

The high and lows of type 2 asthma and mouse models

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Asthma is a severe disease affecting 5% to 10% of the population of many developed countries. However, the term “asthma” does not describe a single illness but rather covers a highly heterogeneous disease of the airways. Asthma therefore encompasses a range of disease phenotypes (clinical, biological, and physiological characteristics) and endotypes (cellular and molecular pathophysiology).¹ The immune dysregulation and airway inflammation that underlies the development of aberrant lung function in patients with asthma may lead to endotypes across a continuous spectrum of immune responses. The in-depth immunologic characterization of patients as well as the advent of biologics targeting type 2 (T2) cytokines has facilitated stratification of some patients with asthma into at least 2 broad endotypes; patients with a high T2 inflammatory response and others with only low or no T2 inflammation (non-T2) (Fig 1).² In the T2 asthma endotype, patients have CD4⁺ T_H cells producing IL-4, IL-5, and IL-13, elevated IgE, airway eosinophilia, and mucus overproduction leading to bronchial hyperresponsiveness.³ The activation of bronchial epithelial cells can release alarmins, such as thymic stromal lymphopoietin, IL-25, and IL-33, to instigate innate circuits via dendritic cells and innate lymphoid cells, promoting the differentiation of adaptive T_H2 cells and production of T2 cytokines. This prevailing T2 paradigm of asthma was supported by certain mouse models that defined critical roles for T_H2 cytokines in the development of IgE, eosinophilia, mucus production as well as airway changes. However, the T2 endotype is observed in only half the patients with asthma and in less than 40% of patients with severe asthma. The non-T2 endotype is typically characterized by the absence of markers of T2 disease, neutrophilic inflammation, and unresponsiveness to corticosteroid therapy. Indeed, in approximately 50% of patients with severe asthma, IFN- γ -producing T_H1 cells are observed. Furthermore, T_H17 cells also play a critical role in non-T2 asthma, with increased levels of IL-17A and IL-17F in the lungs of patients with severe asthma associated with neutrophil

recruitment. Importantly, the chronicity and dynamics of asthma is such that the predominant immune cell population may change over time and therefore patients can move within the spectrum of immune responses, which is simplified here to 2 opposing T2 endotypes (Fig 1. A).

Although the T2 endotype has been studied in detail, in part as alluded to above, facilitated by using mouse models, the underlying mechanisms of the non-T2 endotype remain obscure. In this issue, the Lloyd research group at the Imperial College London sheds some light into the underpinnings of non-T2 airway inflammation using a mouse model.² Branchett et al² describe that IL-10 from effector T cells acts on CD11c⁺ myeloid cells via the IL-10 receptor. These IL-10-activated myeloid cells then suppress exacerbated atypical IFN- γ responses to inhaled house dust mite (HDM) antigens in mice. In their disease model, repeated airway exposure to HDM led to the development of a mixed T1/T2/T17 immune response and IL-10 production from effector T cells. By targeted deletion of IL-10 in T cells, a pathogenic IFN- γ response developed, leading to aggravated airway inflammation. They further identified CD11c⁺ myeloid cells to be the targets of IL-10 activity when they ablated the receptor for IL-10 in CD11c⁺ cells and observed increased damage to the airway epithelium. At first, these results may seem at odds with the dogma of the predominant T2 endotype of asthma that is modeled by HDM challenge in mice, but, at closer inspection, the study highlights the ranges of responses that may influence the genesis of a T2 or non-T2 endotype.

