Asthma Model

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Abstract

Asthma is a chronic inflammatory airway disease. In spite of advances in technology the pathobiology of asthma is still relatively poorly understood. There is no doubt that animal models are indispensable to study detailed aspects of pathogenesis at cellular, molecular, and genetic levels and to develop therapeutic strategies. The range of animal models available is vast, with the most popular models being rodents (inbred mice and rats) and guinea-pigs, which have the benefit of being easy to handle and being relatively cost effective compared with other models that are available. There are a number of issues with current animal models of asthma that must be recognized including the disparity in immunology and anatomy between these species and humans, the requirement for adjuvant during sensitization in most models, the acute nature of the allergic response that is induced and the use of adult animals as the primary disease model. Therefore, careful use of well-defined models in asthma allows researchers to answer specific questions that are otherwise difficult to address.

This review focus on differences of lung structure and function between human and animal models and transgenic mouse model and zebrafish in study of asthma.

Introduction

Asthma is a heterogeneous disease having subgroups defined by etiology, pathology, severity, and physiologic parameters and response to treatment. Characteristic features of asthma are allergen-induced early and late bronchial reactions, airway inflammation, structural changes to the airway wall associated with progressive decline in lung function, and airway hyperresponsiveness (AHR). Although not completely, asthma can adequately be characterized with four of its hallmarks: (i) the usually biphasic allergic airway reaction; (ii) the hyperreactivity of the airways; (iii) the persisting chronic inflammation; and (iv) the remodeling of the airways. That makes it difficult to find a single feature of human asthma which can be used as a universal measure to evaluate the animal models of the disease. Summarizing the present knowledge leads to the following conclusions: (i) there is no animal with a natural disease perfectly mimicking asthma and (ii) no animal model is available that completely reproduces the
multiple features of human asthma. Current models are mainly based on allergic exposure or sensitization. However, even animal models named ‘chronic’ do not reflect many of the well known features of human asthma. Special forms of asthma (non-allergic, aspirin-induced, exercise-induced) are usually not modeled in rodents or small laboratory animals.3)

The challenge of the future is therefore to identify the most relevant animal model for each question to be answered. This challenge requires the communication between medicine with respect to comparative and species-specific aspects of anatomy, physiology, immunology, pathology and clinics related to the specific model required, and the availability and applicability of technical solutions. Animal models have been, and will continue to be, vital in understanding the mechanisms that are involved in the development and progression of asthma. This review focuses on differences of lung structure and function between human and animal models and transgenic mouse model and zebrafish in study of asthma.

**Animal model of asthma**

No animal model completely summarizes all features of the human disease.7) Research has focused primarily on ways to generate allergic inflammation by sensitizing and challenging animals with a variety of foreign proteins, leading to an increased understanding of the immunological factors that mediate the inflammatory response and its physiological expression in the form of AHR.6,7) Small animal models of asthma, using mice, rats and guinea pigs, have proven to be extremely useful for the investigation of potential mechanisms of airway pathophysiology in the intact organism in vivo, as well as in isolated organs and cells ex vivo.6,7) Likewise, animal models have been indispensable for the identification of a vast number of potential drug targets, as well as for efficacy and safety testing of new drugs.6)

There are some examples in the animal world that are similar to asthma; cats may develop a bronchial disease that is similar to human chronic asthma,8) horses may develop a neutrophil dominated airway disease known as emphysema,9) and both sheep10) and dogs11) are known to have a natural susceptibility to some allergens. A majority of animal models of asthma have targeted the classic Th2 asthmatic phenotype, which is characterized by high levels of antigen-specific IgE, airway inflammation dominated by eosinophils and a pattern of Th2 cytokines including IL-4, IL-5 and IL-13.12,13) It is well known that the responses of the airways to such a protocol (sensitization and challenge) can differ substantially between species.12,13) This is due to a number of reasons including the method of sensitization,9) the antigen used14) and species-specific differences including anatomical, physiological and immunological responses.21) The species chosen and method of creating the asthmatic phenotype are highly dependent on the particular aspect of the disease that is of interest with some species or strains being better for modelling particular characteristics of asthma than others.12-14)

**Mouse model**

Currently, the most widely used experimental animal for modelling allergic responses in the airways is the mouse, particularly because of the availability of transgenic and gene targeted animals, as well as the variety of commercially available mouse-specific immunological tools for phenotypic and functional analysis of cells and mediators.4,6) Mice are an ideal species for the study of most diseases.15) Because it is easy to manipulate outcomes using transgenic...
technology, there are numerous commercially available mouse-specific probes for studying allergic outcomes and they are relatively cheap, allowing various studies to be conducted. Mice are easily sensitized to a number of antigens, to which they are not normally exposed, including ovalbumin, which is the most popular, and a number of recognized human allergens such as house dust mite, cockroach antigens, Aspergillus fumigatus and ragweed extracts. Sensitization and subsequent challenge with these antigens result in a clearly defined Th-2 type response in the lungs, with the level of antigen-specific IgE, eosinophilia and responsiveness of the airway to bronchoconstricting agents varying considerably between strains. There are some differences between human and mouse (Table 1), This simple difference in response between strains for a given antigen sensitization and challenge protocol can be seen as an advantage of mice as models of asthma as it allows the identification of cellular and genetic mechanisms of inflammation and AHR.

