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Review

Experimental asthma in rats

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Abstract:
Animal models of asthma have been used for over 100 years. The accuracy of extrapolations from animal models to human asthmatics is highly dependent on the species of animal selected. The rat, in comparison with other animals, demonstrates many features of airway allergy and allergic asthma that are similar to the human conditions. The following features of human asthma can be effectively investigated in a rat model of the disease: cellular infiltration of the lung, antigen-specific IgE production, and a predominant Th2 response. The majority of available models of asthma are restricted to the acute inflammatory response following a short period of allergen exposure. The frequently used model of ovalbumin (OVA) sensitization and challenge replicates the inflammatory process in the airways.

Key words:
asthma, animal model of asthma, rat

Introduction

Animal experiments are widely used to study the pathophysiology of a variety of diseases. There are several reasons why scientists have adopted particular animal models. First, many studies cannot be performed in humans due to ethical concerns. Second, *in vitro* models are far removed from *in vivo* conditions. When we perform *in vivo* studies in certain animal species, we move closer to the conditions seen in humans, though the results should be carefully considered. The extrapolation from animal models to human asthmatics is highly dependent on the species of animal chosen.

Animal models of asthma have been used for more than 100 years [13]. Several species have been used to study respiratory tract allergies. These include guinea pigs, mice, rats, sheep, and dogs. Inbred mice and rats, as well as guinea pigs, are the most popular models for asthma [48]. The guinea pig model was the first described model of asthma and contributed greatly to the development of corticosteroid and β_2 receptor agonist therapies [39]. Currently, the use of guinea pigs is restricted by the lack of genetic modifications and of specific reagents. Sheep and dogs have a different biology from humans and are costly when compared with mice and rats [48]. The most important advantage of the mouse model of asthma is the development of transgenic technology; there are many mouse-specific probes without particular genes which allow the study of genetic factors in the pathology of asthma [3, 5]. Experimental asthma in mice can be developed by adoptive transfer of dendritic cells pulsed with antigens in culture, primed wild-

type T cells, or genetically modified T cells [20, 10, 18]. Nevertheless, the new strategies in asthma therapy proven in mouse models have been not effective in human clinical trials [44].

Rat species

The rat, in comparison with other animals, demonstrates many features of airway allergy and allergic asthma that are similar to those exhibited by humans [31]. The ability to extrapolate from rat studies to humans is one of the paramount advantages. Similarities between rats and humans include immediate and late asthmatic responses after an allergen challenge; responses after a nonspecific challenge with methacholine, acetylcholine, or serotonin; IgE production; and an accumulation of inflammatory cells [4, 46]. Airway hyperresponsiveness, airway inflammation, and obstruction – the main features of asthma – can be easily reproduced in rats. The ability to produce both early- and late-stage asthmatic responses, as well as airway hyperresponsiveness, represents the rat model's significant advantages over the mouse model [48].

Rats are relatively cheap, and their larger size facilitates the measurement of airway and systemic inflammations, due to an increased volume of serum and bronchoalveolar lavage fluid [48]. Additionally, their larger size ensures stability under anesthesia, which is important in measuring airway pulmonary function, hyperresponsiveness, and the acute response to allergen inhalation [48]. Rats are easily sensitized using ovalbumin (OVA) [11, 15, 19], house dust mite extracts [19] or Ascaris antigens [23]. There are an increasing number of reagents available for rat studies; also, transgenic technology applications in rats have recently increased [24].

