

Allergic asthma in mice: what determines the phenotype?

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ASTHMA IS A COMPLEX DISEASE, and its etiology is multifactorial. Both genetic and environmental factors are known to play a role in its pathogenesis. Our understanding of the mechanisms underlying the asthmatic response in the human stems to a large extent from investigations of animal models of the disease (2, 5, 8, 9, 11, 16). Several features of the mouse model that make it suitable for investigations into the pathophysiology and immunology of allergic asthma are outlined in these publications. Features such as cellular infiltrations in the lungs, antigen-specific IgE production, and a predominantly Th2 type of immune response characterized by elevations in the levels of IL-4, IL-5, and IL-13 seen upon allergen sensitization and challenge are similar in the mouse and human (16). In addition, assessment of lung function with relative ease in the mouse has made this an ideal model for determining airway hyperresponsiveness, one of the hallmarks of asthma. Mice develop airway hyperresponsiveness and exhibit both an early- and late-phase response to antigen challenge (3). Understanding the molecular mechanisms of the disease has been greatly facilitated by the availability of specific reagents as well as genetically altered mice (transgenic and specific gene knockout models). In fact, identification of the T lymphocyte as a pathogenic factor in asthma came from studies of the mouse model (12). Several recent studies have been carried out using different mouse models and strains to understand the etiology and pathogenesis of asthma.

To determine the contribution of genetic factors to the inflammatory response and airway hyperresponsiveness, in one of this issue's current articles in focus (Ref. 15a, see p. L32), Whitehead et al. have used nine genetically diverse inbred strains of mice. Airway responsiveness (measured as enhanced pause, Penh) to aerosolized methacholine is measured using whole body plethysmography. In an attempt to determine the dynamics of the disease process, the physiological and inflammatory responses in these genetically diverse inbred mouse strains are examined at different time points following aerosol allergen exposure. The results demonstrate differences in the temporal profile of development of allergen-induced airway disease in the different mouse strains. Airway responsiveness to 10 mg/ml of methacholine after sensitization, but before aerosol challenge, showed clear strain-dependent dif-

ferences. None of the mouse strains exhibited airway hyperresponsiveness to methacholine challenge after allergen sensitization. However, after sensitization and aerosol exposure to the allergen, airway hyperresponsiveness was significant in five of the strains, whereas in four of the strains there was no difference from nonsensitized controls. Although all nine strains exhibited increased cellular responses in the lungs after sensitization and allergen challenge, airway eosinophilia was most pronounced in the C57BL/6J strain, which did not exhibit airway hyperresponsiveness. Examination of the Th2 cytokine profile after allergen sensitization and aerosol challenge also demonstrated some strain differences. Elevated IL-4 levels in the whole lung lavage fluid were noted in six of the mouse strains, with peak levels occurring 24 h postantigen challenge and preceding the maximum eosinophil infiltration. The authors demonstrated elevated levels of IL-5 and eosinophil infiltration in most, but not all, strains. However, the correlation between temporal changes in IL-5 levels and airway eosinophilia was not significant. Mouse strains exhibiting the highest eosinophil infiltration after allergen exposure also had the highest IL-13 levels in the whole lung lavage, suggesting a role for this Th2 cytokine in airway inflammation. This is a comprehensive study involving nine different inbred mouse strains commonly used by various investigators for studies of allergic airway disease.

Although important mechanistic data have been derived from studies of murine allergic asthma models, several factors that potentially contribute to the contradictory results reported in the literature have been recognized. Genetics is an important factor in this regard and can be reflected in the differences in immune reaction to allergen, inflammatory response to allergen sensitization and challenge, and the profile of cytokine production. The strain dependence of outcome of allergen challenge in mice described by Whitehead et al. (15a) can also be influenced by other factors. These include variations in allergen sensitization and challenge protocols used by different laboratories, use of different methods to assess the responses, i.e., physiological, cellular, and antibody to allergen, and the time points of assessment of these changes.

Influence of Allergen Sensitization and Challenge Protocols

Antigen sensitization and challenge protocols used in different studies have been shown to result in varying magnitudes of airway hyperresponsiveness, cellular changes, and the IgE response. A common method of inducing allergic asthma in mice is to sensitize the

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animals on two successive occasions, followed by aerosol challenge with the antigen. This primary allergen challenge results in an asthmatic phenotype. However, to more closely resemble the human disease, secondary allergen challenge, after a prolonged gap, is often used. In a recent study in mice sensitized with ovalbumin and subjected to both primary and secondary aerosol antigen challenge, the investigators demonstrated changes in airway hyperresponsiveness characterized by significant increases in airway resistance and decreases in dynamic compliance to methacholine challenge (6). The mice also exhibited increased numbers of eosinophils, neutrophils, and lymphocytes in the lung compared with nonsensitized controls. However, there appears to be differences in the degree of cellular infiltration between the primary and secondary challenge models. It would be interesting to compare different inbred mouse strains for airway hyperresponsiveness and the nature of cellular infiltration following primary and secondary allergen challenge.

Route and Dose of Allergen Exposure in the Development of the Asthmatic Phenotype

Route of allergen exposure also determines the phenotype of the allergic response in mice. For example, in mice sensitized by systemic antigen exposure with adjuvant, depletion of IgE had no significant protective effect on lung inflammation or pathology (15). On the contrary, in mice sensitized by aerosol exposure to antigen, depletion of IgE significantly reduced lung eosinophilic inflammation and pathology. Similar observations have been made in other studies in which aerosol route of allergen administration results in airway hyperresponsiveness with no clear evidence of airway inflammation (9).

