REVIEW ARTICLE

Use of the Mouse to Unravel Allergic Asthma: a Review of the Pathogenesis of Allergic Asthma in Mouse Models and Its Similarity to the Condition in Humans

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Introduction

Bronchial asthma is a respiratory disturbance caused by obstruction of the bronchi that is accompanied by chronic inflammation of the lower airways. The obstruction is due in part to bronchial hyperreactivity, a tendency towards excessive contraction of the bronchial smooth muscle in response to various stimuli. The reduction in diameter of the bronchi impairs the passage of air to the lungs, and consequently, causes acute dyspnea. Bronchial obstruction is contributed to by chronic inflammation, mucus hypersecretion, and the phenomenon of remodeling, which alters the microstructure of the affected airways. In certain individuals with a genetic predisposition, the described symptoms are largely the result of immunological and biochemical changes.

Various different etiological factors are associated with asthma. Exercise, nonsteroidal antiinflammatories, viral infections, and allergens are among the most frequent. When the asthma is caused by an allergen it is referred to as allergic, atopic, or extrinsic asthma. Although asthma of allergic and nonallergic origin share pathogenic mechanisms, it is likely that allergens generate a specific immune response, especially during the onset of the process. Consequently, and given that it is the most prevalent type of asthma, this article focuses on allergic asthma.

Considering the current prevalence (150 million affected individuals) and annual percentage increase, it is estimated that by 2010 asthma will affect 300 million people worldwide.[1,2] In 80% of affected children and adolescents, the asthma is of allergic origin, and in Spain, 40% of asthma cases in young adults (20-44 years old) have an allergic component, according to preliminary data from studies undertaken as part of the European Community Respiratory Health Survey.[3,4] In agreement with the most fashionable theory, known as the "hygiene hypothesis," this increase in the prevalence of allergic asthma, particularly apparent in developed countries, is attributed, among other causes, to sanitary improvements, such as the lower incidence of viral infections and parasitic infestations, and the incorporation of new vaccines into health programs.[5] The hygiene hypothesis arose from the observation that these improvements are accompanied by an increase in the incidence of atopic diseases, which may be attributable to changes in immunologic mechanisms.[6] Asthma is a disease that is difficult to manage pharmacologically and that, in addition to being a serious health issue, represents a major economic problem due to work absenteeism, pharmaceutical costs, and reduced productivity.[7] The difficulty associated with disease management rests on the fact that current treatment does not resolve the disease and is fundamentally aimed at counteracting episodes of bronchospasm and controlling the underlying inflammation in the chronic process, a phase of the disease that presents particular therapeutic difficulty. In addition to the limitations of treatment, there is the risk of patient death as a result of acute attacks, estimated at 18 per 1 000 000 individuals.[2] The lack of a more effective pharmacological treatment for allergic asthma is due in large part to the fact that the specific immunologic or biochemical changes that cause it remain unknown. Basic research and clinical studies in asthmatic patients are fundamental to the advancement of understanding of the pathogenesis of asthma. However, there are scientific and ethical obstacles that impede the elucidation of certain aspects of the disease. The appearance in the last decade of models of allergic asthma induced in the mouse has stimulated investigation in this field, and offers, without doubt, a valuable addition to studies in asthmatic patients, in vivo studies in other animal models, and in vitro and ex vivo experiments.

In this article, we describe the pathogenic similarities between human allergic asthma and that of models induced in the mouse; we draw attention to the

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particular usefulness of investigating asthma in murine models and present some data from the literature that support their use.

In Vivo Research Into Allergic Asthma

Research in Asthmatic Patients

Various experimental techniques have been developed to evaluate the impact of cellular and molecular changes in the course of asthma in patients. One of these is to analyze tissue samples taken from asthmatic patients in different phases of the disease. Biopsies of the bronchial mucosa are collected by bronchoscopy to evaluate the local cellular response pattern, the expression of mediators, and the possible repercussions for bronchopulmonary function.[8,9] Systemic cellular and molecular fluctuations in the blood are also evaluated, as are possible genetic polymorphisms associated with allergic asthma.[10] In addition, with the aim of collecting samples of bronchial mucosa directly exposed to an allergen, and therefore, to unify the characteristics of the tissue being studied, techniques have been developed for the local exposure (challenge) of the bronchial mucosa of asthmatic volunteers to allergens to which they are known to be hypersensitive,[11] and ex vivo studies have been performed using patient samples.[12] Similar analyses can be performed using bronchoalveolar lavage (BAL) to identify and quantitate the cells and mediators present.[11] Neither bronchial biopsy nor BAL are techniques that are free from risk for the patient, and consequently, their use carries ethical considerations when applied exclusively for experimental purposes.

With appropriate ethical restrictions, these experimental procedures have been very useful in understanding some aspects of the pathogenesis of allergic asthma. However, the following points highlight the reasons for which alternative models are required for the in vivo investigation of the disease: *a)* the difficulty of taking samples from certain organs (eg, pulmonary parenchyma or bronchioles) in asthmatic patients; *b)* the difficulty of performing multiple analyses in parallel in the same patient and of obtaining tissue samples from the respiratory system over time (temporal studies); *c)* problems associated with carrying out simultaneous cellular/molecular studies and functional studies on the respiratory system in humans; *d)* limitations on the size of the sample population when using patients; *e)* heterogeneity of the test (asthmatic) and control (nonasthmatic) populations to be evaluated; and *f)* the legal impossibility of using drugs in patients during the phase prior to clinical trials.

For all of these reasons, numerous species have been tested in which asthma has been induced through a wide variety of protocols with the aim of allowing us to address issues that cannot be answered in studies of asthmatic patients.

Animal Models of Allergic Asthma

The principal functional and pathogenic characteristics of allergic asthma have been observed in a number of animal models. The following species have been used: mouse,[13] rat,[14] guinea pig,[15] rabbit,[16] ferret,[17] dog,[18] cat,[19] sheep,[20] pig,[21] some primates,[22] and even horse.[23] Some of these species develop allergic asthma spontaneously, while in others it is experimentally induced. Of the latter cases, in addition to the mouse, the most commonly used experimental models have been the guinea pig, the sheep, and the monkey. The model of guinea pig sensitized to ovalbumin is characterized, as in human asthma, by an early acute phase and a later chronic phase following contact with the allergen, the existence of eosinophilic lung inflammation, and by bronchial hyperreactivity.[15,24] This was the most frequently used model up until the first models were described in mouse, and has been largely used to evaluate the therapeutic interest of various molecules.[15] However, it does not offer the genetic versatility of the mouse, nor does it offer as many species-specific reagents for the identification of molecular and cellular changes associated with pathogenesis.[25] The sheep model of asthma is also characterized by an early acute phase followed by a later chronic phase, both accompanied by bronchial hyperreactivity.[26] In addition, from an anatomical and functional perspective, the lungs of the sheep under normal conditions are very similar to those of humans.[20,27,28] Although the induction of asthma in sheep has generally been performed using allergens from nematodes, particularly *Ascaris suum*,[29-31] a sheep model has recently been reported that was sensitized to dust mites, an allergen that is a common cause of asthma in patients. In this study, it was shown that, in addition to the changes mentioned, the sheep develop a specific immunoglobulin (Ig) E response with intense eosinophilia.[20] Finally, it is worth mentioning that the primate model has been used for more than 25 years. Although initially animals were studied that were spontaneously sensitized to *A suum*,[32] there now exists an induced model, in which the animals are sensitized to dust mites, that reproduces practically all of the clinical and pathogenic characteristics of human asthma.[22] In spite of the scientific interest, the cost of working with sheep and primates, the length of the experiments, the difficulty of the manipulations, the lack of reagents developed for the evaluation of molecular expression, and the stricter control of their use in the laboratory compared with other species means that these models are rarely used due to their poor scientific exploitability.

For reasons that are fundamentally scientific, but also economic and based on ease of use (housing, manipulation, availability of species-specific reagents), the animal that is nowadays universally used as a model of allergic asthma is the mouse. Compared with other species, another advantage of the mouse is its ability to

be manipulated genetically (transgenics and knockouts) and immunologically (mice with spontaneous or induced immunodeficiencies).

Mouse Models of Allergic Asthma

Many experimental models of asthma induced in the mouse have been proposed. In general, the induction protocols are based on systemic sensitization through injection of an allergen to generate a memory immune response followed by local reexposure of the respiratory system to the same allergen. Sometimes, during sensitization, the allergen is administered along with a coadjuvant or a nonspecific immunopotentiator compound such as aluminum sulfate (alum) to guarantee a sufficiently strong immune response. The following section provides a brief description of some of the factors that should be taken into account in designing a protocol for the induction of allergic asthma in the mouse.

