Allergic Rhinitis Model

Department of Otorhinolaryngology, Seoul National University

Chae-Seo Rhee

Introduction

The increasing prevalence of allergic rhinitis is posing significant socio-economic challenges. The pathogenesis of allergic rhinitis reflects a complex interaction of genetic and environmental factors. The heterogeneity of disease phenotypes challenges the concept of single mechanisms of disease. As human experimentation is limited, animal models have been developed to provide insights into pathogenesis and potential for discovery of novel therapeutics.

Allergic rhinitis animal model has several advantages. Animal models employ a wide range of test allergens, sensitization and provocation protocols, animal strains, and experimental endpoints. It has contributed significantly to the understanding of the genetics and immune-mediated pathophysiology of allergic rhinitis. Recent studies have revealed the effect of blocking antibodies and cytokine in pathophysiology of allergic rhinitis.

Mice, rat and guinea pig can be used as experimental animals in allergic rhinitis model. The results of experiments are reproducible and can be measured objectively with those animals. This cursory review is based on my experience in establishing first allergic rhinitis animal model in Korea.

Allergic rhinitis animal model

1. What is the difference in between allergic rhinitis and asthma model?

Allergic rhinitis and asthma are comorbid diseases and have shared immune pathways. Animal models of allergic rhinitis and those of asthma usually rely on the systemic sensitization and local challenge protocol. A study found out that simultaneous eosinophilic inflammation of both upper and lower airways followed aerosol challenge. Bronchial hyperreactivity of the lower airways was found in association with nasal mucosa thickening, suggesting common airway inflammation occurs after allergen inhalation. McCusker CT et al developed a murine model of allergic rhinitis and asthma using exclusive local sensitization and challenge of the upper airways. They demonstrated evidence of both upper and lower airway inflammation characterized by mucous gland hyperplasia and eosinophilic infiltration, and generation of allergen-specific IgE. Airway hyperresponsiveness was significantly increased following methacholine challenge in both locally and systemically sensitized animals.
Clearly, the anatomic, morphologic, and functional differences in the nasal airways compared with the pulmonary airways render them uniquely sensitive to disparate allergic responses. For example, both upper and lower airways can contribute to airway obstruction, but by quite different mechanisms. Smooth muscle contraction can narrow pulmonary airways, whereas vasodilation of highly vascularized mucosa can narrow nasal airway opening. Mucus overproduction and hypersecretion may also contribute to airway occlusion and contribute to functional obstruction of both nasal and bronchial airways. In general, mucus can be more readily cleared from the nose, but mucus plugging in pulmonary airways is a prominent feature associated with mortality in status asthmaticus. Thus, although a “one-airway” approach may be a useful paradigm in which to frame allergic rhinitis-asthma relationships, animal models that describe each condition separately, as well as in tandem, are needed to understand the pathophysiology of allergic airway disease.

In the separate study design, investigators used diluted ovalbumin more than 50 times intraperitonially divided 4 times. Intranasal ovalbumine was concentrated 5 times and given daily for 7 to 14 days consecutively to be locally sensitized. Awoke rodents were given allergen intranasally with pharyngeal reflex to avoid lower airway distribution of allergen. These kinds of effort make allergic rhinitis model different from asthma model.3)

2. Which animal is used in allergic rhinitis: rat, guinea pig or mice?

It is important to choose the appropriate animal to establish allergic model. Rodents like rat, guinea pig or mice can produce allergen specific antibody using adjuvant. Rats are cheap and most of allergen specific antibody is known to be IgE antibody. Still, intraperitoneal sensitization is inevitable, and adjuvant is needed for the sensitization. Guinea pig, which is frequently used in asthma model, is easy to deal with and can be sensitized with inhalation of allergen and produce airway inflammation. However, immunological study is limited as there is few inbred strain.

Mice are the most frequently used animal among them as there are several advantages over other animals.

1. Well established genetic information
2. Abundant pre-made products such as monoclonal antibodies, polyclonal antibodies, soluble receptors, PCR primers or kit for study
3. Various inbred strain
4. Easy to produce transgenic or gene-targeted mice
5. Mostly produce IgE allergen, like human
6. Relatively cheap

Production of antigen-specific IgE is different among strains of mice. When adjuvant cholera toxin B subunit was added in ovalbumin, BDF1, BALB/c, C3H/He strain showed high, moderate, and low responder, each. When Schistosoma mansoni egg allergen (SEA) was sensitized intranasally, BALB/c, CBA/J, C57BL/6 strain showed high, moderate, and low responder, each.4) House dust mite, which is the commonest allergen in Korea, produces different level of allergen specific IgE according to house dust mite species. For Dermatophagoides pteronyssinus allergen, CBA, C57BL/6 strains were shown to be high responders in that persistent IgE was induced which lasted for several months. In contrast, the C3H/He, BALB/c strains were judged to be poor responders.5) For Dermatophagoides farinae allergen, A/J, BALB/c strains produced high response while C57BL/6 and C3H/H3 strain showed moderate or low response.