Although mice are widely used experimental models in allergy research, they do not spontaneously develop “asthma.” Thus, a number of experimental approaches have been developed in mice to induce pulmonary allergen sensitization, lung-specific inflammation, and airway hyperreactivity (AHR) as models of allergic airway disease (Fig 1, B). A range of clinically relevant allergens have been used in mice to induce allergic airway disease, derived from fungi (*Alternaria alternata*, *Aspergillus fumigatus*), helminth parasites (*Ascaris*, *Schistosoma*), plants (ragweed), food (chicken ovalbumin [OVA]), and environmental cockroach and HDM extracts. In animal models, the most commonly used allergens are OVA and HDM. OVA was widely used, in part because it is readily commercially available and relatively inexpensive, to induce marked T_H2 airway inflammation and AHR. The OVA model is reliant on the use of aluminum hydroxide as a T_H2-skewing adjuvant and requires mice to be first systemically sensitized before the lung challenge to elicit frank T_H2 lung inflammation. The demonstration that administration of OVA to mice with LPS removed, or exogenous LPS added, modulated the genesis of airway T_H1 versus T_H2 inflammation highlights the physiological importance of microbial stimuli associated with allergens as observed in patients with asthma.⁴ In addition, when the airways are directly sensitized with OVA plus LPS, a marked T_H17 response and modest T_H2 responses are elicited in lungs, leading to neutrophilia and AHR.⁵

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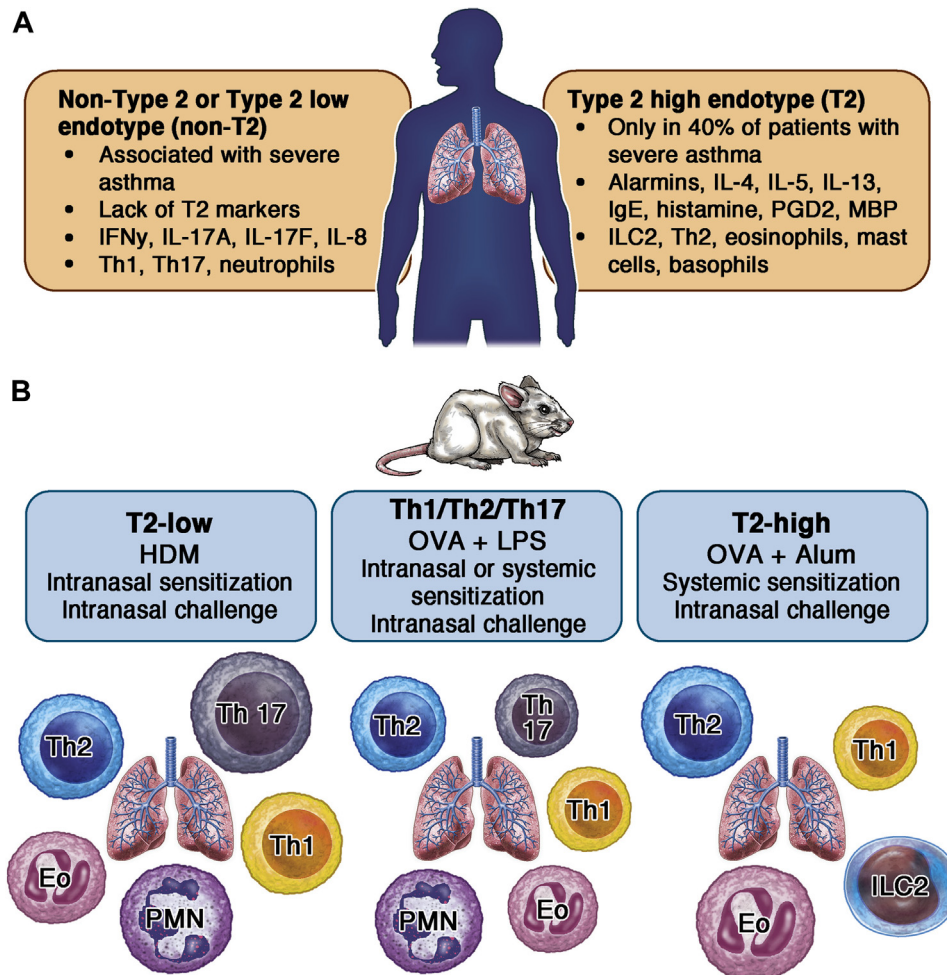


FIG 1. A, Schematic of asthma in man reduced to a simplified T2 and non-T2 endotype. **B**, The generation of allergic airway disease in different mouse models. In the mouse models, distinct pulmonary immune cellular response is evoked. The T2-low HDM mouse model is as described by Branchett et al.² ILC2, Type 2 innate lymphoid cell; MBP, major basic protein; PGD2, prostaglandin D2; PMN, polymorphonuclear neutrophils.