Mouse strain. Comparative studies have demonstrated that not all mouse strains respond similarly to contact with an allergen. Strain differences have been observed in the degree of bronchial hyperreactivity, the production of IgE, and even in the type of cellular immune response. These effects are probably due to genetic differences. In the most commonly used strains, it has been seen, for instance, that sensitized BALBc mice develop higher bronchial hyperreactivity,[33,34] generate 4 times more total IgE and specific IgE,[34] and contain more cells and a higher concentration of cytokines in BAL samples than do sensitized C57BL/6 mice.[33,34] Researchers generally favor those strains with a propensity to display an atopic phenotype; consequently, BALBc is among the most commonly used. Recent studies, however, propose that a chronic model in the A/J strain should be used for the evaluation of structural changes in the bronchi since it displays greater similarity to the changes observed in patients.[35]

Allergen. Of the numerous antigens used in the induction of asthma in mice, the most common has been ovalbumin. However, there is a current tendency to use aeroallergens that cause spontaneous asthma in humans. These include dust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*)[13] and pollens, in particular that of ragweed,[36] a common plant in northern Europe and North America (in Spain, the predominant pollens responsible for allergy come from grasses, masterwort, and the olive tree; to our knowledge, however, no models have been established with these allergens). Natural aeroallergens offer the advantage of being compounds suspended in the air that enter the body naturally via the respiratory airways. This is in contrast to ovalbumin, which would normally enter the body spontaneously through the alimentary canal, thereby triggering allergies that originate in the digestive system. The biotechnological identification and production of allergens allows mice to be exposed to highly purified molecules rather than allergen extracts. This is true for the dust mite, for which at least 2 components (*Der p* 1 and *Der p* 5) are known to be partly responsible for the activation of the immune response and the subsequent induction of asthma.[37] Recently, a new approach to induction has been investigated, namely the sensitization of mice to 2 recombinant antigens, one from dust mites (*Der f* 1) and the other from cockroaches (*Bla g* 2). This combination has been observed to produce greater lung inflammation and epithelial damage than either antigen provided separately.[38] In terms of the dose of allergen that should be used, the range of concentrations administered is very wide, and varies according to the phase of the induction protocol and the allergen. For systemic sensitization, amounts between 1 and 800 µg of antigen have been used, although a study comparing different doses of ovalbumin found that 10 µg was sufficient to induce asthma in mice.[39] In the challenge or activation phase, since they are normally used as aerosols, ovalbumin solutions are commonly administered at a concentration of between 1% and 5%. In contrast, using one of the principal allergens from dust mites (*Der p* 1), 1 µg is sufficient to sensitize mice.[40] Nevertheless, the concentrations of allergen to which animals are exposed are generally high, an issue that is slowly being corrected with the introduction of chronic models.

Route of administration or exposure to the allergen. In the majority of models described, the sensitization of the animal is achieved intraperitoneally, while reexposure to the antigen (activation or challenge) is normally performed intranasally, either by direct injection into the nostrils or by nebulization.[41,42] The most recent and most refined protocols propose that both phases should use the airways, in an effort to imitate the normal process of exposure in asthmatic patients.[13,36]

Duration of asthma induction in mice. The induction of allergic asthma in mice requires between days and months, depending on the protocol. Models of short duration (or short exposure to the allergen) have principally been used in which animals are exposed to high concentrations of allergen over a relatively short period (between days and weeks), giving rise to the so called "acute asthma model." Short exposure or acute models do not reproduce some aspects of allergic asthma that are observed in chronic asthmatics. This shortcoming led to the development of long exposure or chronic models. In efforts to induce models of chronic asthma with ovalbumin, it was observed that nebulization of the allergen over long periods without prior systemic sensitization led to immunologic tolerance; in other words, the immune system stopped responding to ovalbumin after successive exposures.[43,44] This phenomenon of tolerance in murine models of asthma would suggest that the immune response of the respiratory mucosa is self-limiting, and that this may be one of the mechanisms used by the immune system to

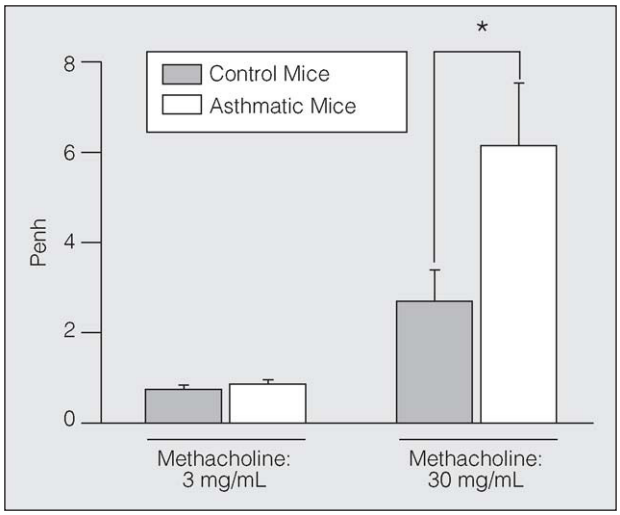

Figure 1. Bronchial reactivity in mice with asthma induced by dust mite extract and control animals (nonasthmatic). Measurements were made using a plethysmograph (Buxco Europe Ltd, Winchester, UK) in conscious animals after airway exposure to 2 concentrations of the bronchoconstrictor methacholine. At the lower concentration of methacholine (3 mg/mL) the bronchial reactivity in asthmatic mice is the same as that of controls, while at the higher concentration (30 mg/mL), the asthmatic mice display bronchial hyperreactivity. *P<.05.

prevent the progression of allergic inflammation in the lung; consequently, patients with chronic asthma would have had a prior defect in the regulation of this process.[45] In other experiments, it was observed that prolonged administration of ovalbumin to sensitized animals did not succeed in maintaining "asthmatic characteristics," and resulted in a reduction of either lung inflammation,[46] or bronchial hyperreactivity.[42,47] In addition, models in which there is no distinction between a sensitization phase and a phase of activation or challenge have begun to be developed based on continued exposure to low doses of aeroallergens. Most notable is the chronic model of allergic asthma to dust mite extracts recently described by Johnson et al,[13] the results of which are very encouraging because they reveal characteristics of chronic asthma that are not observed in acute models, such as the sustained remodeling of the tissues subsequent to inflammation. This similarity may also be due to the fact that the induction protocol is in many ways similar to the natural exposure to allergens.

The choice of whether to use a model of allergic asthma that is based on long or short exposure to the allergen will depend upon what we want to study. Thus, models of acute asthma offer practical advantages for the investigation of the mechanisms of the inflammatory process and bronchospasm, whereas chronic models allow the analysis of long-term structural changes and their influence on bronchial obstruction.

Clinical and Pathophysiological Characterization of Allergic Asthma in the Mouse

The models of induced allergic asthma in the mouse are generally characterized by the existence of bronchial hyperreactivity, bronchopulmonary inflammation, increased serum concentration of IgE, and mucus hypersecretion. In addition, chronic models present structural changes of the respiratory airways. Below, we review these characteristics and the similarities between the diseases in the 2 species.

Bronchial Hyperreactivity and Bronchospasm

Bronchospasm is the main symptom of asthma in patients, and is the clinical manifestation of the underlying cellular and molecular immunologic changes responsible for bronchial hyperreactivity. Similarly, the majority of mouse models of allergic asthma present bronchial hyperreactivity. One of the motives that led to the development of mouse models of asthma was precisely the possibility of measuring the bronchial response in these animals. Respiratory function can be evaluated in asthmatic mice using methods that will be described briefly below.

Ex vivo techniques for the evaluation of bronchial reactivity. In the organ bath method, the trachea of sensitized animals is isolated, introduced into the bath, and its smooth muscle stimulated with electric fields or through the addition of bronchoconstrictors such as histamine or methacholine.[48] Using this procedure it is possible to perform isolated measurements without the interference caused by arterial oxygenation or cardiovascular elements; for instance, it is possible to measure the muscle reactivity of the principal respiratory airways, which is believed to be largely responsible for pulmonary dysfunction in human asthma.[49] However, this technique does not allow reproduction of the impact of bronchial obstruction caused by edema of the bronchial mucosa, mucus, and narrowing of the airways. Consequently, it is often preferable to use in vivo models.

