Animal Models of Allergic Asthma

Heidi Isenberg-Feig, MD, J. Paul Justice, PhD, and Andrea Keane-Myers, PhD

Address
Laboratory of Allergic Disease, Eosinophil Biology Section, NIAID/NIH, Room 200E, Twinbrook II, 12441 Parklawn Drive, Rockville, MD 20852, USA.
E-mail: akeane@niaid.nih.gov

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We are fortunate to have many species of animals that can serve as adequate models for allergic disease in humans. This review is focused mostly on models of allergic airway disease, and some major categories of animals used for asthma research are discussed, including rodents and non-human primates. Furthermore, evidence that supports and criticizes the use of animal models of asthma is provided. There is no animal model that exactly reproduces the pathology of human asthma. However, these models are necessary for the development of novel therapies and an understanding of the detailed pathogenesis of the response of mammals to respirable allergens.

Introduction

The oldest mention of asthma may date back as early as the 26th century BC in the *Canon of Internal Medicine* of "The Yellow Emperor" in ancient China. It describes an ailment in which "man (has) noisy breathing" and "wheezing", making him "unable to walk," and his pulse "large and full" [1]. The "Ebers Papyrus" may contain sections that date back to the 30th century BC, and describes "remedies to eradicate asthma," such as bird dung, beer, figs, grapes, and frankincense [2]. Despite its early description, asthma remains one of the most complex disorders in both treatment and pathogenesis.

In spite of advances in technology and hygiene, and the introduction of vaccines and modern medications, the prevalence of asthma has continued to increase in the past 30 years. The explanations for this increase are not completely understood, but many have been proposed, including both intrinsic and extrinsic factors. The extrinsic factors, such as proteinaceous allergens (ragweed, pollens, cockroach, dust mite), or chemical irritants (cigarette smoke, ozone, diesel exhaust fumes) are compounded by intrinsic factors such as genetic variations in beta-2 adrenergic receptors, interleukin-10 (IL-10) promoter polymorphisms, and other variations. However, the increasing incidence of asthma is likely due to a combination of our changing environment and its effect on the immune system, particularly in genetically predisposed individuals.

Human asthma is characterized by bronchial hyperreactivity, reversible airflow obstruction, increased pulmonary mucus production, and airway inflammation, followed by chronic changes in airway architecture and sub-basement membrane collagen deposition known as airway remodeling [3•]. Researchers have investigated the treatment of allergic asthma from several angles and mechanisms of action. However, a true "cure" for asthma seems out of reach. Currently, the most effective treatments are directed toward the endpoints of the disease (*eg*, corticosteroids for T-cell dysregulation, beta agonists for bronchospasm). The National Heart Lung and Blood Institute has instituted several "asthma study centers" around the country, which focus on the immunology and pathogenesis of atopic disease in humans. These groups, as well as other smaller groups, have utilized animal models to help describe the basic science behind human disease. The advancement of these models has improved our understanding of the disease process and has allowed for the development of several new classes of therapeutic compounds that may alleviate disease severity.

Characteristics of Human Asthma

As we investigate the immunologic mechanisms involved in human asthma, it has become difficult to identify a single cell type or factor that plays the dominant predisposing element in the development of allergic asthma. Over the past 40 years, several cell types have been suggested as the primary mediator in this disease process. Of these cell types, eosinophils have most often been implicated as their abundance within the airway in status asthmaticus has suggested an important role in the disease process. Eosinophil research has led to the identification of a series of cell-specific granule proteins that have cytotoxic properties, including major basic protein (MBP), eosinophil-derived neurotoxin (EDN), eosinophil-derived peroxidase (EPO), and other eosinophilic cationic proteins (ECP) [4]. The presence of eosinophils within the airways can lead to cytotoxic cell death and subsequent damage to normal airway architecture. However, the extent to which this effect leads to labored breathing is controversial. In fact, there are several studies that report eosinophil-independent experimental asthma, and recent trials pertaining to eosinophils do not implicate these cells as the major contributor to histamine-induced airway hyperreactivity in human asthma [5].

The recruitment of eosinophils to the airways following exposure to aerosolized antigens appears to be a T-cell mediated event and indicates that eosinophil influx likely represents the endpoint rather than the cause of the cascade of events representing the disease process. This finding has led investigators to suspect that T cells may contribute to the development of allergic asthma. Gonzalo *et al*. [6] indicated the important role of T cells in experimental asthma after observing the absence of eosinophils in the lungs of CD4+-deficient mice [6]. Furthermore, T cells are required for the development and expansion of allergen-specific B cells and subsequent immunoglobulin E (IgE) and IgG antibody production. The role of T cells in allergic inflammation is also supported by the effectiveness of corticosteroid treatment in allergic disease, as corticosteroids are known to inhibit both T-cell proliferation and cytokine production.