**Another animal models**

Mammalian tracheo-bronchial airways are complicated and cannot be defined by one idealized branching system. The branching pattern of the conducting airways is significantly asymmetrical in the human, and asymmetry is believed to have an important effect on air flow. Three idealized branching systems are commonly recognized in different species (Table 2): (i) monopodial (at the branching point a small segment may branch from the main, or parent, stem), (ii) dichotomous (the parent segment may divide into two equal daughter segments), and (iii) polychotomous (the parent segment may divide into many daughter segments).

Due to the anatomy of extrathoracic airways and the length of the tracheo-bronchial tree, the ratio between dead space volume and tidal volume (Vd/Vt) differs between different species. There are some differences of various

### Table 1. Comparison of human asthma with mouse models

<table>
<thead>
<tr>
<th>Feature</th>
<th>Human (exacerbation)</th>
<th>Mouse (short-term models)</th>
<th>Mouse (chronic model)</th>
<th>Mouse (exacerbation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distal airway inflammation</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Peak eosinophil recruitment</td>
<td>?</td>
<td>24 h</td>
<td>4 h</td>
<td>4 h</td>
</tr>
<tr>
<td>Eosinophils in BAL fluid</td>
<td>++</td>
<td>+++</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Lymphocytes in BAL fluid</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>AHR</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Chronic inflammation in tissue</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Airway wall remodeling</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

### Table 2. Airway branching in different animal species

<table>
<thead>
<tr>
<th>Species</th>
<th>Branching system of tracheo-bronchial airways</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>Irregularly dichotomous</td>
</tr>
<tr>
<td>Mouse</td>
<td>Monopodial</td>
</tr>
<tr>
<td>Rat</td>
<td>Monopodial</td>
</tr>
<tr>
<td>Hamster</td>
<td>Strictly monopodial</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Primarily irregular dichotomous</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>Irregular to the pulmonary region but regularly dichotomous thereafter</td>
</tr>
<tr>
<td>Dog</td>
<td>Monopodial (within a lobe) follows an irregular dichotomized pattern with fractal features</td>
</tr>
<tr>
<td>Rhesus monkey</td>
<td>Baboon irregularly dichotomous</td>
</tr>
</tbody>
</table>
Table 3. Functional and inflammatory response to allergen exposure

<table>
<thead>
<tr>
<th></th>
<th>Early BDR response</th>
<th>Late BDR response</th>
<th>Bronchial hyperresponsiveness</th>
<th>BAL, brushing, biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat</td>
<td>◯</td>
<td>◯</td>
<td>◯</td>
<td>Eosinophils and neutrophils</td>
</tr>
<tr>
<td>Dog</td>
<td>◯</td>
<td>◯</td>
<td>◯(-50%)</td>
<td>Eosinophils and neutrophils</td>
</tr>
<tr>
<td>Sheep</td>
<td>◯</td>
<td>◯</td>
<td>◯</td>
<td>Eosinophils and neutrophils</td>
</tr>
<tr>
<td>Pig</td>
<td>◯</td>
<td>◯</td>
<td>◯</td>
<td>Neutrophils</td>
</tr>
<tr>
<td>Cattle</td>
<td>×</td>
<td>×</td>
<td>◯</td>
<td>Eosinophils and neutrophils</td>
</tr>
<tr>
<td>Horse</td>
<td>×</td>
<td>◯</td>
<td>◯</td>
<td>Neutrophils</td>
</tr>
<tr>
<td>Monkey</td>
<td>×</td>
<td>◯</td>
<td>◯</td>
<td>Eosinophils and neutrophils</td>
</tr>
</tbody>
</table>

animals in terms of inflammatory responses to allergen exposure (Table 3).

