

Allergy Series: Junior Member Affiliates Forum

Mouse models of global airway allergy: what have we learned and what should we do next?

Recent epidemiological and clinical data indicate that allergic rhinitis and asthma coexist and should be considered as one airway allergy syndrome. In spite of the importance of this new concept of global airway allergy, it has not fundamentally changed our daily diagnostic and therapeutic strategies so far because of the lack of essential clues to understand the correlation between allergic inflammation in upper and lower airways. Because of the resemblance of experimentally induced allergic airway inflammation in mice to inflamed airways of allergic patients, mouse models can enhance our insight into mechanisms underlying the global airway allergy syndrome. We here review data generated in mice that are relevant for understanding the development of airway allergy and provide new options for research on the so-called 'united airway disease'.

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Allergen inhalation in sensitized patients initiates an immunoglobulin (Ig)E-mediated inflammatory cascade in the airways, characterized by the influx of Th2 lymphocytes and eosinophils. The clinical manifestation of this response primarily in the nose, bronchi or both, has led to diagnostic and therapeutic strategies focusing primarily on upper or lower airway disease in isolation. However, recent epidemiologic and clinical studies have illustrated that allergic inflammation in the nose and bronchi of sensitized patients should be seen as a continuum of airway inflammation and that the clinical dichotomy between allergic rhinitis and asthma is arbitrary (1). Indeed, both allergic airway disorders share the same pathophysiology and increasing prevalence, and respond to similar treatment strategies. As a consequence of these insights, the concept of global airway allergy with nasal and/or bronchial symptoms is now becoming more and more accepted as a dogma, and resulted in the publication of the ARIA document in 2001 (2). However, many clues to fully understand the interaction between nasal and bronchial inflammation are missing. Indeed, the endogenous and/or exogenous factors involved in the progression from sensitization to allergic disease in nose and/or bronchi are not fully characterized, and hence prevention of the so-called *allergic march* on an individual base is not possible. In addition, it still remains unclear why some sensitized patients present with mainly upper and others with mainly lower airway symptoms. It is not known to what extent and via which mechanisms the inflammatory response in the nose contributes to lower

airway inflammation and vice versa. The source of the systemically released interleukin (IL)-5 after allergen inhalation, and the relative contribution of bone marrow eosinopoiesis, influx of eosinophils into the airway mucosa and differentiation of CD34⁺ eosinophilic progenitors in airway tissue to the chronic airway eosinophilia, remain speculative. It also remains a matter of debate what beneficial effects nasal therapy may have on lower airway inflammation and vice versa. Taking these considerations into account, unraveling the interaction between inflammation in the nose and bronchi is mandatory in order to provide a better diagnostic and therapeutic approach for sensitized patients with airway symptoms.

Beside clinical and *in vitro* studies, mouse models of allergic airway disease may provide us with useful information concerning upper and lower airway inflammation. Indeed, experimental asthma in mice largely mimics human asthma and allows us to dissect immunologic processes *in vivo*. For instance, the causal link between systemic IL-5 and eosinophil recruitment toward the airways (3), or the primary role of IL-13 in the phenotype of allergic airway inflammation (4), have been first highlighted in mice. Moreover, mice are attractive tools for immunologic research purposes because of their well-characterized immune system, the availability of research products and transgenic mice, and the advantage of measuring bronchial tone for evaluation of bronchial hyperresponsiveness. Finally, research in mice allows us to perform relatively invasive experimental procedures,

e.g. bronchial biopsy, bone marrow puncture and analysis of peribronchial lymph nodes, which is less feasible in patients.