So, it seems to be appropriate to select BALB/c mice in study with ovalbumin allergen, and C57BL/6 mice in study
with house dust mite allergen.

3. How to make allergic rhinitis animal model

There are some differences in allergic responses of action according to allergen and adjuvant, the dosage and the sensitization duration. For allergen, proteins such as ovalbumin (OVA), bovine serum albumin (BSA), ascaris extract, or SEA is frequently used. For adjuvant, aluminum hydroxide gel (alum), complete Freund’s adjuvant (CFA), lipopolysaccharide (LPS) or bordetella pertussis vaccine is commonly used. IgE antibody is produced later than IgG antibody. However, when allergen is injected with adjuvant, high dose IgE is produced.

In murine experimental models to investigate the regulation of IgE synthesis in vivo, administration of antigens via the natural route is desirable because administration of antigen via other routes results in different degrees of IgE production. Therefore, many trials of intranasal or aerosolized sensitization were performed to investigate the regulation of IgE production in murine airway allergy. However, attempts with intranasal or aerosolized sensitization resulted in either transient induction of antigen-specific IgE or none at all, causing tolerance rather than sensitization. In order to generate measurable production of IgE, antigens are usually inoculated with adjuvant. Adjuvant increases Th2 priming and allergic response. If adjuvant is injected intraperitoneally without allergen, non-specific Th2 cytokine is induced. If allergen is injected with allergen, allergen-specific Th2 response is augmented.\(^4\)

Delivery route is also affecting the allergic response in sensitization. Usually, human related allergen injected intranasally does not produce allergic response without adjuvant. So, for sensitization, either intraperitoneal or subcutaneous injection is preferred. The highest levels of IgE and eosinophil infiltration were achieved after systemic sensitization with allergen (plus adjuvant) followed by repeated airway challenge. Passive sensitization with allergen-specific IgE followed by limited airway challenge induced a modest eosinophilic inflammatory response in the airways despite high levels of serum IgE. Exposure to allergen exclusively via the airways also resulted in a modest serum IgE response and a limited eosinophilic inflammatory responses.\(^6\)

Sensitized animals are then challenged by either inhalation using aerosol, intranasal, or intratracheal routes. For allergic rhinitis model, intranasal (IN) inoculation using micropipette or paper disk is frequent way to be used.
Allergic stimulus is characterized by early phase response (EPR) and late phases response (LPR). EPR occurs within minutes of exposure to the allergen and tend to produce sneezing, itching, and clear rhinorrhea; this episode lasts for 5 to 30 minutes, and then wanes. The LPR occurs 6 to 24 hours after local allergen challenge of subjects characterized by congestion, fatigue, and irritability. Evidence for both animal and human clinical studies showed that preformed mediators released from mast cells and basophils (specifically histamines, tryptase, cysteinyl leukotrienes, and platelet activating factor) promote the initial irritation and sneeze reflex. During the LPR, other performed and synthesized mediators, predominately prostaglandins, interleukins, and chemoattractants, elicit the recruitment of eosinophils and neutrophils into nasal tissues.

In the allergic rhinitis mice model, enumerating the frequency of nasal rubbing and sneezes is a subjective but useful measure, especially for testing the EPR and therapies involving histamine- and leukotriene-dependent pathways. A change in respiratory frequency is a physiologically relevant parameter that has recently been used to estimate nasal obstruction. Decreases in respiratory frequency have been used as a surrogate marker in the EPR and LPR as mice are obligate nasal breathers.  

**Table 1. Requirements for IgE in development of AHR**

<table>
<thead>
<tr>
<th>Mode of sensitization</th>
<th>Strain</th>
<th>Eosinophils</th>
<th>IgE</th>
<th>Eosinophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>Passive</td>
<td>BALB/c</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Airway</td>
<td>BALB/c</td>
<td>+</td>
<td>++</td>
<td>++</td>
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<tr>
<td>Systemic</td>
<td>BALB/c</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
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<tr>
<td></td>
<td>C57BL/6</td>
<td>++</td>
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1. Altered airway function detected in tracheal smooth-muscle preparations in response to electrical field stimulation.
2. Altered airway function detected in tracheal smooth-muscle preparations as well as in vivo to inhaled MCh.