Another important cell type that is thought to contribute to the effects of asthma is the mast cell. Mast cells affect connective tissue and mucosal surfaces via antibody-mediated histamine release. It is speculated that the release of soluble factors such as cysteinyl leukotrienes and platelet-activating factor may play a significant role in the precipitation of more advanced and chronic inflammation [7]. Inhibitors of mast cell granule contents, such as antihistamines and cysteinyl leukotriene receptor antagonists are effective therapies for the treatment of allergic inflammation, but may not prove as effective in the treatment of airway hyperresponisveness.

Why Use Animal Models?

We are fortunate to have many species of animals that can serve as adequate models for allergic disease in humans. This review focuses mostly on models of allergic airway disease, and some major categories of animals used for asthma research, including rodents and nonhuman primates, are discussed.

Because asthma is a multifactorial and multifaceted disease, some models are more suitable for studying aspects of human disease than others. Experimental asthma is characterized by both early- and late-phase bronchoconstriction, airway infiltration with inflammatory cells (*eg*, CD4+ T cells, eosinophils, mast cells, and basophils), mucus production, peripheral blood eosinophilia, goblet cell metaplasia and hyperplasia, and bronchial hyperresponsiveness. Many of these findings mimic those found in human asthma, thus providing an important tool in the study of the disease.

Although protocol designs vary among investigators, the basic model is consistent. The animal receives systemic sensitization of an antigen (intraperitoneally or subcutaneously) either with or without an adjuvant to develop immunologic memory. In animal models, adjuvants (*eg*, aluminum hydroxide, incomplete Freund's adjuvant) are used to both control and enhance the induction of immunologic memory for a given antigen. Later, the animal is exposed to antigen via its airway by an intratracheal or intranasal bolus or by nebulization. Many antigens have been used for experimental asthma protocols, including experimental (ovalbumin [OVA]) and environmental allergens (*eg*, ragweed, dust mite, and *Aspergillus*). In addition, parasitic egg antigens such as *Ascaris suum* and *Schistosoma mansoni* have also been used. Some of these are discussed in further detail later in this paper.

Measuring physiologic airway parameters such as airway hyperreactivity in animals is a unique tool in studying experimental asthma. This can be implemented in several ways, using both in vitro and in vivo methods. In vitro measurement of airway responsiveness is generally achieved through electrical-field stimulation using a tracheal bath apparatus and administering electrical stimuli to presensitized animal airways. Upper-airway smooth muscle constriction is increased in tissue from animals presensitized with OVA, compared with those that were not presensitized [8]. Furthermore, agents such as methacholine or endothelin can be added to the bath as constrictor agonists, and isoproterenol may be used as a constrictor antagonist to investigate the dynamic properties of the upper airways. This method is relevant, because it allows assessment of the major conducting airways, which are believed to be the site of pulmonary dysfunction in human asthma. However, because the natural structure of the trachea is maintained by cartilaginous rather than muscular elements, it is resistant to large fluctuations in diameter in vivo. This in vitro measurement is merely an artificial means of isolating segments of the airway and measuring smooth muscle contractility. This method excludes interference of in vivo factors such as artificial ventilation, arterial oxygenation, and cardiovascular elements commonly encountered with other methods of measuring airway reactivity in animals. A more detailed discussion of this method has been described by Fedan *et al.* [9]

There are several methods of in vivo airway measurements that are utilized in animal models of pulmonary disease. Noninvasive, whole-body plethysmography (BUXCO system, Troy, NY) provides a rough estimate of airway responsiveness by measuring the Penh (enhanced pause), which can be described using both early and late expiratory box flow as well as inspiratory box flow [8]. With this method, nebulized constrictor agonists (*eg*, methacholine) can be administered and results interpreted with relative ease without requiring intubation. This method also allows for re-use of animals for both control and treatment purposes. However, nonintubated, whole-body plethysmography is limited, because it does not distinguish between reactivity of upper and lower airways in experimental asthma. A detailed description of murine whole-body plethysmography as it relates to mouse models of asthma has been described by Hammelman *et al.* [10].