Allergic bronchoconstriction in rats seems to be primarily mediated by serotonin [31]. It should be highlighted that the rat is a weak bronchoconstrictor: more agonist is necessary to produce a narrowing of the airways as compared to the doses required in guinea pigs [31]. Thus, the rat model of asthma is focused mainly on inflammatory processes [21, 31, 48]. There are, however, significant differences between respective rat strains. The Brown Norway strain is naturally atopic and presents a more pronounced IgE and inflammatory response to allergen challenges following allergen sensitization [25]; it is the most appropriate strain for studying allergic inflammation [11]. The common features of Brown Norway rats and human asthma include high IgE levels; early and late allergen responses (the latter can be adoptively transferred with CD4+ and CD8+ T cells); eosinophilia; Th2 cytokine production; a susceptibility to respiratory viral bronchiolitis; and interferon-γ deficiencies contributing to atopic status [21]. The early response after an allergen challenge in Brown Norway rats is mediated by serotonin and cysteinyl leukotrienes (cys-LT); it is blocked by methysergide and cys-LT receptor antagonists. The late response depends on leukotriene E4 (LTE4) and is attenuated by cys-LT receptor antagonists [12, 22, 36]. The late asthmatic response can also be blocked by corticosteroids or by anti-adhesion antibodies (anti-VLA-4) [32].

Wistar rats can also be sensitized and challenged with OVA, producing similar but less pronounced effects as compared to those observed in Brown Norway rats [15, 42]. In contrast, Sprague Dawley rats do not develop an allergic reaction or an increase in IgE production under the same conditions [27, 40] and usually serve as a control group for Brown Norway rats [2]. In Fisher and Lewis rats there is no pulmonary inflammation and no rise in IgE after an allergen challenge [38].

Animal studies are the first step in discovering new pathways and mediators that are important in the progression of a disease. The modification of certain pathways, cytokines, or chemokines facilitates the development of new drugs and new strategies for asthma pharmacotherapy. This includes inhibitors of phosphodiesterase 4 (PDE4); inhibitors of kinases [IKK, MAK (p38)]; inhibitors of signal transducers; activators of transcription 6; antibodies against cytokines (anti-tumor necrosis factor α); chemokines; and anti-VLA-4. *In vivo* animal models are the most effective tool for studying the effects of a drug because they involve intact immune and respiratory systems [48]. The rat shares many clinical features of human asthma [31]. The rat model of asthma is a standard model for testing new drugs and therapies before new drugs enter clinical trials.

An ovalbumin-induced model of asthma

No laboratory animal is known to spontaneously develop a disease with features that could be considered

asthma [43]. Experimental asthma can be induced by OVA-sensitization followed by an OVA challenge [11, 15, 49]. There are many modifications of this model, primarily concerning the method of OVA administration, the number of OVA injections, the number of OVA challenges, and the administration of adjuvants.

As mentioned above, the rat is a species that can be easily sensitized. We performed an experimental model of asthma in Wistar rats, the animal species often used in pharmacological experiments. OVA sensitization was done by intraperitoneal injection, and OVA was precipitated with aluminium hydroxide. This injection was repeated four times. On the first day a foot pad injection of heat-killed Bordetella pertussis was given as an adjuvant. The allergen challenge with OVA continued for seven consecutive days. In asthmatic animals, OVA sensitization followed by the OVA challenge produces a significant increase in total IgE and typical histological changes in the airways [15].

Adjuvants

In asthma models, adjuvants are used to prime the immune system to react in the desired fashion. In our experimental model, we used both aluminium hydroxide and heat-killed Bordetella pertussis [15]. Aluminium hydroxide, administered together with antigen exposure, promotes the Th2 phenotype [1]. Furthermore, lipooligosaccharide from Bordetella pertussis drives a Th2-biased response [8]. There are also adjuvants that promote a Th1 response, such as Freund's complete adjuvant [26]. The disadvantage of adjuvant use is the influence on the immune response, preventing a direct comparison between humans and animals after exposure to a certain allergen. This asthmatic response of the lungs has been demonstrated by intranasal sensitization of mice, without adjuvants, but the effect was strain specific [7].

A model of inflammation

Asthma is characterized by recruitment and activation of inflammatory cells; chemotaxis; bronchoconstriction; increased airway secretion and mucus cell hyperplasia; plasma exudation; neural effects; hyperplasia; hypertrophy of airway smooth muscle cells; and increased airway hyperresponsiveness [31]. The elements of the airway wall are very important to determine the response to an allergen and they are organized in epithelial-mesenchymal trophic units [6]. The progress of the disease is probably caused by the interaction of epithelial, interstitial, nervous and immunological factors [31]. Recruitment of the inflammatory and structural cells in the airways causes the release of a variety of mediators, aggravating the inflammatory response. Mediators may act synergistically to enhance their own effect, or one mediator may increase the release of another.