For induction of experimental airway allergy, different experimental protocols are being applied by different research groups. Most frequently, a biphasic protocol consisting of a systemic sensitization phase followed by an airway challenge phase, is used for the induction of allergen-specific IgE production, bronchial eosinophilia and hyperresponsiveness in mice (5). In the more 'chronic' mouse models, in which the airways of sensitized mice are repeatedly exposed to the allergen, mucus hypersecretion, epithelial cell hyperplasia and thickening of the basement membrane are found in the bronchi in response to repeated airway challenges with the allergen (6). In this way, most hallmarks of human asthma are induced in mice. Evaluation of upper airway inflammation in mice has been made possible by means of studying nasal lavage fluids, histologic examination and radiologic imaging of the nasal mucosa (7, 8). Studies on experimental allergic rhinitis have shown that the inflammatory response in the nose largely resembles human allergic rhinitis, is dependent on the functional interaction of CD28-CTLA-4 molecules on T lymphocytes with B7 molecules on monocytes (9), correlates with the presence of IL-5 (10, 11), and can occur in the absence of IgE (8). It is interesting to mention here that, beside systemic sensitization, intranasal applications of OVA could also induce allergen-specific IgE production and eosinophilic infiltration in murine bronchi, even though the distribution of the allergen particles appeared to be only in the nares without reaching the bronchi (12).

So far, research in mice has mainly been focusing on upper or lower airway allergy in isolation, without investigating upper airway inflammation in the context of the lower airways. We here review data generated in a mouse model of global airway allergy and discuss them in the light of the concept of global airway allergy.

Lessons from mouse models of global allergic airway inflammation

We recently reported that an allergic inflammatory response is induced in both upper and lower airways of mice after exposure of spontaneously breathing, OVA-sensitized animals to nebulized OVA (7). The experimental protocol used here is relevant for human asthma in that both the chronic as well as the airborne character of exposure of allergic patients to allergens is mimicked in sensitized mice. By comparing similar parameters of upper and lower airway inflammation, i.e. cellular contents and cytokine levels in lavage fluids and histologic sections, a simultaneous induction of allergic inflammation in both upper and lower airways was found in the course of repeated allergen exposures. In parallel with the induction of allergic airway inflammation, thickening of

the nasal mucosa was found on radiologic images of the murine skull and bronchial hyperresponsiveness measured by whole body plethysmography. Taken together, these findings reinforce that statement of the coexistence of allergic rhinitis in asthma and support the notion of 'one airway, one disease'.

An interesting observation in mice with experimental asthma is the fact that the severity of the inflammatory response in bronchi is more pronounced than in the nose. In the nasal mucosa of mice with experimental airway allergy, inflammatory cells are scattered in the nasal epithelial and subepithelial layers whereas dense eosinophilic infiltrates are found in the peribronchial tissue (cf. Fig. 1). Similar observations are reported in patients with asthma, where the extent of eosinophilic inflammation, the thickening of the basement membrane and the epithelial shedding is greater in bronchial than nasal tissue (13). The question that arises then is where the deposition of inhaled particles takes place. As mice are obligatory nose-breathers, there is no debate to what extent breathing through the nose or mouth may account for the observed difference in degree of inflammation in mice. In order to know the distribution of inhaled allergens, we studied the deposition of airborne particles by exposing spontaneously breathing mice to nebulized radio-actively labeled albumin particles and found that the majority of inhaled particles (83%) was deposited onto the nasal mucosa, whereas only a minority of inhaled particles reached the lower airway mucosa (13%, unpublished observation). Under certain circumstances however, higher amounts of allergens may reach the bronchi. When administered intranasally in mice with hyperextended neck in supine position, up to 31% of administered particles have been reported to reach the lower airways (14). In contrast, Mc Cusker et al. (12) found no allergens in the bronchi after intranasal administration, which could be explained by the fact that particles on nasal mucosae are immediately transported toward the hypopharynx by the mucociliary clearance system rather than reaching the lower airways passively via the inhalatory route (15). Whatsoever, we find that the method of intranasal allergen deposition contrasts with the more physiologic inhalatory route as used in our experiments. We should mention here that the above-mentioned experiments on distribution of inhaled particles were performed in nonsensitized mice and therefore raise the question whether inflammation in the nose may alter the distribution of inhaled particles. However, Takeuchi et al. (16) reported that there was no alteration of median total nasal airflow during the pollen season in pollen-allergic rhinitis patients. Hence, one does not expect a major shift in the distribution pattern of allergens in individuals with allergic rhinitis.

