

Chapter 15

Review of Mouse Models Applied to the Study of Asthma

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Abstract

The diversity of asthma phenotypes increases its complexity. Animal models represent a useful tool to elucidate the pathophysiological mechanisms involved in both allergic and nonallergic asthma, as well as to identify potential targets for the development of new treatments. Among all available animal models, mice offer significant advantages for the study of asthma. In this chapter, the applications of mouse models to the study of asthma will be reviewed.

Key words Animal models, Asthma, Challenge, Mouse, Sensitization

1 Introduction

Asthma is a complex and heterogeneous disease characterized by chronic inflammation of the airway, hyperresponsiveness, and recurrent symptoms such as sneezing, coughing, and breathing difficulty [1]. This complexity is caused by multiple environmental as well as genetic factors [1], which defines different forms of the disease expression as phenotypes (observable clinics characteristics) and endotypes (different pathogenic mechanisms) [2].

Currently, multiple animal models have been developed, not only for the study of asthma but also for other allergic diseases such as atopic dermatitis [3], allergic conjunctivitis [4], food allergy, anaphylaxis [5], and allergic rhinitis [6]. These mouse models are important to elucidate the pathophysiological mechanisms of asthma, as well as to evaluate both safety and efficacy of therapies in preclinical phase, before starting clinical phases in humans. In this chapter, we will focus on the study of different mouse models applied to asthma.

2 Mechanism of Asthma in Mouse

Animal models of asthma have been extensively used to examine mechanisms of the disease. Many advances in the understanding of the pathophysiology of asthma are based on these models [7]. The different cytokine profiles associated to asthma were initially described in mice [8]. Indeed, most of the mechanisms that are discussed today derive from studies conducted in animal models. As an example, the classical Th2 paradigm involving interleukin-4 (IL-4) or interleukin-5 (IL-5) was discovered using animal models [9]. However, these models only reflected allergic asthma. The identification of the interleukin 17 (IL-17), neutrophils that participate in the severe asthma or steroid resistant asthma, suggested that asthmatic disease was much more complex than what could be described by via Th1/Th2 paradigm [10]. These findings needed the development of appropriate mouse models that simulate the characteristics of these forms of asthma.

Respiratory viral infections can trigger asthma. The sendai virus (related to the human parainfluenza virus) has been administered to reach a chronic lung disease associated with airway hyper-reactivity (AHR) in mouse [1]. Asthma is also triggered by pollution. Mouse models have been exposed to ozone (a major component of aerial pollution) to develop AHR [11].

For the study of intrinsic asthma, the strain A/J mice has been used. These mice spontaneously develop AHR without manipulation [12]. The intrinsic AHR has been associated to a chromosomal region containing *adam33* gene. This association was firstly identified in a mouse model [13]. The identification of genes associated with asthma in mice before they do in humans reveals that animal models may be useful for the study of human asthma.

In summary, these models allow us to study specific pathways or genes related to different forms of asthma. This reductionist approach greatly simplifies the study of such a heterogeneous disease, enabling the understanding of mechanisms that would otherwise be difficult to elucidate.

3 Is Mouse the Ideal Animal Model?

A wide variety of animal species have been used for the study of asthma [14]. Mouse, horse, rat, dog, sheep or monkey, have been used for studies of airway inflammation [14–17]. Each has advantages and disadvantages as asthma model (Table 1). Besides the mouse, the species most used for experimentation in asthma are the guinea pig [18], sheep [19], and monkey [20].

Despite the variety of available animal, mouse is the most used model. The widespread use of the mouse for studies of asthma is

Table 1
Advantages and disadvantages of different animal models for the study of asthma

	Advantages	Disadvantages
Mouse	Short gestational period Easy manipulation Reaction IgE-mediated Small and cheap	Not spontaneous AHR Anatomic differences Limited airway musculature
Rat	Reaction IgE-mediated Late airway response	Immunological reagents not abundant Need adjuvants for sensitization
Rabbit	Reaction IgE-mediated Late airway response	Difficult manipulation

due to the advantages over other animals. The choice of the mouse as a basic model is mainly based on scientific and economic reasons.

3.1 Scientific Reasons

Mouse is the most used model in multiple human diseases. This is due to the extensive knowledge we have obtained from the multiple genetic studies previously conducted [19] as well as the ease handling for the generation of transgenic animals [21]. With regard to allergic diseases, these animals are easily sensitized, using allergens such as the ovalbumin (OVA), and House Dust Mite (HDM) [22], or molds [23].

In addition, the existence of different mouse strains, which do not behave in the same way from the same allergen, is an advantage for identifying the mechanisms [24, 25] of inflammation and airway hyperresponsiveness.