Rats due to large size\(^{33}\) makes it easier to measure the classic characteristics of allergic airways disease such as airway and systemic markers of inflammation due to an increase in the volume of serum and BAL fluid that can be obtained. Rats are relatively cheap, and their larger size facilitates the measurement of airway and systemic inflammations, due to an increased volume of serum and BAL fluid.\(^{4}\) Additionally, their larger size ensures stability under anesthesia, which is important in measuring pulmonary function, AHR, and the acute response to allergen inhalation.\(^{3}\) Rats are easily sensitized using ovalbumin,\(^{34-36}\) house dust mite extracts\(^{36}\) or *Ascaris* antigens. There are an increasing number of reagents available for rat studies. Transgenic technology applications in rats have recently increased. Sensitized rats have been shown, using appropriate methods for measuring lung mechanics, to not only demonstrate increased responsiveness to non-specific bronchoconstricting agents\(^{17}\) but also acute responses to allergen inhalation.\(^{38,39}\) Rats are often used as a standard model for testing new drug therapies; in particular, the efficacy and toxicity of new drugs are often tested in rats before proceeding with clinical trials.\(^{40}\) As with mice, one major criticism of rat models of asthma is the tolerance that develops with increasing allergen challenges following sensitization, which essentially, prevents the development of a chronic allergic response and the associated changes in lung structure and function seen in asthmatics.\(^{37-40}\)

Guinea-pigs\(^{41}\) are limited in terms of mechanistic studies, particularly those involving genetics, due to the low number of inbred strains and lack of guinea-pig-specific reagents available. The guinea-pig is the most widely used model and test system for contact hypersensitivity to chemical irritants and proteins.\(^{41}\) Guinea-pigs are also often used as a screening model for drugs that act through particular pathways that are seen to be relevant to human asthma and have been useful in the development of drugs such as corticosteroids and b2 receptor-agonists.\(^{41}\) Guinea-pigs are useful as models of immediate hypersensitivity to irritants and have pharmacological responses similar to humans, although care must be taken when applying this to all pathways in the human lung.\(^{41}\)

Larger animal models such as dog and sheep, etc. These models, in terms of the quantity of studies that have been conducted, are nowhere near as popular as the previous models but this is probably simply due to their cost and the lack of specific probes available for studying their allergic responses.

**Transgenic models in allergic responses**

The rapid expansion in transgenic technology in recent years makes mice ideal for mechanistic studies, whereby a single molecular pathway can be switched off, suppressed or up-regulated\(^{4,16}\) in order to understand the importance
of this pathway in the development of the asthmatic phenotype. Such studies in mice have highlighted the importance of the cytokines IL-4, IL-5 and IL-13, \(^{42,43}\) which are thought to be critical in Th2-driven allergic reactions in the airways in a large portion of human asthmatics. The term transgenic animal\(^{44}\) refers to an animal in which there has been a deliberate targeted modification in the genome - the material responsible for inherited characteristics - in contrast to spontaneous mutations as documented e. g. in the S(pontaneous)C(ombined) I(mmuno) D(eficiency) mice. Moreover, the transgenic model provide reliable models for the preclinical approval of therapy for allergic asthma to develop more efficient compounds and functional antibodies.\(^{44}\) Transgenic and knockout murine models of transcription factors and airway remodeling provided very important information for the human disease.\(^{44}\) The use of transgenic mouse models is a powerful tool to dissect tissue specific function of several genes important for the pathogenesis of allergic asthma and furthermore, germline transgenics offer the opportunity to verify the acute and chronic state of the disease while producing stable transgenesis and same genetic conditions even in different models of investigation.\(^{32-44}\)

A Drosophila asthma model

Although asthma is typically complex one, that has an important genetic component. Genome-wide association studies have provided us with a relatively small but comprehensive list of asthma susceptibility genes that will be extended and presumably completed in the near future.\(^{45}\) To identify the role of these genes in the physiology and pathophysiology of the lung, genetically tractable model organisms are indispensable and murine models were the only ones that have been extensively used.\(^{45}\) An urgent demand for complementary models is present that provide specific advantages lacking in murine models, especially regarding speed and flexibility.\(^{45}\) Among the model organisms available, only the fruit fly \textit{Drosophila melanogaster}\(^{40}\) shares a comparable organ composition and at least a lung equivalent. It has to be acknowledged that the fruit fly Drosophila has almost completely been ignored as a model organism for lung diseases, simply because it is devoid of lungs.\(^{45}\) Nevertheless, its airway system shows striking similarities with the one of mammals regarding its physiology and reaction towards pathogens, which holds the potential to function as a versatile model in asthma-related diseases.\(^{45}\)

Conclusion

Animal models of allergic asthma have been applied to the investigation of the mechanisms of acute and chronic AHR. Despite the concerted efforts of scientists and clinicians, the pathobiology of asthma is still relatively poorly understood. The recognition that asthma is a heterogeneous disease involving a number of pathways, and the ethical issues associated with the required studies in humans, has required the development of tools to assist in mechanistic studies of asthma. The tool that allows the most effective investigation of disease mechanisms and progression involving an intact respiratory and immune system is the animal model of asthma. There are a wide array of models available for study both within and between species. Each model has its own limitations and advantages which must be recognized when designing studies and interpreting the results that are obtained. Issues that need to be considered include immune and anatomical differences between the animal and humans.
References

23. Takeda K, Haczku A, Lee JJ, Irvin CG, Gelfand EW. Strain dependence of airway hyperresponsiveness reflects