Considering the above-mentioned observations in mice, it is interesting to find that most allergic inflammation is induced in the bronchi, i.e. the place where only a minority of allergens are deposited. Therefore, we

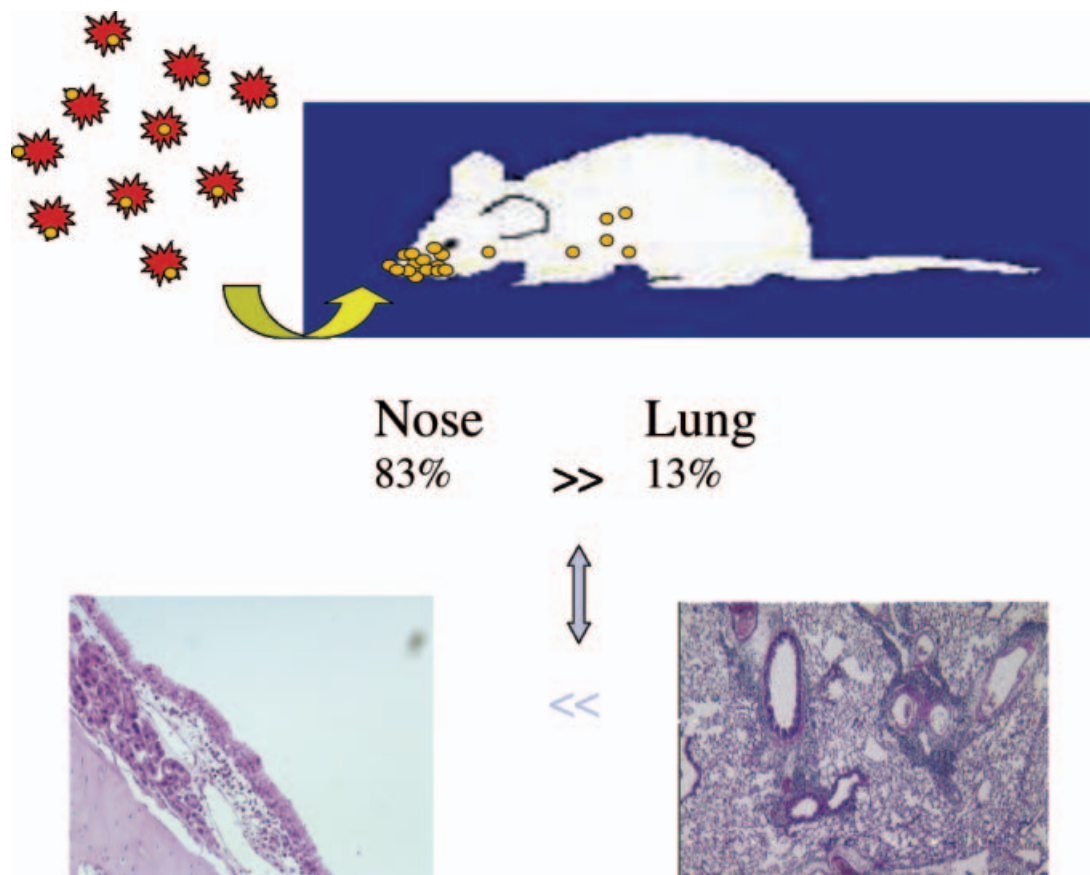


Figure 1. Inhalation of radioactively labeled albumin by spontaneously breathing mice showed that the majority of inhaled particles were deposited onto the nasal mucosa whereas only 13% reached the lower airways. This observation contrasts apparently with the finding of dense peribronchial and perivascular inflammatory infiltrates in bronchi of experimental asthma whereas the degree of inflammation in the nose is much less pronounced.

speculate that lower airways are more sensitive to minute amounts of inhaled allergen and/or that other mechanisms than allergen deposition and local initiation of the immunologic cascade, are responsible for the induction of lower airway inflammation. So far, murine data are in favor of a systemic allergic response after allergen inhalation.