OVA sensitization and challenge causes an inflammatory response in the airways. This model is suitable for the study of acute inflammatory events. The infiltration of inflammatory cells involves mainly eosinophils, mast cells, neutrophils, and lymphocytes [15, 48]. The eosinophylic component is substantially triggered by an allergen. Based on allergic disease models, it is known that Th1 and Th2 responses are present in models of allergic inflammation. In sensitized rats, both CD4+ and CD8+ cells are activated in response to an allergen challenge; they express Th2 cytokines (18). The Th2 response typically involves an increase in interleukin (IL)-4, IL-5, and IL-13 [17]. The involvement of Th1 cytokines may explain IgE-independent mechanisms of allergic inflammation [37, 47].

A model of tolerance

In humans, repeated allergen exposure causes pathological structural changes in the airways and leads to airway remodeling. In animals such as the rat, sensitization and challenge with OVA cause profound morphological changes in the lungs. However, in both mice and rats, tolerance develops with increasing allergen challenges following sensitization [16, 48]. Repeated exposure to an allergen using the continuous exposure protocol induces an increase in IgE in the absence of an inflammatory response in the airway, suggesting that tolerance depends on local mechanisms, not systemic lymphocyte clonal deletion or anergy [31]. Allergen tolerance makes it impossible to develop a chronic allergic response; therefore, an ani-

mal model of asthma cannot be used to study the mechanisms of chronic asthma. One possible solution would be using a low dose for OVA challenge [45], but further studies are needed to clarify this phenomenon. Notably, tolerance should also be considered as an important model, especially to study the role of regulatory T cells, which are known to control the suppression of allergic responses [41].

A model of chronic asthma

Asthma is a chronic disease. One methodological limitation of animal models of asthma concerns the chronic stage of disease [9]. Epithelial disruption and desquamation are the most critical steps for the progression of the disease. However, as mentioned above, repeated allergen exposure results in tolerance rather than the progression of asthma. There have been several attempts to reproduce the chronic stage of the disease. In rats and mice, the pulmonary overexpression of cytokines essential to lung remodeling represents one possibility, but re-challenge protocols seem to be more promising [14, 28, 29]. Nevertheless, a model of chronic asthma needs to be established.

A model of airway remodeling

Anatomically, the rat airways constitute a lower percentage of the lung (5.7%) compared to airways in mice (11%) [31]. However, mice have a low proportion of airway smooth muscle in their airways, resulting in augmented airway constriction [13]. Therefore, the rat is a good experimental model to study airway remodeling that involves smooth muscle cells. Brown Norway rats in particular can be used to examine the contribution of airway smooth muscle cells in the remodeling process. After allergen sensitization, a rat requires at least three challenges before it is possible to see an increase in airway smooth muscle [35]. The increase in airway smooth muscle is caused mainly by hyperplasia, proliferation, and the inhibition of apoptosis [30, 33, 34].

Conclusions

Studies of rat asthma experimental models contribute greatly to our understanding of disease pathophysiology. The following features of human asthma can be profitably investigated in the rat model of the disease: cellular infiltration of the lung, antigen-specific IgE production, and predominant Th2 response. Of all currently applied animal models of asthma, none reflects all features of human asthma. The majority of available models of asthma are restricted to the acute inflammatory response following a short period of allergen exposure. The most frequently used model of OVA sensitization and challenge replicates the inflammatory process in the airways. The available models of asthma are limited by their lack of chronicity and by the use of adjuvants. These limitations should prompt investigators to find more appropriate methods that allow valid comparison with humans. Thus, experiments involving an animal model of asthma should precisely define which aspect of complex disease is the focus.

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