In vivo techniques for the evaluation of bronchial reactivity. To analyze the majority of the phenomena that can lead to bronchial obstruction in the same model, it is preferable to work with in vivo models. Given the current availability of both invasive and noninvasive methods with which to evaluate bronchospasm, either anesthetized or conscious mice can be used. The most commonly used invasive technique consists of intubating the animal with an intratracheal cannula and using a plethysmograph to directly measure bronchopulmonary resistance to airflow.[50,51] This technique is useful but complex, because it requires the difficult cannulation of the tail vein (caudal) or the subclavian vein and entails the drawbacks that the antigen cannot be administered while respiratory pressure is being determined, that methacholine is administered intravenously, and that the animal is sacrificed at the end of the measurement, making it impossible to reevaluate respiratory function in the same animal. In contrast, in the noninvasive method the conscious animal is introduced into a chamber and

respiratory function is measured through a variable called Penh (Figure 1) using a whole body plethysmograph (WBP). Because it is not necessary to anesthetize the animal and there is no disturbance or physical damage, various measurements can be performed in the same mouse during different phases. Although some authors recommend confirmation of the Penh value using invasive methods to assess parameters that directly reflect respiratory obstruction,[52] Hamelmann et al[41] demonstrated the correlation between bronchial hyperreactivity to methacholine measured using WBP and lung resistance and intrapleural pressure evaluated through other procedures. Some of the doubts about WBP arise from the fact that the bronchial reactivity measured using this method lacks units, since it is derived from the mathematical processing of empirical respiratory signals.[53-55] Nevertheless, the demonstration that the Penh value obtained with WBP reflects the hyperreactivity has led to an increase in the number of research groups that adopt WBP as the method of choice for the evaluation of bronchopulmonary function in asthmatic mice, in spite of the high initial investment. This is borne out by the increasing number of high-quality publications in which this procedure is cited.[56-60] Our own experience with WBP allows us to affirm that it is a simple, rapid, and reproducible method with which to compare the bronchial hyperreactivity of sensitized mice with that of normal animals in response to the bronchoconstrictor methacholine. Although this measurement is normally made 24 hours after the last exposure of the animal to the allergen, with WBP it can also be performed at other stages in the process in order to follow its evolution. For instance, bronchopulmonary function can be evaluated before and after induction of asthma, during the acute and chronic phases of the process, or before and after a treatment. Figure 2 shows the main components of our WBP equipment (Buxco Europe Ltd, Winchester, UK) and describes its operation.

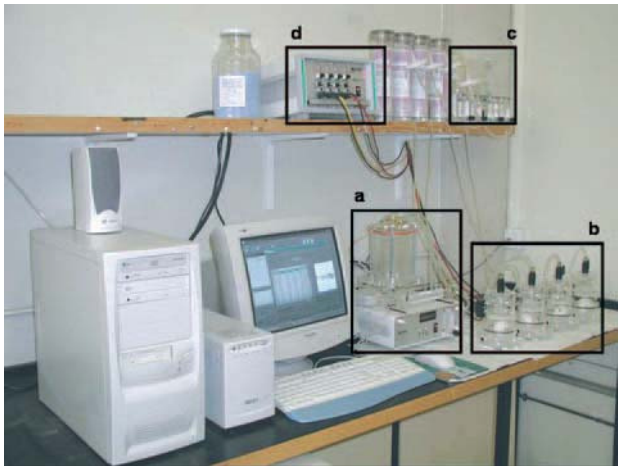

Figure 2. Equipment for the measurement of respiratory function in nonimmobilized, conscious mice (Buxco Europe Ltd, Winchester, UK). The 4 main parts of the equipment are indicated: *a)* nebulizer, which delivers the methacholine (or elected bronchoconstrictor) as an aerosol into each chamber at a preselected, constant flow rate; *b)* whole body plethysmograph with 4 mouse chambers, each of which has a coupled transducer that transforms the respiratory signal into an electrical signal and sends it to the amplifier; *c)* airflow regulator, which maintains a constant flow of drug-free air in the interior of the plethysmographic chambers; and *d)* amplifier, which receives the signals captured by the transducer in each chamber, amplifies them, and translates them into data for visualization and analysis using Biosystem XA software from Buxco.

Bronchopulmonary Histopathology in Asthmatic Mice

One of the determining factors in the establishment of a parallel between asthma in patients and asthma in murine models is the histopathological characterization of the airways and lung parenchyma. Notable in human asthma is the presence of chronic bronchopulmonary inflammation with abundant eosinophils, even localized in the epithelium, the presence of other inflammatory cells, and characteristic structural changes that follow the inflammatory process (remodeling) in airway walls.

Bronchovascular inflammation. In models of allergic asthma induced in the mouse, lung inflammation can be evaluated using 2 procedures: BAL and histologic analysis of sections of the lung.[13,61,62] BAL is used to quantify both the total number of cells in the tracheobronchial tree that can be extracted through lavage and the proportions of the different cell populations. Normally, in human and mouse, the number of inflammatory cells found in BAL is correlated with the type and grade of inflammation observed in the lung parenchyma by pathology.[13,61-63] Although the latter quantification procedure is not as simple as with BAL, it allows the elements detected to be localized in the tissue. Histologic sections of the lung stained with hematoxylin and eosin display bronchovascular inflammation distributed in small, dense foci—as shown in Figure 3—or more extensive areas, the latter infiltrates being generally more diffuse. All inflammatory processes in asthmatic mice are associated, to greater or lesser degree, with the presence of eosinophils (between 30% and 80%, depending on the model), and both the main and smaller airways can be affected. The differences in cell composition, grade of inflammation, and localization of inflammatory foci depend on the immunization protocol and the mouse strain used.[13,61,62,64] In more natural models of induction, such as, for instance, those in which the mouse is exposed to the allergen without a coadjuvant like alum, there is a tendency to generate an inflammatory process that is more similar to that observed in biopsies and autopsy of the bronchi of asthmatic individuals.

Inflammation in the lungs of asthmatic patients is characterized particularly by infiltration of T lymphocytes and eosinophils into the bronchial mucosa, and the presence of mast cells.[25] The majority of the recruited lymphocytes are CD4+ T helper cells or T helper 2 cells (Th_2), a cell subtype normally associated with pathologic processes of allergic origin that, once activated, attract and stimulate principally eosinophils. It was initially observed in mouse models of asthma

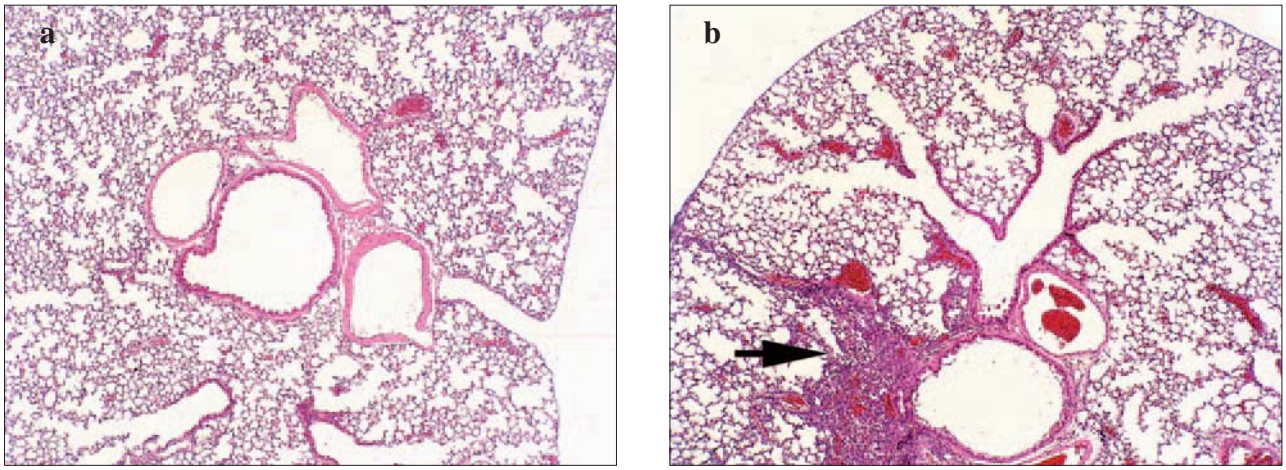

Figure 3. Photomicrograph (□100) of a histologic section of lung from *a)* control (nonasthmatic) mouse, and *b)* mouse with asthma induced by ovalbumin (intraperitoneal sensitization with ovalbumin and aluminium sulfate and challenge with nebulized ovalbumin at 1%). The sections are stained with hematoxylin-eosin. The arrow indicates an inflammatory focus of the bronchovascular infiltration typical of the mouse model of asthma.

that the immunological "memory" that is responsible for the development of asthma in a sensitized animal that reencounters the allergen is mainly due to the persistence of infiltrated T lymphocytes in the lung parenchyma.[62] The recruited and activated eosinophils may be, in part, responsible, along with mast cells and other effector cells that are activated in situ, such as pulmonary macrophages and the epithelial cells of the respiratory mucosa, for the damage and tissue remodeling via the release of multiple mediators.[65] In contrast to humans, in mouse models of asthma the eosinophils do not localize to the epithelium of the bronchial mucosa; nor have they been observed to be degranulated following contact with the allergen.[66] Notable among the mediators released by the eosinophils are proteins and highly reactive oxygen radicals that are able to cause damage.[67] Consequently, it is initially surprising that mouse models have been obtained in which allergic asthma is induced without eosinophils in the airways,[37] although it has begun to be suggested that perhaps the eosinophils have a regulatory function more than an effector or proinflammatory role in chronic asthma; this could include, for example, helping to maintain the Th_2 response.[68-70]