In recent years, the rapid expansion of transgenic technology has allowed the development of mouse models in which the selective expression of a gene is inhibited (Knock-out), or induced (Knock-in) [26]. These tools allow us to more clearly understand the molecular pathways involved in the development of asthma [27].

3.2 Economics Reasons

Besides the previously exposed scientific reasons, there are also economic reasons that favor the use of mice as asthma model. Firstly, there are numerous and relatively cheap commercially available mouse-specific probes for studying allergic outcomes that allow conducting large studies. In addition, due to the small size and easily handling, many mice can be maintained in small areas.

Mouse also has a short life cycle. Its gestation period is 21 days that facilitates the rapid procurement of animals for experiments. In this short period, it has large litters (6–8 mice) that rapidly provide a lot of animals. In addition, it reaches sexual maturity in a relatively short period of time (6–8 weeks).

In summary, mouse appears to be a good model for conducting a variety of experiments aimed at elucidating the mechanisms involved in asthma.

4 Limitations of Animal Models in Asthma

Despite the undoubted advantages of animal models for the study of asthma, they also have limitations that must not be forgotten. These limitations must be always taken into account before choosing the model due to the influence that may have on the results obtained. The limitations relate to data extrapolation to humans, adjuvants used, chronicity of the disease, or anatomical differences, among others.

4.1 Extrapolation to Humans

The question that arises after obtaining data on animal models of diseases, including asthma, is how to extrapolate the results to humans. Animal models used in the laboratory do not spontaneously develop asthma; this is why different protocols are performed. The technique variations are particularly important as can have a significant impact on the outcome and are often overlooked when comparing responses between studies.

The general technique for inducing asthma in animals involves sensitizing to a previously unseen antigen and subsequently challenging the airways with the same antigen, in order to study the cascade of events or an outcome parameter of interest [28]. This is an experimental asthma induced in the airway of the animal that has to be compared to the naturally developed human asthma.

4.2 Adjuvants

Besides the allergen, an adjuvant is usually added [8]. This molecule modulates the immune response. It acts as immune enhancer, ensuring a sufficiently intense immune response. One of the more used adjuvants is aluminum hydroxide or Alum [29]. Other adjuvants, used although to a lesser proportion, are heat-killed *Bordetella pertussis* [30] or the complete Freund's adjuvant [28].

The main problem of using adjuvants is that they may alter the mechanism of sensitization to the allergen under consideration, and modify the immune response [26]. To avoid these problems, adjuvant-free models [31] or models that inoculate previously stimulated immune cells such as T lymphocytes [32] have been developed.

4.3 Chronicity of Asthma

Besides the inflammatory process, a remodeling of the airway occurs as a result of the chronicity in asthma. This remodeling involves goblet cell metaplasia and hyperplasia [33], mucus hyper secretion [34], and thickening of airway smooth muscle [35] due to the repeated exposure to the allergen. In addition, animal models initially respond to the intranasal allergen provocation, but when provocation is prolonged, the animal may develop tolerance [36]. To avoid this, the provocation can be done with low doses main-

tained over time. With this strategy, mouse models that express the characteristics of chronic asthma have been developed [37].

4.4 Asthma in Early Life

The animals used for the study of asthma are normally adults. However, human asthma can appear early in life. At that point, there are situations such as in uterus environment [38], viral infection [39], exposure to allergens [40], smoking, and pollution [41] or pets [42] that should be considered, therefore, models that allow studying asthma in early life are needed.

4.5 Anatomical Differences

It is important to highlight that the anatomical structure of the airway is different in rodents than humans. Animals are quadrupeds and due to gravity, this position may influence the effort made by the lungs to inside moving the air [43]. This situation is aggravated by the airflow limitation due to the asthma reaction.

Another important aspect is the morphology and arrangement of the bronchial tree that affects the penetration of the allergen into the lung. It has been reported an inverse relationship between body size and relative airway caliber in rodents [44]. In addition, mice exhibit a thin smooth muscle layer that causes an easier constriction of the airway [45] and a high number of goblet cells [46].

4.6 Size

The size of the animal greatly influences lung function. For long time, to evaluate lung function in small animals has been challenging. Currently there are three methods:

- Noninvasive methods: the most widely used is whole body plethysmography. The animal is placed in a chamber and the respiratory parameters are indirectly analyzed before and after methacholine administration.
- Invasive methods: it is considered the gold standard. The animal is anesthetized and a tracheal tube is introduced to directly measure lung volumes before and after the methacholine administration.
- Electrical field stimulation (EFS): although the in vivo response to inhaled methacholine is the most widely used method of assessing the AHR, it was initially limited due to the difficulty of delivering an aerosol to the mouse airways. EFS has been described as an alternative method to assess AHR in mouse models.

