changes.

Thirdly, the sensitization and exposure procedures used in the different models also largely vary.

Finally, we must remember that important differences exist between different mouse strains, in both the functional role of components of the immune system and the baseline degree of airway responsiveness (13–16). These different issues have to be taken into account, not only when comparing the results from different experiments, but especially when trying to extrapolate data from murine models to human asthma.

In addition, although gene knockout technology has obvious advantages, it has to be taken into account that

most currently available knockout models are constitutional deletions, thus precluding the evaluation of the gene of interest in a specific phase of the disease process. As the technology further improves, the possibilities for conditional deletion increase, thus allowing us to time the switching off of the gene of interest. For example, this will allow us to evaluate in adult life the role of gene products that are essential during the early stage of development. In addition, conditional deletions will also enable us to analyze the role of gene products specifically during the primary antigen sensitization, or during the secondary antigen exposure to memory cells (17–19).

Investigation of the functional role of Th2-like cytokines

The shortcomings of these models notwithstanding, gene knockout models of asthma have allowed some interesting observations. Th2-like cells are currently thought to play a central role in the pathophysiology of asthma through the release of a range of cytokines, IL-4 and IL-5 in particular (20). Among its many effects, IL-4 induces IgE production, whereas IL-5, despite possible redundancy with other cytokines such as GM-CSF and IL-3, is considered of major importance in the development of allergen-induced airway eosinophilia. Both the IgE/mast-cell axis and the IL-5/eosinophil axis have been assigned a major role in the pathogenesis of BHR. The exact functional role of Th2 cells, IL-4, IgE, and IL-5 in the pathogenesis of allergen-induced airway inflammation and airway hyperresponsiveness has been further evaluated in a number of gene knockout models. We have developed a model of allergen (ovalbumin)-induced airway changes in C57Bl/6 mice. In this model, IL-4 is essential for the development of ovalbumin (OA)-induced eosinophil influx into the airways, IgE production, and increase in airway responsiveness (21, 22). More recent studies show very similar results in mice deficient in signal transducer and activator of transcription 6 (Stat-6), which is considered to be a central signalling pathway for mediating the biologic response to IL-4 (23, 24). In our model, mast-cell-deficient mice still developed airway eosinophilia in response to OA exposure, whereas MHC class II-deficient mice, lacking effective antigen presentation to CD4 cells and hence functionally active Th2 cells, did not. This indicates, as has been confirmed by others (25), that the crucial role of IL-4 lies in its effect on Th2 cell development, rather than on the induction of IgE synthesis and subsequent mast-cell degranulation. This is in line with a number of

other investigations. Takeda et al. have shown that mast-cell-deficient mice or WT littermates develop a comparable increase in both airway eosinophil numbers and airway responsiveness when sensitized and exposed to OA (26). Similarly, Mehlhop et al. reported that in IgE knockout mice sensitization and exposure to *Aspergillus* extracts induced a comparable degree of airway inflammation and methacholine responsiveness to WT animals (27). Notably, the baseline degree of airway responsiveness is higher in IgE knockout mice than in their WT littermates. We made similar observations in IL-4 knockout mice (22). Finally, with B-cell-deficient mice, it was shown that the absence of any immunoglobulin production does not prevent development of allergic airway inflammation (28, 29). However, in these studies, *in vivo* airway responsiveness was not measured. These observations generally indicate that the IgE/mast-cell axis does not play a major role in the induction of allergen-induced airway inflammation and BHR. These experiments do not allow us to exclude a possible modulatory role of IgE by enhancing T-B-cell interaction (30, 31).

At the same time, this illustrates the central role of T cells in these models. Further studies with different approaches, including pretreatment with anti-CD4 antibodies and passive transfer of cells, confirmed the functional importance of CD4 cells and, more specifically, Th2 cells (32–36). Rankin et al. showed that lung-specific expression of IL-4 in transgenic mice causes epithelial hypertrophy and some degree of peribronchial inflammatory changes. However, this was not accompanied by increased airway responsiveness, suggesting that IL-4 in itself is insufficient to induce all the changes characteristic of asthma, and that other Th2-derived cytokines are also involved (37). An obvious candidate in this respect is IL-5, which, by causing eosinophil influx into the airway mucosa, would lead to airway hyperresponsiveness (38). Indeed, Foster et al. have shown that OA sensitization and exposure in IL-5 knockout mice of the C57Bl/6 strain do not cause BHR as opposed to WT mice, and that the eosinophil influx can be restored by reconstituting IL-5 production (39). In a subsequent study, they showed that passive transfer of IL-5-secreting CD4⁺ T cells from OA-sensitized WT animals to unsensitized IL-5 mice led to airway eosinophilia and increase in airway responsiveness when these animals were exposed to aerosolized OA (34). They also claimed that IL-4 is not essential in C57Bl/6 mice for the induction of IL-5-producing cells, as OA exposure induced a similar increase in airway responsiveness in WT and IL-4 knockout mice with similar eosinophil infiltration around the bronchi. The OA-induced

airway changes were inhibited by pretreatment with anti-CD4 or anti-IL-5 (40).

These data seem somewhat divergent from our own data. At least in part, this could be attributed to differences in the methodology used, again illustrating the difficulty in directly comparing results from different groups. In contrast to our model, Foster et al. used boosters with alum. It has been shown that IL-4-deficient mice treated with alum, although not producing IgE, continue to produce IL-5 (41), a fact which could explain the apparent discrepancies observed between both models. In addition, experiments using other antigen sources (parasites) or other mouse strains (mainly Balb/c mice) illustrate that, although IL-5 seems to be crucial to induce eosinophil infiltration into the airways, modulation of airway responsiveness can occur independently of IL-5 and airway eosinophilia (36, 42–44).