Mast cells are considered to be principally effector cells in the pathogenesis of the early phase of asthma.[71] It has been observed that the airways of asthmatic patients contain a large number of mast cells and mast cell mediators released following activation.[72] The concentration of these mediators has also been correlated with an increase in pulmonary obstruction[73] and with bronchial hyperreactivity in patients with asthma.[74,75] Many lines of evidence indicate that mast cells also participate in the late phase of chronic asthma. Firstly, cytokines that are known to be produced by mast cells, such as interleukin (IL) 4 and IL13, could promote or contribute to the Th_2 immune response characteristic of asthma. Secondly, mast cells participate in the recruitment of inflammatory cells, especially Th_2 lymphocytes and eosinophils, through the action of prostaglandin D2,[76] a mediator associated with experimental models of asthma in the mouse.[77] Furthermore, in asthma induction protocols in which systemic immunization is omitted and, therefore, in which Th_2 cells do not participate, mast cells must be present and activated for pulmonary eosinophilia to be observed.[56,78] This reflects the probable importance of mast cells in the establishment of inflammation in models of asthma, in addition to their participation in earlier phases. No mouse model of chronic asthma has yet been developed in which mast cells are observed to infiltrate the respiratory mucosa as they do in asthmatic individuals.[79]

Tissue remodeling. One of the most important characteristics of chronic asthma in patients is tissue remodeling, which occurs as a consequence of an abnormal tissue repair process that follows chronic inflammation. The histologic observations specific to remodeling are epithelial hypertrophy/hyperplasia, subepithelial fibrosis, hyperplasia of the mucus-producing goblet cells (and, consequently, mucus hypersecretion), and hyperplasia of the bronchial smooth muscle.[80] These structural changes lead to a thickening of the wall of the respiratory mucosa and, consequently, narrowing of the respiratory airways, which is manifested by a chronic reduction in lung function that aggravates the bronchial hyperreactivity.[81] It has been possible to induce remodeling in nonasthmatic transgenic mice that overexpress Th_2-type cytokines,[82-84] an observation that highlights the relevance of these cytokines in the remodeling process. However, it was not possible to reproduce remodeling in mouse models of allergic asthma until chronic models were established. Current chronic models will, therefore, help to explain aspects of how this phenomenon is brought about and in what way it contributes to bronchial obstruction. To date, these

models show many of the histologic characteristics of remodeling described previously,[13,42,85] although none reproduce them in their entirety. One model that imitates the process quite faithfully is, as mentioned earlier, that of Johnson et al,[13] in which a natural aeroallergen such as the dust mite is used. In this model, the use of systemic immunization and coadjuvants is omitted, an allergen is used that causes asthma in patients (aeroallergen), and the route of respiratory sensitization is very probably the same as that used naturally. It is also worth mentioning that the model of Johnson et al,[13] like the spontaneous disease, involves exposure of the mouse to continuous contact with the allergen rather than alternating contact or contact in cycles. This latter point is of particular interest because it allows the development of symptoms to be studied and the pathogenesis of asthma to be evaluated at various points, from a few days to 7 or 8 weeks after initiating sensitization. In the new chronic models it is possible to determine, in addition to remodeling, the evolution of processes such as lung inflammation and bronchial hyperreactivity.

Mediators in Experimental Asthma

Cytokines. A number of groups have investigated the importance of cytokines in the various different mouse models of asthma. In order to establish their relevance, local and systemic expression of cytokines has been studied, and asthma has been induced in mice that are genetically deficient in a particular cytokine. In addition to local expression, the serum expression of many of these cytokines has also been determined. These immune mediators fulfill an immunoregulatory role in the allergic response, and some of them act as inducers of the bronchovascular inflammatory process. The expression pattern of the cytokines suggests that the majority of murine models of allergic asthma exhibit a predominant Th_2-type response, as occurs in humans[13,62,86]; in other words, the CD4+ Th_2 lymphocytes that recognize the antigen are activated and differentiate into a cell subtype that is characterized by the release of a certain range of cytokines, notable among which are IL4, IL5, and IL13. The consequence of a bias towards this type of immune response is that a specific clone of B lymphocyte that produces and releases IgE is activated, and in addition, mainly eosinophils are recruited and activated. In mouse, the relevance of IL5[87-89] and IL13[89-92] in asthma has been described, and it has been pointed out that both mediators, as well as other Th_2 cytokines such as IL4, exert various modulatory effects on bronchial hyperreactivity, inflammatory infiltration of eosinophils, and lung remodeling.[82-84,92-94]

Eicosanoids. Since the 1970s, associations have been observed between the products of arachidonic acid metabolism, or eicosanoids, and asthma. These molecules, both those of the cyclooxygenase (COX) pathway, the prostaglandins (Pg), and those of the lipoxygenase pathway (leukotrienes), are currently considered to be important.

For instance, inhalation of PgE_2 is known to attenuate the asthmatic response induced in patients by aeroallergens. Consistent with this, some investigators have also demonstrated that airway cells have a lower capacity for PgE_2 production,[95-97] possibly due to an anomaly in COX expression, as described in patients with nonallergic asthma.[98] COX is a molecule known, paradoxically, for its marked proinflammatory effect. Some of the data showing activity of COX2 and production of PgE_2 in asthma have also been obtained in studies using murine models of asthma. For instance, asthmatic mice lacking COX (knockouts) exhibit more intense inflammation of the respiratory airways and greater bronchial hyperreactivity on contact with the allergen than wild type (normal) animals; these changes are accompanied by reduced production of PgE_2.[99] Likewise, Peebles et al,[89,100] using selective and nonselective inhibitors of COX in a mouse model of asthma, described that the treatment favored the asthmatic response and that this was also accompanied by a reduction in PgE_2 and an increase in leukotrienes. This view of the detrimental effect of inhibiting COX on the inflammatory process and lung function in asthma is supported by recent proposals that the enzyme could fulfill antagonistic, proinflammatory and antiinflammatory roles, depending on the phase of the disease.[101] Consistent with these results, we have obtained preliminary, unpublished data in a model of asthma induced by ovalbumin[102] in mice treated with rofecoxib, a selective inhibitor of COX2, that indicate that pharmacological blockade of the enzyme leads to a more intense inflammatory process and a stronger bronchial hyperresponse than in untreated asthmatic mice. As mentioned, in addition to PgE_2, other molecules such as PgD_2 are linked to the asthmatic process in mice.[77]

Although it is known that there is an increase in leukotrienes in human asthma,[103] and that antagonists of these mediators offer improvements in asthma,[104] there has been little investigation of their relevance in the asthmatic process induced in mice. As in humans, some results point to an increase in their production in asthmatic mice,[89,99] and others also indicate the participation of leukotrienes in airway remodeling.[105] It would nevertheless be worth establishing more clearly the dynamics of these mediators in bronchial hyperreactivity and in the inflammatory process of different models of asthma in the mouse.

Immunoglobulins in Allergic Asthma in the Mouse

The first contact of an atopic individual with an allergen causes sensitization, or in immunological terms, the production of specific anaphylactic antibodies against the allergen. The main anaphylactic antibody is IgE, a molecule whose levels are increased in practically all asthmatic individuals.[106] The predominance of Th_2

cells in asthmatic individuals mentioned earlier is probably the determining factor in this phenomenon, since the cytokines IL4 and IL13, specific to the Th_2 response, contribute to the prioritized production of IgE over other classes of antibodies. The clinical significance or the cause and effect relationship between increased IgE levels and bronchial hyperreactivity, the inflammatory process, or airway remodeling, particularly in chronic processes, remains unknown. It is known, however, that the IgE that is produced binds mast cells of the respiratory system (in the pulmonary parenchyma and in the airway mucosa) and possibly other cells that express receptors for this immunoglobulin. Sensitized mast cells with IgE on their surface that are activated through a new contact with the allergen can contribute as effector cells to both bronchospasm and induction of the inflammatory process through the release of a wide variety of mediators. It is notable that some cells that do not have the IgE receptor in normal individuals express it in asthmatic individuals (dendritic cells, macrophages, eosinophils, etc).[107,108] In most models of asthma induced in the mouse an increase in total IgE and IgE specific to the antigen is also detected in the serum. In some models, there is also a direct relationship between serum IgE and bronchospasm.[109] Evidence showing the importance of elevated levels of IgE in asthmatic patients has led to the design of a new therapeutic focus, namely blockade of IgE with antibodies to prevent it binding effector cells.[110] All of this points to the probable role of this antibody in the pathogenesis of asthma.