Murine data that favor the concept of a systemic allergic response

Beside induction of airway inflammation, allergen inhalations by sensitized mice cause a boost of allergen-specific IgE production and an increase in eosinophil counts in the blood (5). This elevated eosinophilia is accompanied by an increase in the number of bone marrow eosinophil colony forming units. The stimulation of bone marrow eosinopoiesis after allergen contact occurs rapidly after mucosal allergen contact (17) and is suggestive of a systemic link between airway inflammation and bone marrow biology. At present, most studies

point toward the important role of IL-5 in bone marrow eosinopoiesis (11, 14). Apart from stimulating eosinopoiesis, systemic IL-5 appears to play, in concert with eotaxin, a crucial role in the migration of eosinophils toward the inflamed airways in allergic asthma (3, 18) and rhinitis (11). The fact that an increase in serum levels of IL-5 is found after allergen inhalation in mice (7) as well as in men (19), is an intriguing finding that links the deposition of allergens onto the airway mucosa with stimulation of bone marrow eosinopoiesis, and illustrates that IL-5 acts both in an autocrine way as well as at a distance after allergen contact.

Why are upper airways of mice are less attractive research objects than lower airways?

Most research groups working in the field of allergic airway disease concentrate on experimental asthma in mice when it comes to unraveling mechanisms of allergic inflammation *in vivo*. For several reasons, studying the lower airways in mice is more attractive than studying the

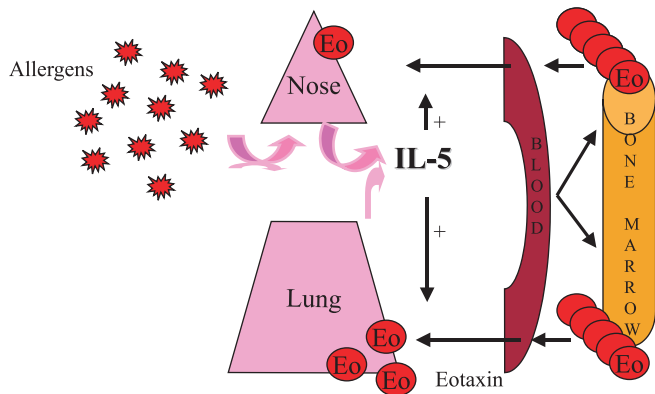


Figure 2. Scheme on series of events taking place after allergen inhalation, with systemic release of IL-5 playing a key role in both the synthesis as well as the recruitment of eosinophils towards the airways.

upper counterpart. Unlike the bronchi, the murine nose shows a baseline inflammation characterized by the presence of inflammatory cells, mainly neutrophils and monocytes, in nasal lavage fluids (7). One may thus suppose that, in spite of the housing of mice in relatively pathogen-free conditions and handling under laminar flow conditions, the baseline nasal inflammation is elicited by exposure to exogenous particles, e.g. dust in the cages, feces, and/or contact with the paw of littermates. In contrast to the nose, bronchi of these mice are in an inflammatory quiescent state without inflammatory cells in the broncho-alveolar lumen. Hence, the inflammatory response that is induced in experimental airway disease reflects a pure allergen-driven inflammation in bronchi, whereas a mixture of baseline and allergen-induced inflammation is found in the nasal cavity. It should be mentioned here that, in contrast to nasal lavages, hardly any inflammatory cell is present in the nasal mucosa under basal circumstances (Hellings P. W., Hessel E. M., van den Cord J., Kasran A., Van Hecke P., Ceuppens J. L., unpublished observation). Secondly, the eosinophilic inflammation that is observed in the nose of mice with experimental airway inflammation, is limited compared with the overt inflammatory response of the lower airways. As a consequence, the degree of inflammation in the tissue is quantified by counting individual inflammatory cells in the nasal mucosa in a defined region whereas the thickness of peri-bronchial inflammatory infiltrates is measured in lower airways. From a technical point of view, performing a nasal lavage in a mouse is more tricky and time-consuming than a broncho-alveolar lavage. Hence the inter-individual variability of cell counts of mice in one group is expected to be higher in nasal than in broncho-alveolar lavage fluids. In order to quantify the inflammatory state of the nose and bronchi *in vivo*, we measured the thickness of the nasal mucosa on magnetic resonance imaging (MRI) scans and investigated bronchial responsiveness to nebulized metacholine

using whole body plethysmography (7). When comparing these evaluations, radiologic imaging of the nasal mucosa in an especially designed MRI-scanning device is technically more demanding than measurements of bronchial hyperresponsiveness in a commercially available whole body plethysmograph (Buxco®; EMKA Technologie, Paris, France). Finally, sneezing, as used by some authors (9, 10) as a parameter of nasal inflammation *in vivo*, represents to our opinion a rather subjective parameter. Taken together, studying allergic inflammation in the upper airways of mice is not as attractive as studying lower airway inflammation.