5 Asthma Model Design

Currently, there are a variety of mouse models for the study of asthma. The general outline is based on an initial systemic sensitization followed by an aerial local elicitation. Depending on the hypothesis, several aspects must be taken into account, the mouse strain, type of allergen, route of administration, and induction time among others.

5.1 Mouse Strain

The availability of a high number of different mouse strains is an advantage. Mouse strains can be classified according to the capacity of developing airway inflammation and AHR. There are responder strains, such as A/J and AKR/J with high levels of AHR to methacholine [47] or non-responder strains, as the C3H/HeJ or DBA/2, that are resistant to allergen-induced AHR [48]. However, the most used are the BALB/c and C57B/6 strains because their immune response is well characterized. The BALB/c respond occurs via Th2 that typically induces allergic parameters, such as IgE production, AHR, and eosinophilic inflammation of the airway; however, C57CL/6 has limitations in developing allergic airway response because the immune response occurs via Th1. This strain is used in many genetically manipulated mice.

Different behaviors of mouse strains are mainly due to genetic characteristics. Genetic manipulation has favored the generation of mice to study molecular mechanisms involved in asthma. Thus, mice that do not express a particular gene (knock out) or conversely overexpress the gene of interest (knock in) have been developed. A third model is the conditional knockout mouse in which the expression of the gene can be manipulated to the necessary extent.

5.2 Type of Allergen

A variety of allergens have been used in animal models. One of the most used allergen has been ovalbumin (OVA) in both sensitization and challenge phases. It is cheap, well characterized, and can be produced in large quantities [49]. As already described, the continuous administration of the allergen can trigger tolerance in various mouse strains. At the stage of sensitization, OVA is usually injected with an intraperitoneal adjuvant. The challenge phase is performed by air without adjuvant. The allergic induction caused by OVA is not the same to be obtained by allergens usually present in the environment.

The generation of asthma models that are more approximate to human requires allergens such as pollen, mods, or HDM, naturally present in the environment. These aeroallergens, suspended in the air, directly reach the airway, while OVA normally access to the body through the digestive tract. The progress of biotechnological techniques is focused in generating isolated epitopes responsible for the asthmatic phenotype to achieve more specific and potent responses than the obtained with extracts. Other models combine two or more allergens getting stronger inflammatory responses [50]. Finally, allergen concentration varies depending on the phase of the reaction and type of allergen.

5.3 Route of Allergen Administration

The route of allergen administration depends on the phase of the experiment. Thus, in the sensitization phase, the intraperitoneal via is commonly used, while in the provocation phase the allergen is introduced by air to generate a local response. Nowadays, in the

sensitization phase, it is common to replace the intraperitoneal route by the intubation, resembling what happens in human asthma [51].

There are different strategies in the elicitation phase. Nebulization implies that the concentration of allergen does not penetrate far enough into the airway remaining in the upper respiratory tract. Other possibility is the application of allergen directly in the airway [52, 53], on the nostrils of the mouse, via intra-tracheal or depositing the allergen into the lungs with a bronchoscope. The choice of the method to be used is determined by the availability of material, as well as technical skill at the laboratory.

5.4 Induction Time

In mice, the induction of asthma can be achieved in short exposures to the allergen, days or weeks, or in longer periods of months. According to the exposure time, there are two different models, acute or short-term and chronic or long-term model. In acute models, high concentrations of allergen are used to obtain the asthmatic response in a short period. These models are useful to study airway inflammation but do not reflect all changes that occur in the human response.

However, in chronic models, the exposure to the allergen for longer periods produces inflammation and airway remodeling as in human asthma. The main problem of chronic models is tolerance that can be avoided by using low doses of allergen, as previously mentioned.

6 Mouse Models of Asthma

Most mouse strains do not spontaneously develop allergic airway inflammation or AHR; for this reason, different inducing agents are used. The mouse models of asthma can be grouped according to the phenotypes of human asthma. The models that have been conventionally developed are those aimed to study allergic asthma in acute or chronic models as previously seen.

Other models to study asthma phenotypes have been designed. In these models, molecules like ozone, cigarette smoke, diesel particles, or infectious agents can induce the response as respiratory virus although models of intrinsic asthma such as the strain A/J can be used. In addition to these classic models, genetically modified mice help to better understand the metabolic pathways involved in asthma.

Finally, different types of purified blood cells from asthmatic patients can be transferred to nude mice SCID (Severe Combined Immunodeficiency) simulating human asthmatic reactions in mice. All these models can also be used for the identification of new therapeutic targets for the disease as well as for the development of new drug.

In summary, the mouse models represent an opportunity to study the mechanisms involved in asthma and find targets to develop new treatments. The challenge in this field is to develop models that more closely approximate to human asthma, reflecting all changes that occur in the disease.

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