However, the apparent relevance of IgE in the pathogenesis of asthma and its consistent increase in asthmatic mice does not always reflect a clear functional implication. Thus, some publications indicate that even in the absence of IgE it is possible to induce some of the models of asthma in mice.[111-114] For instance, mice deficient in IgE or IL4 (a cytokine that induces the production of IgE by specialized B lymphocytes) display bronchial hyperreactivity, and the inflammatory response is only partly reduced.[111,115] Furthermore, animals that are deficient in B cells (and therefore unable to produce antibodies)[116] or lack mast cells (c-*kit* negative)[51] develop asthma, although without inflammatory infiltration of eosinophils. These are surprising results that, in principle, question the role played by IgE and mast cells in some experimental models. The interpretation of these results in terms of their repercussions for allergic asthma in humans is difficult. However, it is important to note that, in general, the studies in which the importance of IgE is questioned have been undertaken in ovalbumin models and, with some exceptions,[42] in models involving short exposure to the antigen. Thus, it is possible that the most recently described chronic models that use natural allergens will yield different results.

In addition to IgE, the presence of specific IgG has been described in individuals with asthma. The role that can be attributed to this immunoglobulin is less clear. In some mouse models of allergic asthma, IgG_1 has also been detected,[13,62] but its relevance is likewise unknown at this time.

Limitations of the Mouse Models of Asthma

As mentioned, there is no ideal experimental model in which each and every one of the pathogenic processes of asthma can be studied.[117,118] The election of an appropriate model depends on the hypothesis to be investigated. The mouse models of asthma offer a wide range of experimental possibilities, but their suitability has to be evaluated in terms of the experimental objectives and taking into account certain limitations, many of which are also applicable to other disease models in mice.

One aspect that differentiates models of asthma induced in the mouse from spontaneous asthma in patients is that, generally, all mice exposed to the allergen develop the disease. The lack of an apparent individual genetic factor introduces an element of uncertainty over the suitability of these animals for use in studies of genetic polymorphism, although some have been undertaken.[119] However, it should be noted that there are mouse strains that can be described as more susceptible than others due to their atopic phenotype in terms of the tendency to produce IgE.[33]

On the other hand, perhaps certain immunologic differences between species explain why, unlike in humans, the majority of experimental models require high concentrations of allergen. The appearance of chronic models in murine species has, to some degree, allowed this limitation to be addressed. As mentioned, although in principle IgE and mast cells are considered to be key factors in the early phase of allergic asthma in humans, this does not appear to be confirmed in some of the murine models. In those models, specific IgE exhibits a clear increase, but the functional implications of this change are less obvious. Another difference is the apparent lack of eosinophil degranulation or activity in the majority of mouse models,[66,120,121] although it has been observed in some studies.[48,105] This is in contrast to what is seen in asthmatic individuals, where eosinophils recruited by the respiratory system present signs of activation.[122] However, it must be said in support of the experimental models that there has been no unequivocal demonstration that degranulation is necessary for the induction of bronchial hyperreactivity in humans. To these genetic and/or immunologic differences we should add anatomic and physiologic differences between the respiratory systems of mouse and human. Consequently, emphasis should not be placed on differences in the functional mechanisms associated with the development of bronchospasm in the two species. For instance, while asthmatic individuals present bronchial hyperreactivity to methacholine even during asymptomatic periods, this hyperreactivity is transitory during the period of exposure to the allergen in mouse models, as indicated in the recent review of Epstein.[123]

It is important to remember that while limitations are inherent to any experimental model, they should be taken into consideration when evaluating the effectiveness of the induction of allergic asthma in achieving the stated aims, and when interpreting the results obtained. Finally, it is important to emphasize the fact that the effort to induce chronic models will, to a large extent, allow these limitations to be overcome.

Conclusions: the Asthmatic Mouse Is a Valuable Addition

There is no doubt that mouse models of asthma occupy a unique position in research into the mechanisms of allergic asthma, essentially through a combination of 4 factors: *a)* most of the phenomena and key immunologic and histologic processes in the pathogenesis of human asthma are reproduced with increasing accuracy in the mouse; *b)* equipment is available with which to measure respiratory change in the mouse; *c)* it is possible to investigate in greater detail and with a wider perspective aspects that would be too extensive in asthmatic patients; and *d)* the mouse offers experimental advantages over other species in terms of the induction of disease models that are also applicable to models of asthma, such as the ability to perform genetic manipulations (transgenics and knockouts) and the availability of species-specific reagents.

The mouse, therefore, is a complementary tool in asthma research, and is clearly a valuable addition to this field. Thus, it is recommendable to perform parallel studies of the mechanisms of asthma from different experimental perspectives; the combination of studies in human patients and mouse models will probably be the most fruitful, because the mouse can both indicate phenomena to be studied in patients and allow a deeper understanding to be developed of aspects observed in asthmatic individuals.

Despite the value of all of the mouse models of asthma and the consistency of many of the findings, there is a certain degree of variability among them. This is largely attributable to the genetic background of the mouse strains used, differences in the induction procedures, the different techniques used to evaluate the response, and the point at which evaluations are undertaken. It is not the aim of this review to draw particular attention to any of these, but we would like to suggest some guidelines, discussed in greater detail earlier, that we consider important. The choice of the mouse strain, the allergen and its route of exposure, and the duration of the sensitization/activation protocol —distinguishing between short (days/weeks) and long (months) periods of exposure—are fundamental. In terms of the allergen, although induction with ovalbumin has been, and continues to be, very useful, given the importance of allergen structure in the type of immune response, it seems preferable to move over to the use of natural allergens. If, in addition, contact with the allergen occurs through the respiratory airways, both in the initial phase of sensitization and in subsequent phases, and is continuous (daily or almost daily) rather than in cycles, it will better imitate the natural exposure in patients, and therefore, may achieve a more faithful model. It is of particular interest to make at least two models of asthma available in the laboratory that can be induced via the airways using the same natural allergen: an acute model based on short exposure with which to investigate the inflammatory and functional events associated with the first few days of the process, and a chronic model based on extended exposure in which it is possible to reproduce remodeling of the bronchial wall and subsequent functional respiratory alterations.

The reproducibility of basic pathophysiological characteristics of human allergic asthma, such as bronchial hyperreactivity, eosinophilic inflammation, mucus hypersecretion, and remodeling, in the mouse, and the possibility of studying bronchopulmonary function in this species indicate that these models of allergic asthma are suitable to address many of the questions raised in the pathogenesis and treatment of the disease.

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REFERENCES

1. Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martínez F, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. Eur Resp J. 1995; 8:483-91.
2. Office of Press and Public Relations. WHO: Bronchial asthma. Geneva: World Health Organization, 2000.
3. Martínez-Moratalla J, Almar E, Sunyer J, Ramos J, Pereira A, Payo F, et al. Estudio Europeo del Asma. Identificación y tratamiento de individuos con criterios epidemiólogicos de asma en adultos jóvenes de cinco áreas españolas. Arch Bronconeumol. 1999;35:223-8.
4. Basagana X, Sunyer J, Zock JP, Kogevinas M, Urrutia I, Maldonado JA, et al. Incidence of asthma and its determinants among adults in Spain. Am J Respir Crit Care Med. 2001;164: 1133-7.
5. Nolte H, Backer V, Porsbjerg C. Environmental factors as a cause for the increase in allergic disease. Ann Allergy Asthma Immunol. 2001;87:7-11.
6. Kim DS, Drake-Lee AB. Infection, allergy and the hygiene hypothesis: historical perspective. J Laryngol Otol. 2003;117: 946-50.
7. Nieto A, Álvarez-Cuesta E, Boquete M, Mazón A, de la Torre F. The cost of asthma treatment in Spain and rationalizing the expense. J Invest Allergol Clin Immunol. 2001;11:139-48.
8. Jarjour NN, Peters SP, Djukanovic R, Calhoun WJ. Investigative use of bronchoscopy in asthma. Am J Respir Crit Care Med. 1998;157:692-7.