Fig. 7. Histopathologic changes (A) and eosinophilia (B) of nasal cavity 48 hours after last challenge. Subepithelial thickening and infiltration of inflammatory cells including eosinophils (arrow) and lymphocytes are prominent in all groups compared with control. *p<0.05 compared with control (H&E stain, original magnification × 400. The most right inferior one : magnification of AR group).
4. Estimation of allergic response in allergic rhinitis animal model

① Eosinophil infiltration in nasal mucosa: Mice were killed 24 hours after the last challenge. After perfusion fixation, the head was removed and fixed in 4% paraformaldehyde (PFA). Nasal tissue was decalcified, embedded in paraffin, and sectioned coronally about 5 mm from the vestibule. Each section was stained with Hematoxylin & Eosin and the number of eosinophils of the septal mucosa was counted.

② Serum was obtained from retro-orbital vein of mice and centrifuged in 3000 rpm for 10 minutes. Serum level of allergen-specific IgE was measured by solid-phase enzyme-linked immunosorbent assay (ELISA).

③ From the centrifuged serum and nasal lavage, Th2 cytokines (IL-4, IL-5, IL-13) and Th1 cytokines (IFN-γ, IL-12) levels were measured by ELISA.

5. Study designs of animal model in allergic rhinitis

① Allergic rhinitis mice model using ovalbumin

BALB/c mice were given 300 μl of phosphate-buffered saline (PBS) with 25 μg OVA and alum 1 mg intraperitoneally for systemic sensitization. From day 21, mice were intranasally inoculated with 30 μl of PBS with 500 μg OVA on 7 consecutive days. After 24 hours from the last nasal challenge, nasal symptom is induced with 30 μl of PBS with 500 μg OVA and then sacrificed.⁸)

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⁸) This refers to a previous study by the same author or another study that supports the methodology described.
② Allergic rhinitis mice model using house dust mite allergen

C57BL/6 mice were given 100 $\mu$l of PBS with 100 $\mu$g Der f with 100 $\mu$g of CFA subcutaneously at tail base for systemic sensitization. Then, 300 ng of Pertussis toxin were injected intraperitoneally on day 1 and 3. On day 7, 100 $\mu$g Der f with 100 $\mu$g of Incomplete Freund’s Adjuvant (IFA) were injected subcutaneously at tail base. After systemic sensitization, 20 $\mu$g of Der f was diluted with 20 $\mu$l of PBS and then, instilled intranasally for 6 times every week for challenge. After the last inoculation, allergic response was induced with same amount of allergen via intranasal inoculation with micropipette.9)

6. Limitations in allergic rhinitis animal model

Although much has been learned from these investigations, there are limitations when these models are translated to the human diseases. The murine model and human nose have distinct gross structural differences and distribution of epithelium. Marked differences in airflow patterns among mammalian species are primarily due to variation in the shape of nasal turbinates. The human nose has three turbinates: the superior, middle and inferior. These structures are relatively simple in shape compared to turbinates in most laboratory animals that have complex folding and branching patterns. In laboratory rodents, evolutionary pressures concerned chiefly with olfactory function and dentition have defined the shape of the turbinates and the type and distribution of the cells lining the turbinates. In the proximal nasal airway, the complex nasoturbinates and maxilloturbinates of small laboratory rodents probably provide better protection of the lower respiratory tract than the simple middle and inferior turbinates of the human nose. Mucosal swelling in turbinates, especially where they are in close opposition to the septum and lateral wall, can impede both airflow and mucus drainage through the nasal cavity.10) It is also known that chronic models have been difficult to establish in the mice. Acute and transient model reflects acute inflammation though it cannot show airway remodeling. Chronic model such as polyp model in mice takes about 2-3 months, which is shorter than human changes in nasal mucosa which takes more than several years. As the mice model usually does not develop spontaneously, the allergic response using adjuvant can be quite different from human allergic response including IgE production.

Conclusions

Mice model of allergic rhinitis is a great tool that allows in vivo studies to be conducted in the state of an intact immune and respiratory system. This model can provide many chances to find important signaling pathways and therapeutic target molecules in allergic rhinitis. Though some limitations exist, allergic rhinitis mice model will promise to unravel new horizon in the field of allergic rhinitis.

References


5. Stewart GA, Holt PG. Immunogenicity and tolerogenicity of a major house dust mite allergen, Der p I from Dermatophagoids pteronyssinus, in mice and rats. Int Arch Allergy Appl Immunol 1987;8:44-51