Future options for research on global airway allergy in mice

In spite of the progress in our understanding of the pathology underlying allergic rhinitis and asthma, many clues are still missing to fully comprehend the correlation between rhinitis and asthma. Indeed, it still remains unclear in patients why sensitization to airborne allergens leads to allergic symptoms primarily in the nose, bronchi or both in affected patients. Undoubtedly, a mixture of factors like end organ sensibility, genetic and environmental factors, upper airway infections and breathing patterns, are involved in the clinical manifestation of airway allergy in patients. Future research on global airway allergy in mice could help us in answering some of these questions. In order to delineate genetic factors in the manifestation of rhinitis and asthma, it will be of interest to apply the same experimental protocol to different murine strains and study the outcome of inflammation after intranasal or intratracheal allergen application. Studying the pattern of chemokine secretion, e.g. eotaxin, regulated on activation, normal T-cell expressed and secreted (RANTES), and expression of adhesion molecules, e.g. intracellular adhesion molecule-1 and vascular cell adhesion molecules (VCAM), and growth factors like GM-CSF, in both nose and bronchi during the initiation of allergic inflammation and after allergen provocation may yield relevant information. In addition, studying the migration of allergen-specific CD4⁺ cells and/or eosinophils from the systemic circulation toward the nose and/or bronchi after allergen inhalation, will learn us where inflammatory cells preferentially home after allergen contact. Another interesting issue will be to determine the relative contribution of eosinophilic influx and differentiation of CD34⁺ cells in the tissue to the initiation and chronicity of airway eosinophilia. As a consequence of the finding of systemic release of IL-5 after allergen inhalation, it will be interesting to pinpoint the cellular source of systemic IL-5 and to study the mechanisms and location of absorption of allergens into the systemic circulation. It will also be necessary to study the influence of external factors like pollutants and/or microorganisms on the clinical phenotype of inflammation in nose and

bronchi and on the penetration of allergens deposited onto the nasal and bronchial epithelium into the mucosa. Concerning the epithelial hyperplasia in lower airways of asthmatic patients, it will be interesting to look on cellular level to differences in epithelial responses between nose and bronchi in a mouse model of global airway allergy, as these cells also represent a source of pro-inflammatory mediators (20) and may hence contribute to the overt inflammation in lower airways of asthmatic patients. In general, the role of eosinophils in the allergic phenotype still remains ill defined and can be further explored in murine models by performing experiments with granulocyte-depleted mice (21).

Another interesting question relates to the way how individuals get sensitized to inhaled allergens. Recently, McCusker et al. (12) compared intranasal vs intraperitoneal sensitization, and found that the route of administration of allergens greatly affects the characteristics of the subsequent immune response. It will be of interest to investigate how the sensitization process in the upper airway takes place, how this differs from sensitization via lower airways, and what external factors, e.g. pollution,

cigarette smoke and/or microorganisms, may influence the sensitization process. To all the above-mentioned purposes, mice are attractive research tools and will further refine our insight into mechanisms of airway allergy.

Conclusion

Murine data point to simultaneous induction of allergic inflammation in the whole airway, in parallel with systemic cytokine release and bone marrow changes after allergen inhalation. Hence, murine data reinforce epidemiological and experimental evidence for the existence of global airway allergy in patients with allergic airway inflammation. In spite of the disadvantages of studying upper airway inflammation in mice, ongoing studies on the correlation between upper and lower airway inflammation in mice will yield information that is relevant for understanding the phenotype of airway allergy and will ultimately lead to optimize both diagnostic and therapeutic strategies for affected patients.

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