9. Kavuru MS, Dweik RA, Thomassen MJ. Role of bronchoscopy in asthma research. Clin Chest Med. 1999;20:153-89.
10. Hoffjan S, Ober C. Present status on the genetic studies of asthma. Curr Opin Immunol. 2002;14:709-17.
11. Brown JR, Kleimberg J, Marini M, Sun G, Bellini A, Mattoli S. Kinetics of eotaxin expression and its relationship to eosinophil accumulation and activation in bronchial biopsies and bronchoalveolar lavage (BAL) of asthmatic patients after allergen inhalation. Clin Exp Immunol. 1998;114:137-46.
12. Jaffar Z, Roberts K, Pandit A, Linsley P, Djukanovic R, Holgate ST. B7 costimulation is required for IL-5 and IL-13 secretion by bronchial biopsy tissue of atopic asthmatic subjects in response to allergen stimulation. Am J Respir Cell Mol Biol. 1999;20:153-62.
13. Johnson JR, Wiley RE, Fattouh R, Swirski FK, Gajewska BU, Coyle AJ, et al. Continuous exposure to house dust mite elicits chronic airway inflammation and structural remodeling. Am J Respir Crit Care Med. 2004;169:378-85.
14. Motta A, Peltre G, Dormans JA, Withagen CE, Lacroix G, Bois F, et al. Phleum pratense pollen starch granules induce humoral and cell-mediated immune responses in a rat model of allergy. Clin Exp Allergy. 2004;34:310-4.
15. Toward TJ, Broadley KJ. Early and late bronchoconstrictions, airway hyper-reactivity, leucocyte influx and lung histamine and nitric oxide after inhaled antigen: effects of dexamethasone and rolipram. Clin Exp Allergy. 2004;34:91-102.
16. Gascoigne MH, Holland K, Page CP, Shock A, Robinson M, Foulkes R, et al. The effect of anti-integrin monoclonal antibodies on antigen-induced pulmonary inflammation in allergic rabbits. Pulm Pharmacol Ther. 2003;16:279-85.
17. Aoki M, Fukunaga M, Kitagawa M, Hayashi K, Morokata T, Ishikawa G, et al. Effect of a novel anti-inflammatory compound, YM976, on antigen-induced eosinophil infiltration into the lungs in rats, mice, and ferrets. J Pharmacol Exp Ther. 2000;295:1149-55.
18. Barrett EG, Rudolph K, Bowen LE, Muggenburg BA, Bice DE. Effect of inhaled ultrafine carbon particles on the allergic airway response in ragweed-sensitized dogs. Inhal Toxicol. 2003;15: 151-65.
19. Norris CR, Byerly JR, Decile KC, Berghaus RD, Walby WF, Schelegle ES, et al. Allergen-specific IgG and IgA in serum and bronchoalveolar lavage fluid in a model of experimental feline asthma. Vet Immunol Immunopathol. 2003;96:119-27.
20. Bischof RJ, Snibson K, Shaw R, Meeusen ENT. Induction of allergic inflammation in the lungs of sensitized sheep after local challenge with house dust mite. Clin Exp Allergy. 2003;33:367-75.
21. Fornhem C, Peterson CG, Dahlback M, Scheynius A, Alving K. Granulocyte function in the airways of allergen-challenged pigs: effects of inhaled and systemic budesonide. Clin Exp Allergy. 1996;26:1436-48.
22. van Scott MR, Hooker JL, Ehrmann D, Shibata Y, Kukoly C, Salleng K, et al. Dust mite-induced asthma in cynomolgus monkeys. J Appl Physiol. 2004;96:1433-44.
23. Turlej RK, Fievez L, Sandersen CF, Dogne S, Kirschvink N, Lekeux P, et al. Enhanced survival of lung granulocytes in an animal model of asthma: evidence for a role of GM-CSF activated STAT5 signalling pathway. Thorax. 2001;56:696-702.
24. Underwood S, Foster M, Raeburn D, Bottoms S, Karlsson JA. Time-course of antigen-induced airway inflammation in the guinea-pig and its relationship to airway hyperresponsiveness. Eur Resp J. 1995;8:2104-13.
25. Isenberg-Feig H, Justice J, Keane-Myers A. Animal models of allergic asthma. Curr Allergy Asthma Rep. 2003;3:70-8.
26. Ali H, Leung KB, Pearce FL, Hayes NA, Foreman JC. Comparison of the histamine-releasing action of substance P on mast cells and basophils from different species and tissues. Int Arch Allergy Appl Immunol. 1986;79:413-8.
27. Wanner A, Mezey RJ, Reinhart ME, Eyre P. Antigen-induced bronchospasm in conscious sheep. J Appl Physiol. 1979;47:917-22.
28. Abraham WM, Delehunt JC, Yerger L, Marchette B. Characterization of a late phase pulmonary response after antigen challenge in allergic sheep. Am Rev Respir Dis. 1983;128:839-44.
29. Chen W, Alley MR, Manktelow BW. Airway inflammation in sheep with acute airway hypersensitivity to inhaled Ascaris suum. Int Arch Allergy Appl Immunol. 1991;96:218-23.
30. Chen W, Alley MR, Manktelow BW. Morphological and morphometric studies of the airways of sheep with acute airway hypersensitivity to inhaled Ascaris suum. Int J Exp Pathol. 1991;72:543-51.
31. Bosse J, Boileau R, Begin R. Chronic allergic airway disease in the sheep model: functional and lung-lavage features. J Allergy Clin Immunol. 1987;79:339-44.
32. Johnson HG, Stout BK. Late phase bronchoconstriction and eosinophilia as well as methacholine hyperresponsiveness in Ascaris-sensitive rhesus monkeys were reversed by oral administration of U-83836E. Int Arch Allergy Immunol. 1993;100:362-6.
33. Brewer JP, Kisselgof AB, Martin TR. Genetic variability in pulmonary physiological, cellular, and antibody responses to antigen in mice. Am J Respir Crit Care Med. 1999;160:1150-6.
34. Herz U, Braun A, Ruckert R, Renz H. Various immunological phenotypes are associated with increased airway responsiveness. Clin Exp Allergy. 1998;28:625-34.
35. Shinagawa K, Kojima M. Mouse model of airway remodeling: Strain differences. Am J Respir Crit Care Med. 2003;168:959-67.
36. Cates EC, Gajewska BU, Goncharova S, Álvarez D, Fattouh R, Coyle AJ, et al. Effect of GM-CSF on immune, inflammatory, and clinical responses to ragweed in a novel mouse model of mucosal sensitization. J Allergy Clin Immunol. 2003;111:1076-86.
37. Tournoy KG, Kips J, Schou C, Pauwels R. Airway eosinophilia is not a requirement for allergen-induced airway hyperresponsiveness. Clin Exp Allergy. 2000;30:79-85.
38. Sarpong SB, Zhang L-Y, Kleeberger SR. A novel mouse model of asthma. Int Arch Allergy Immunol. 2003;132:346-54.
39. Sakai K, Yokoyama A, Kohno N, Hiwada K. Effect of different sensitizing doses of antigen in a murine model of atopic asthma. Clin Exp Allergy. 1999;118:9-15.
40. Clarke AH, Thomas WR, Rolland JM, Dow C, O'Brien RM. Murine allergic respiratory responses to the major house dust mite allergen Der p 1. Int Arch Allergy Immunol. 1999;120:126-34.
41. Hamelmann E, Schwarze J, Takeda K, Oshiba A, Larsen GL, Irvin CG, et al. Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmography. Am J Respir Crit Care Med. 1997;156:766-75.
42. Temelkovski J, Hogan SP, Shepherd DP, Foster P, Kumar RK. An improved murine model of asthma: selective airway inflammation, epithelial lesions and increased methacholine responsiveness following chronic exposure to aerosolised allergen. Thorax. 1998;53:849-56.
43. Holt PG, Batty JE, Turner KJ. Inhibition of specific IgE responses in mice by re-exposure to inhaled antigen. Immunology. 1981;42:409-17.
44. Sedgwick JD, Holt PG. Down-regulation of immune responses to inhaled antigen: studies on the mechanism of induced suppression. Immunology. 1985;56:635-42.
45. Roberts K, Jaffar Z. Regulation of the adaptive immune response to inhaled allergens [editorial]. Clin Exp Allergy. 2002;32:343-4.
46. Swirski FK, Sajic D, Robbins CS, Gajewska BU, Jordana M. Chronic exposure to innocuous antigen in sensitized mice leads to suppressed airway eosinophilia that is reversed by GMC-SF. J Immunol. 2002;169:3499-506.
47. McMillan SJ, Lloyd CM. Prolonged allergen challenge in mice leads to persistent airway remodelling. Clin Exp Allergy. 2004; 34:497-507.
48. Hamelmann E, Takeda K, Oshiba A, Gelfand EW. Role of IgE in the development of allergic airway inflammation and airway hyperresponsiveness-a murine model. Allergy. 1999;54:297-305.
49. Fedan JS, van Scott MR, Johnson AR. Pharmacological techniques for the in vitro study of airways. J Pharmacol Toxicol Methods. 2001;45:159-74.
50. Martin TR, Gerard NP, Galli SJ, Drazen JM. Pulmonary responses to bronchoconstrictor agonists in the mouse. J Appl Physiol. 1988;64:2318-23.
51. Takeda K, Hamelmann E, Joetham A, Shultz LD, Larsen GL, Irvin CG, et al. Development of eosinophilic airway inflammation and airway hyperresponsiveness in mast cell-deficient mice. J Exp Med. 1997;186:449-54.
52. Drazen JM, Finn PW, De Sanctis GT. Mouse models of airway hyperresponsiveness: physiological basis of observed outcomes and analysis of selected examples using these outcome indicators. Annu Rev Physiol. 1999;61:593-625.