Recently, HDM became more widely used to induce AHR in mouse models because it has immunogenic properties—hence there is no need for the use of an adjuvant. The intrinsic enzymatic properties (eg, peptidases and proteases) of HDM reflect a shared characteristic of many clinically relevant allergens. These enzymes lead to immune activation through degradation of extracellular matrix or direct interference with immune effector molecules, thus promoting a T_H2 -polarized response. Importantly, the inherent endotoxin “contamination” in HDM preparations is also a natural adjuvant, which makes it even more clinically relevant because HDM sensitization in humans occurs in nonsterile environments. Indeed, in acute HDM exposure, single-cell sequencing of CD4 cells in bronchoalveolar lavage revealed a distinct T_H2 profile accompanied by a significant proportion of non- T_H2 responders and a T_H2 -like cell population responding to type I IFNs in a TLR4- and MyD88-dependent manner.⁶ As exemplified in the Lloyd study,² the levels of endotoxin in HDM used in mouse studies should always be assessed and stated in publications to promote reproducibility. Further studies are required to dissect the relative contribution of the enzymatic and endotoxin

properties of HDM in stimulating allergic inflammation in the lungs of mice.

In the study by Branchett et al.,² the experimental approach to establish a T2-low mouse model of allergic airway disease is notable. Here, the authors applied HDM intranasally, 5 times per week for 3 weeks, leading to a pulmonary $T_H17 > T_H2 > T_H1$ response with lung eosinophilia and some neutrophil infiltration leading to AHR.² In contrast to systemic sensitization, immune activation originates in the lung factoring in the pulmonary antigen-presenting cell network. Especially in light of the ever-expanding universe of myeloid antigen-presenting cell subpopulations and the unique tissue oxygen environment of the lung, the lung sensitization approach may prove clinically significant and warrants further study. Their findings regarding the mechanism regulating airway inflammation provides important insights into potential therapeutic options: Although limiting IL-10 signaling reduced airway eosinophilia, overall disease was not ameliorated but shifted toward the T2-low endotype. Antagonizing the recruitment of inflammatory effectors via CXCR3 and its ligands CXCL9 and CXCL10 (both increased in the T2-low endotype) may prove more successful in patients with non-T2

asthma. The authors' choice of mice on a C57BL/6 background is also noteworthy. Traditionally, mice on a BALB/c background have been preferred in allergy research because these strains are more prone to a T_H2-biased immune response. The sex of mice also plays a critical role for the outcome of the modeled disease. Although the authors herein commendably used comparable proportions of male and female mice for all transgenic experiments to rule out sex-specific artifacts,² the use of a single sex of mice can damage translatability. In humans, there are distinct disease characteristics in male and females with asthma. Furthermore, male mice have fewer mature T2 innate lymphoid cells and develop less severe airway inflammation in response to HDM compared with female mice.⁷

The inclusion of specific details on the mouse models, such as mouse strain and sex as addressed above, used in allergy research must be included in publications. In this regard, *The Journal of Allergy and Clinical Immunology* has always taken the lead, setting criteria for mouse allergic airway models for authors to adhere.⁸ This is exemplified by Finkelman's formal definition on appropriate use of mouse pulmonary function tests: "that measurement of airway responsiveness by unrestrained, single-chamber barometric plethysmography (the Penh method) must be confirmed by invasive techniques."⁹ The asthma community has recently paused and reflected toward defining the correct nomenclature toward guidelines for the future.¹⁰ It seems timely for researchers using allergic airway mouse models also to implement and adopt new standards. Some relatively innocuous changes by authors are warranted to help readers grasp the experimental basis for the studies' findings. In the article title "mouse" or "mice" should be formally stated if conclusions were drawn from animal

experiments. Furthermore, mouse models of "asthma" need to be described as "allergic airway disease" models. Only the strict adherence to reporting guidelines and experimental rigor will allow us to translate preclinical findings from mice into practice. Finally, it should always be remembered when using animal experimental models that "Man is a bad model of the mouse."

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