IL-4 and IL-5 are not the only cytokines produced by Th2 cells that can influence airway responsiveness. In a very elegant study, Cohn et al. recently reported in an OA T-cell receptor transgene model that passive transfer of Th2 cells from IL-4 knockout mice can induce airway hyperresponsiveness and pulmonary eosinophil infiltration upon OA exposure in the recipient mice. The passively transferred T cells produced high levels of not only IL-5, but also other TH₂ cytokines, including IL-13 (35). It has recently been shown that IL-13 contributes substantially to allergen-induced airway changes, independently of IL-4, through interaction with the α subunit of the IL-4 receptor (45, 46). IL-9 is yet another cytokine that could mediate Th2-induced effects on airway responsiveness (47). Lung selective expression of IL-9 in transgenic animals has been shown to induce airway hyperresponsiveness, in addition to morphologic changes similar to those in asthma (48). The overall message that emerges from these various lines of experiments is that T cells can alter airway responsiveness in their own right, most probably through production of a cytokine cocktail, the composition of which can vary under different experimental conditions.

Investigation of the mechanisms underlying cell trafficking

The selective recruitment of inflammatory cells from the bloodstream into airway tissue is a complex phenomenon that involves the contribution of a variety of molecules including chemokines and adhesion molecules. The exact functional importance of each of the ever-increasing number of the different chemokine families and their receptors is

largely unknown (49). To date, the β -chemokine family has mainly been evaluated in models of allergic airway inflammation, as this family is known to have chemotactic activity toward eosinophils. The results available so far point toward a sequential role of several chemokines in the allergen-induced recruitment of eosinophils into the tissue (50–52). However, this area clearly needs further study. Similarly, the contribution of the various adhesion molecules to cell recruitment and altered airway behavior also remains to be elucidated. Current evidence suggests that despite a compensatory upregulation of VCAM-1, allergen-induced eosinophil recruitment and development of airway hyperresponsiveness are largely reduced in ICAM-1-deficient mice (53, 54). However, this effect could be at least partly attributed to deficient T-cell development, as ICAM-1 is one of the cofactors involved in T-cell activation.

Investigation of the functional role of immunomodulatory cytokines

The development of Th2 cells as opposed to Th1 cells during the primary immune response is also known to be influenced by, among other factors (55), the surrounding cytokine milieu. Whereas IL-4 stimulates Th2 cell development, IL-12 and, to a lesser extent, IFN- γ favor development of Th1 cells (56). Initial studies have shown that exogenous administration of IL-12 during the primary antigen presentation suppresses allergen-induced Th2-cell development (57, 58), thus inhibiting IgE production, airway eosinophilia, and increased responsiveness. Concordantly, airway inflammation has been shown to be enhanced in IL-12 receptor (p40)-deficient mice (59). Interestingly, IL-12 also retains the capacity to inhibit allergen-induced eosinophilia and airway hyperresponsiveness, even when administered only during secondary antigen exposure (57, 60). It has been hypothesized that this effect could be mediated through the secondary release of immunomodulatory cytokines such as IL-10 and IFN- γ . Data obtained with exogenously administered IL-10 and experiments in our model using IL-10 knockouts illustrate that IL-10 inhibits allergen-induced eosinophil infiltration and BHR (61, 62). Similarly, administration of exogenous IFN- γ prevents the development of airway eosinophilia and hyperresponsiveness after allergen

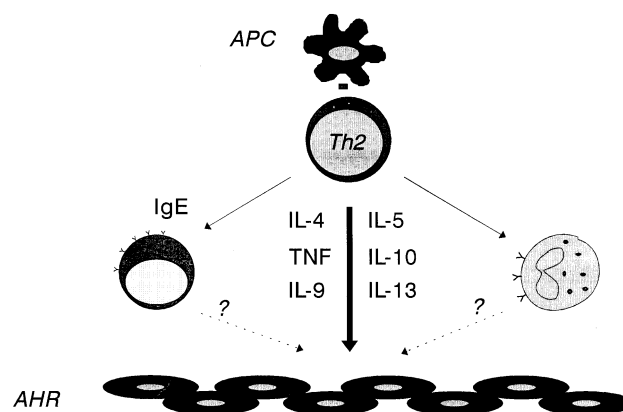


Figure 1. Central role of Th2 cells in pathophysiology of airway hyperresponsiveness.

exposure (63, 64). Others have shown that IFN- γ receptor knockout mice develop prolonged airway eosinophilia in response to allergen (65). However, we have shown that IL-12 retains its immunomodulatory effect when given during secondary antigen exposure in IFN- γ receptor-deficient mice, indicating that these effects are not due to the endogenous release of IFN- γ (66). Whether IL-12 mediates these effects through the secondary release of IL-10 remains to be further evaluated.

Conclusion

From the currently available gene knockout models of asthma, it emerges that T cells themselves are the main determining factor establishing the degree of airway responsiveness, and that altering the Th1/Th2 cell balance, irrespective of exogenous factors, does have an effect on airway behavior (Fig. 1). This concept is further illustrated by the studies of de Sanctis et al. showing that the transfer of T cells from mice with a different genetically determined baseline airway responsiveness can alter methacholine responsiveness in recipient strains, without causing notable inflammatory airway changes (67). It would appear that neither the influx of eosinophils nor the production of IgE causes BHR. It remains to be fully established to what extent these two factors might modulate the T-cell-determined degree of airway responsiveness in these models.

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