53. Lundblad LK, Irvin CG, Adler A, Bates JHT. A reevaluation of the validity of unrestrained plethysmography in mice. J Appl Physiol. 2002;93:1198-207.
54. Hantos Z, Brusasco V. Assessment of respiratory mechanics in small animals: the simpler the better? J Appl Physiol. 2002;93:1196-7.
55. Mitzner W, Tankersley C. Interpreting Penh in mice. J Appl Physiol. 2003;94:828-32.
56. Williams MMC, Galli SJ. Mast cells can amplify airway reactivity and features of chronic inflammation in an asthma model in mice. J Exp Med. 2000;192:455-62.
57. Kraneveld AD, van der Kleij HP, Kool M, van Houwelingen AH, Weitenberg AC, Redegeld FA, et al. Key role for mast cells in nonatopic asthma. J Immunol. 2002;169:2044-53.
58. Carey MA, Germolec DR, Bradbury JA, Gooch RA, Moorman MP, Flake GP, et al. Accentuated T helper type 2 airway response after allergen challenge in cyclooxygenase-1–/– but not cyclooxygenase-2–/– mice. Am J Respir Crit Care Med. 2003; 167:1509-15.
59. Whitehead GS, Walker JK, Berman KG, Foster WM, Schwartz DA. Allergen-induced airway disease is mouse strain dependent. Am J Physiol Lung Cell Mol Physiol. 2003;285:L32-L42.
60. Melgert BN, Postma DS, Geerlings M, Luinge MA, Klok PA, van der Strate BW, et al. Short-term smoke exposure attenuates ovalbumin-induced airway inflammation in allergic mice. Am J Respir Cell Mol Biol. 2004;30:880-5.
61. Jungsuwadee P, Dekan G, Stingl G, Epstein M. Recurrent aerosol antigen exposure induces distinct patterns of experimental allergic asthma in mice. Clin Immunol. 2002;102:145-53.
62. Mojtabavi N, Dekan G, Stingl G, Epstein M. Long-lived Th2 memory in experimental allergic asthma. J Immunol. 2002; 169:4788-96.
63. Grootendorst DC, Sont JK, Willems LN, Kluin-Nelemans JC, van Krieken JH, Veselic-Charvat M, et al. Comparison of inflammatory cell counts in asthma: induced sputum vs bronchoalveolar lavage and bronchial biopsies. Clin Exp Allergy. 1997;27:769-79.
64. Takeda K, Haczku A, Lee J, Irvin CG, Gelfand EW. Strain dependence of airway hyperresponsiveness reflects differences in eosinophil localization in the lung. Am J Physiol Lung Cell Mol Physiol. 2001;281:L394-L402.
65. Lambrecht BN, van Rijt LS, Kuipers H. Immunology of eosinophilic airway inflammation: what the animal models teach us. In: Lambrecht BN, Hoogsteden HC, Diamant Z, editors. The immunological basis of asthma. New York: Marcel Dekker; 2003. p. 343-65.
66. Stelts DS, Egan RW, Falcone A, Garlisi CG, Gleich GJ, Kreutner W, et al. Eosinophils retain their granule major basic protein in a murine model of allergic pulmonary inflammation. Am J Respir Cell Mol Biol. 1998;18:463-70.
67. Banner KH, Paul W, Page CP. Ovalbumin challenge following immunization elicits recruitment of eosinophils but not bronchial hyperresponsiveness in guinea-pigs: time course and relationship to eosinophil activation status. Pulm Pharmacol. 1996;9:179-87.
68. MacKenzie JR, Mattes J, Dent LA, Foster P. Eosinophils promote allergic disease of the lung by regulating CD4+Th2 lymphocyte function. J Immunol. 2001;167:3146-55.
69. Mattes J, Yang M, Mahalingam S, Kuehr J, Webb DC, Simson L, et al. Intrinsic defect in T cell production of interleukin (IL)13 in the absence of both IL-5 and eotaxin precludes the development of eosinophilia and airways hyperreactivity in experimental asthma. J Exp Med. 2002;195:1433-44.
70. Alam R, Busse WW. The eosinophil–Quo vadis? J Allergy Clin Immunol. 2004;113:38-42.
71. Saini SS. Immune functions of mast cells and basophils. In: Lambrecht BN, Hoogsteden HC, Diamant Z, editors. The immunological basis of asthma. New York: Marcel Dekker; 2003. p. 121-45.
72. Koshino T, Arai Y, Miyamoto Y, Sano Y, Itami M, Teshima S, et al. Airway basophil and mast cell density in patients with bronchial asthma: relationship to bronchial hyperresponsiveness. J Asthma. 1996;33:89-95.
73. Jarjour NN, Calhoun WJ, Schwartz LB, Busse WW. Elevated bronchoalveolar lavage fluid histamine levels in allergic asthmatics are associated with increased airway obstruction. Am Rev Respir Dis. 1991;144:83-7.
74. Casale TB, Wood D, Richerson HB, Trapp S, Metzger WJ, Zavala D, et al. Elevated bronchoalveolar lavage fluid histamine levels in allergic asthmatics are associated with methacholine bronchial hyperresponsiveness. J Clin Invest. 1987;79:1197-203.
75. Broide DH, Gleich GJ, Cuomo AJ, Coburn DA, Federman EC, Schwartz LB, et al. Evidence of ongoing mast cell and eosinophil degranulation in symptomatic asthma airway. J Allergy Clin Immunol. 1991;88:637-48.
76. Hirai H, Tanaka K, Yoshie O, Ogawa K, Kenmotsu K, Takamori Y, et al. Prostaglandin D2 selectively induces chemotaxis in T helper type 2 cells, eosinophils, and basophils via seven-transmembrane receptor CRTH. J Exp Med. 2001;193:255-61.
77. Matsuoka T, Hirata M, Tanaka H, Takahashi Y, Murata T, Kabashima K, et al. Prostaglandin D2 as a mediator of allergic asthma. Science. 2000;287:2013-7.
78. Kung TT, Stelts DS, Zurcher JA, Jones H, Umland SP, Kreutner W, et al. Mast cells modulate allergic pulmonary eosinophilia in mice. Am J Respir Cell Mol Biol. 1995;12:404-9.
79. Kumar RK, Foster P. Murine model of chronic human asthma. Immunol Cell Biol. 2001;79:141-4.
80. Bousquet J, Jeffery PK, Busse WW, Johnson M, Vignola AM. Asthma. From bronchoconstriction to airways inflammation and remodeling. Am J Respir Crit Care Med. 2000;161:1720-45.
81. Busse WW, Elias J, Sheppard D, Banks-Schlegel S. Airway remodeling and repair. Am J Respir Crit Care Med. 1999;140: 1745-53.
82. Rankin JA, Picarella DE, Geba GP, Teman UA, Prasad B, DiCosmo B, et al. Phenotypic and physiologic characterization of transgenic mice expressing interleukin-4 in the lung: lymphocytic and eosinophilic inflammation without airway hyperreactivity. Proc Nat Acad Sci. 1996;93:7821-5.
83. Lee J, McGarry MP, Farmer SC, Denzler KL, Larson KA, Carrigan PE, et al. Interleukin-5 expression in the lung epithelium of transgenic mice leads to pulmonary changes pathognomonic of asthma. J Exp Med. 1997;188:1307-20.
84. Zhu Z, Homer RJ, Wang Z, Chen Q, Geba GP, Wang J, et al. Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. J Clin Invest. 1999;103: 779-88.
85. Kumar RK, Herbert C, Foster P. Expression of growth factors by airway epithelial cells in a model of chronic asthma: regulation and relationship to subepithelial fibrosis. Clin Exp Allergy. 2004;34:567-75.
86. Garlisi CG, Falcone A, Kung TT, Stelts D, Pennline KJ, Beavis AJ, et al. T cells are necessary for Th2 cytokine production and eosinophil accumulation in airways of antigen-challenged allergic mice. Clin Immunol Immunopathol. 1995;75:75-83.
87. Foster PS, Hogan SP, Ramsay AJ, Matthaei KI, Young IG. Interleukin 5 deficiency abolishes eosinophilia, airways hyperreactivity, and lung damage in a mouse asthma model. J Exp Med. 1996;183:195-201.
88. Hogan SP, Koskinen A, Matthaei KI, Young IG, Foster PS. Interleukin-5-producing CD4+ T cells play a pivotal role in aeroallergen-induced eosinophilia, bronchial hyperreactivity, and lung damage in mice. Am J Respir Crit Care Med. 1998;157: 210-8.
89. Peebles RSJ, Dworski R, Collins RD, Jarzecka K, Mitchell DB, Graham BS, et al. Cyclooxygenase inhibition increases interleukin 5 and interleukin 13 production and airway hyperresponsiveness in allergic mice. Am J Respir Crit Care Med. 2000;162:67681.
90. Grunig G, Warnock M, Wakil AE, Venkayya R, Brombacher F, Rennick DM, et al. Requirement for IL-13 independently of IL-4 in experimental asthma. Science. 1998;282:2261-3.
91. Mattes J, Yang M, Siqueira A, Clark K, McKenzie J, McKenzie AN, et al. IL-13 induces airways hyperreactivity independently of the IL-4R alpha chain in the allergic lung. J Immunol. 2001; 167:1683-92.
92. Kumar RK, Herbert C, Yang M, Koskinen AM, McKenzie AN, Foster P. Role of interleukin-13 in eosinophil accumulation and airway remodelling in a mouse model of chronic asthma. Clin Exp Allergy. 2002;32:1104-11.
93. Cohn L, Homer RJ, Marinov A, Rankin J, Bottomly K. Induction of airway mucus production by T helper 2 (Th2) cells: a critical role for interleukin 4 in cell recruitment but not mucus production. J Exp Med. 1997;186:1737-47.