The method of sensitization and challenge and the dose of antigen used also influence the development of the asthmatic phenotype in mice. In this regard, Temelkovski et al. (14) showed that when sensitized mice are exposed by inhalation to carefully controlled mass concentrations of aerosolized antigen over an 8-wk period, the animals developed airway-specific acute-on-chronic inflammation, airway remodeling, and airway hyperresponsiveness, with no evidence of parenchymal inflammation. This chronic antigen challenge recruits eosinophils into the airways more rapidly after antigen challenge than the short-term protocol of sensitization used by many investigators. It also appears from a recent report that C57BL/6 mice do not exhibit any airway lesions or airway hyperresponsiveness to chronic low-level exposure to aerosolized antigen (8). This is in contrast to observations in the same strain in response to short-term, high-level exposure to antigen.

Relationship of Cellular Infiltrates to the Asthmatic Phenotype

An important factor contributing to strain dependence of airway hyperresponsiveness is localization of eosinophils in the lungs (13). For example, in a model of primary allergen challenge after sensitization, these

authors have demonstrated accumulation of eosinophils predominantly in the peribronchial and peripheral lung tissue in BALB/c mice. In the C57BL/6 mice, accumulation is much more diffuse in the peripheral lung tissue. This difference in eosinophil localization may account for variations in other features of asthma. For example, the antibody responses and airway responsiveness to methacholine in BALB/c mice are significantly higher than in C57BL/6 mice (13).

Cholinergic responsiveness of the airways after allergen sensitization in mice is genetically determined (2). The results of the present study by Whitehead et al. (15a) also demonstrate strain dependence of cholinergic responsiveness. Furthermore, in mouse strains such as C57BL/6 that respond poorly to methacholine, T lymphocytes from the hyperresponder A/J strain can confer increased methacholine responsiveness (4). This suggests that differential recruitment of T lymphocytes into the airways and/or consequent changes may account for the differential methacholine responsiveness. There is evidence that in inbred mouse strains, there is significant genetic variability in the development of airway responsiveness, eosinophil recruitment into the airways, and elevation of antigen-specific IgE levels (2). In the different mouse strains subjected to repeated aerosol allergen exposure after sensitization, there is a strong association between airway hyperresponsiveness and degree of bronchoalveolar lavage and lung tissue eosinophilia. However, a similar association between airway hyperresponsiveness and antigen-specific IgE levels was not seen. The finding that serum IgE levels measured after aerosol allergen exposure varied over a broad range, from barely detectable to very high concentrations, in the different inbred strains, supports this conclusion.

Assessment of Airway Function: Penh vs. Airway Resistance and Dynamic Compliance

The methods used to assess airway reactivity to bronchoconstrictor agents may have an impact on the outcome of studies involving allergen-sensitized mice models of asthma. In this context, assessment of airway function in mice has been greatly facilitated by the introduction of whole body plethysmographic methods involving unrestrained animals. This unrestrained whole body plethysmography allows for rapid screening of large numbers of mice and provides a measure of airway resistance (R_L) known as Penh. In a recent review, Lundblad et al. (10) provide a detailed reevaluation of the validity of unrestrained whole body plethysmography in mice in the assessment of bronchial responsiveness. More importantly, the authors describe the criteria that must be met for pressure changes within the chamber to accurately reflect changes in R_L . The conclusion of this elegant study is that unrestrained plethysmography can be used to characterize changes in R_L if the effects of gas conditioning are eliminated and both functional residual capacity and tidal volume are also measured. The chamber gas preconditioned to body temperature and

humidity appears to greatly reduce changes in gas pressure within the box.

An alternative method to assess airway reactivity involves the use of mice anesthetized, tracheostomized, mechanically ventilated, and challenged with aerosolized bronchoconstrictor agents. With this approach, it is possible to obtain measures of R_L and dynamic compliance (C_{dyn}). This method provides a direct and quantitative assessment of lung function. Depending on the method used to assess airway reactivity, the results obtained even within a given mouse strain may be different. For example, using Penh as readout of airway reactivity, Whitehead et al. (15a) demonstrate little, if any, hyperresponsiveness in C57BL/6J mice. With a similar sensitization protocol, other investigators have shown airway hyperresponsiveness in this mouse strain by measuring R_L and C_{dyn} (1, 7). Therefore, mouse strain differences in airway hyperresponsiveness after allergen sensitization and challenge must be interpreted in the context of the method used to assess lung function.

In conclusion, in models of mouse allergic asthma, several factors can influence the phenotype, including genetic factors. The strain-dependent differences in the temporal profile of airway hyperresponsiveness and inflammation that Whitehead et al. (15a) have reported can be used in future studies to delineate the genetics of allergic asthma in mice and perhaps in humans. The study also underscores the necessity to standardize allergen sensitization and challenge protocols, inclusion of different postallergen challenge time points, and inclusion of multiple genetically inbred strains of mice in studies of allergic airway disease. Finally, assessment of airway hyperresponsiveness to inhaled bronchoconstrictors in different mouse inbred strains should be carried out using unrestrained plethysmography as well as by direct measurement of R_L and C_{dyn} . Inclusion of different sensitization and challenge protocols in these studies is also required.

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