94. Foster PS, Ming Y, Matthei KI, Young IG, Temelkovski J, Kumar RK. Dissociation of inflammatory and epithelial responses in a murine model of chronic asthma. Lab Invest. 2000;80:65562.
95. Kowalski ML, Pawliczak R, Wozniak J, Siuda K, Poniatowska M, Iwaszkiewicz J, et al. Differential metabolism of arachidonic acid in nasal polyp epithelial cells cultured from aspirin-sensitive and aspirin-tolerant patients. Am J Respir Crit Care Med. 2000; 161:391-8.
96. Mullol J, Fernández-Morata JC, Roca-Ferrer J, Pujols L, Xaubet A, Benítez P, et al. Cyclooxygenase 1 and cyclooxygenase 2 expression is abnormally regulated in human nasal polyps. J Allergy Clin Immunol. 2002;109:824-30.
97. Chambers LS, Black JL, Ge Q, Carlin SM, Au WW, Poniris M, et al. PAR-2 activation, PGE2, and COX-2 in human asthmatic and nonasthmatic airway smooth muscle cells. Am J Physiol Lung Cell Mol Physiol. 2003;285:L619-L27.
98. Picado C, Bioque G, Roca-Ferrer J, Pujols L, Mullol J, Benítez P, et al. Nuclear factor-kappa B activity is down-regulated in nasal polyps from aspirin-sensitive asthmatics. Allergy. 2003;58: 122-6.
99. Gavett SH, Madison SL, Chulada PC, Scarborough PE, Qu W, Boyle JE, et al. Allergic lung responses are increased in prostaglandin H synthase-deficient mice. J Clin Invest. 1999; 104:721-32.
100. Peebles RSJ, Hashimoto K, Morrow JD, Dworski R, Collins RD, Hashimoto Y, et al. Selective cyclooxygenase-1 and -2 inhibitors each increase allergic inflammation and airway hyperresponsiveness in mice. Am J Respir Crit Care Med. 2002;165:1154-60.
101. Colville-Nash PR, Gilroy DW. Potential adverse effects of cyclooxygenase-2 inhibition: evidence from animal models of inflammation. BioDrugs. 2001;15:1-9.
102. Kobayashi T, Miura T, Haba T, Sato M, Serizawa I, Nagai H, et al. An essential role of mast cells in the development of airway hyperresponsiveness in a murine asthma model. J Immunol. 2000;164:3855-61.
103. O'Byrne PM. Leukotriene bronchoconstriction induced by allergen and exercise. Am J Respir Crit Care Med. 2000;161: S68S72.
104. Hamilton A, Faiferman I, Stober P, Watson RM, O'Byrne PM. Pranlukast, a cysteinyl leukotriene receptor antagonist, attenuates allergen-induced early- and late-phase bronchoconstriction and airway hyperresponsiveness in asthmatic subjects. J Allergy Clin Immunol. 1998;102:177-83.
105. Henderson WRJ, Tang LO, Chu SJ, Tsao SM, Chiang GK, Jones F, et al. A role for cysteinyl leukotrienes in airway remodeling in a mouse asthma model. Am J Respir Crit Care Med. 2002;165: 108-16.
106. Burrows B, Martínez FD, Halonen M, Barbee RA, Cline MG. Association of asthma with serum IgE levels and skin-test reactivity to allergens. N Engl J Med. 1989;320:212-7.
107. Humbert M, Grant JA, Taborda-Barata L, Durham SR, Pfister R, Menz G, et al. High-affinity IgE receptor (FcepsilonRI)-bearing cells in bronchial biopsies from atopic and nonatopic asthma. Am J Respir Crit Care Med. 1996;153:1931-7.
108. Campbell AM, Vachier I, Chánez P, Vignola AM, Lebel B, Kochan J, et al. Expression of the high-affinity receptor for IgE on bronchial epithelial cells of asthmatics. Am J Respir Cell Mol Biol. 1998;19:92-7.
109. Mayr SI, Zuberi RI, Zhang M, De Sousa-Hitzler J, Ngo K, Kuwabara Y, et al. IgE-dependent mast cell activation potentiates airway responses in murine asthma models. J Immunol. 2002;169:2061-8.
110. Milgrom H, Fick RBJ, Su JQ, Reimann JD, Bush RK, Watrous ML, et al. Treatment of allergic asthma with monoclonal antiIgE antibody. rhuMAb-E25 Study Group. N Engl J Med. 1999;341: 1966-73.
111. Mehlhop PD, van de Rijn M, Goldberg AB, Brewer JP, Kurup VP, Martin TR, et al. Allergen-induced bronchial hyperreactivity and eosinophilic inflammation occur in the absence of IgE in a mouse model of asthma. Proc Nat Acad Sci. 1997;94:1344-9.
112. Hogan SP, Mould A, Kikutani H, Ramsay AJ, Foster P. Aeroallergen-induced eosinophilic inflammation, lung damage, and airways hyperreactivity in mice can occur independently of IL-4 and allergen-specific immunoglobulins. J Clin Invest. 1997;99:1329-39.
113. Korsgren M, Erjefalt JS, Korsgren O, Sundler F, Persson CGA. Allergic eosinophil-rich inflammation develops in lungs and airways of B cell-deficient mice. J Exp Med. 1997;185:885-92.
114. Wilder JA, Collie DD, Wilson BS, Bice DE, Lyons CR, Lipscomb MF. Dissociation of airway hyperresponsiveness from immunoglobulin E and airway eosinophilia in a murine model of allergic asthma. Am J Respir Cell Mol Biol. 1999;20:1326-34.
115. Brusselle GG, Kips JC, Taverner JH, Van der Heyden JG, Cuvelier CA, Pauwels RA, et al. Attenuation of allergic airway inflammation in IL-4 deficient mice. Clin Exp Allergy. 1994; 24:73-80.
116. MacLean JA, Sauty A, Luster AD, Drazen JM, De Sanctis GT. Antigen-induced airway hyperresponsiveness, pulmonary eosinophilia, and chemokine expression in B cell-deficient mice. Am J Respir Cell Mol Biol. 1999;20:379-87.
117. Gelfand EW. Pro: mice are a good model of human airway disease. Am J Respir Crit Care Med. 2002;166:5-6.
118. Persson CG. Con: mice are not a good model of human airway disease. Am J Respir Crit Care Med. 2002;166:6-7.
119. Zhang Y, Lefort J, Kearsey V, Lapa e Silva JR, Cookson WO, Vargaftig BB. A genome-wide screen for asthma-associated quantitative trait loci in a mouse model of allergic asthma. Hum Mol Genet. 1999;8:601-5.
120. Malm-Erjefalt M, Persson CG, Erjefalt JS. Degranulation status of airway tissue eosinophils in mouse models of allergic airway inflammation. Am J Respir Cell Mol Biol. 2001;24:352-9.
121. Denzler KL, Borchers MT, Crosby JR, Cieslewicz G, Hines EM, Justice JP, et al. Extensive eosinophil degranulation and peroxidase-mediated oxidation of airway proteins do not occur in a mouse ovalbumin-challenge model of pulmonary inflammation. J Immunol. 2001;167:1672-82.
122. Persson CG, Erjefalt JS. Degranulation in eosinophils in human, but not in mouse, airways. Allergy. 1999;54:1230-2.
123. Epstein M. Do mouse models of allergic asthma mimic clinical disease? Int Arch Allergy Immunol. 2004;133:84-100.