More invasive techniques can also be utilized including the intubated plethysmograph system (intubated;

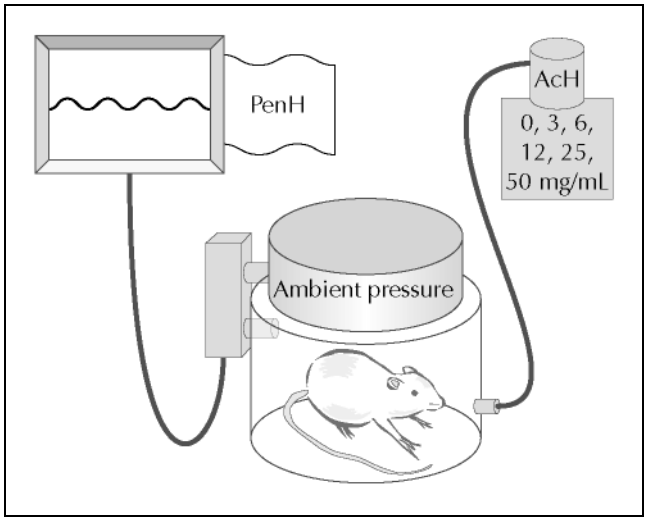


Figure 1. Whole-body plethysmography. A noninvasive method of airway assessment that approximates pulmonary function in the animal models of human disease. Reactivity is measured as Penh (enhanced pause), which is a dimensionless value derived from both inspiratory and expiratory box airflow. This allows reuse of animals and multiple treatments.

BUXCO, Troy, NY). More recently, the method of high-frequency oscillatory ventilation has come into favor. This method utilizes high-frequency, low-amplitude ventilation to determine airway resistance, compliance, and conductance in both upper and lower airways allowing for a more detailed assessment of lower versus upper-airway disease (Flexivent; SCIREQ, Montreal, Canada) [11]. Using these two techniques, the investigator can localize (conductive versus respirative airways) the alterations in pulmonary functions more accurately (Fig. 1).

What Are Animal Models?

There is no animal model that exactly reproduces the pathology of human asthma. However, these models are necessary for the development of improved treatments and understanding of the detailed pathogenesis of asthma (Table 1). Although researchers have used several animal models for studying allergic asthma, the most widely used model is the mouse asthma model. Mouse models have been found to be versatile and easily manipulated. Larger rodent models, such as rats, guinea pigs, and rabbits, have also frequently been used. Additionally, ferrets, cats, dogs, sheep, pigs, and horses have been studied, but are not discussed in this paper because their use is relatively uncommon. Finally, the nonhuman primate model is interesting in that although they are the closest genetic relation to humans, it remains to be seen whether they indeed are the most applicable model to study human airway disease [12].

How to Choose the Appropriate Model

The advantage of using one animal species over another for exploring experimental asthma is further elucidated in the body of this paper. Many aspects must be considered when choosing a species for a given protocol. Cost, convenience, and the desire for specific outcome measures are some of the obvious considerations. The initial cost of experimental animals ranges from $8 per animal for mice to upwards of $10,000 each for nonhuman primates. Furthermore, the upkeep, housing, methods, and materials for the use of one primate may cost as much as that of 20 small rodents (M.R. Van Scott, PhD, personal communication). Therefore, depending on the research question, it may be more economical to use a rodent model. The ability to genetically and immunologically manipulate animal subjects is also a strong consideration, as we are learning more every day about the role of cytokines and genetic markers in allergic inflammation.

Mouse Models of Asthma

Of all of the experimental asthma models, the mouse model is the most commonly used and reported. Several sensitization and challenge protocols in mouse models have been used to evaluate experimental asthma outcomes. Our group uses a protocol for murine allergic asthma that we have found very effective. In this model, we sensitize mice with 50 μg of OVA or ragweed with adjuvant (aluminum hydroxide) intraperitoneally on day 0, and then boost with a second injection 7 to 10 days later. On day 21, we challenge the animals via the intratracheal route with 50 μg of antigen in 30 μl aliquots. We repeat the challenge on days 22 and 23, and measure airway parameters on day 24 followed by tissue collection on either day 24 or 25. (Fig. 2). This schedule and dose pattern allows optimal responses in both inflammatory infiltrate and airway hyperresponsiveness (AHR).

Gelfand *et al.* [8] developed a model including two intraperitoneal sensitizations with 1 mg of OVA in alum on days 1 and 14 of the protocol. On days 28, 29, and 30, the animals were challenged with aerosolized OVA, and airway assessments were made on day 32. Alternatively, they describe a model using daily administration of nebulized OVA for 10 days followed by assessment on day 12 without prior sensitization. Control animals were given phosphate-buffered saline instead of OVA with the same time intervals.

Other protocols employ only one sensitization without adjuvant followed by challenge with 10 μg OVA or vehicle alone at day 14. In this model, AHR was measured using intravenous acetylcholine challenges [13]. An additional group sensitized mice with ragweed antigen and alum on days 0 and 4 followed by intratracheal administration of ragweed on day 11. This group utilized three methods of airway reactivity evaluation including intubated, nonintubated, and in vitro measurements [14]. Although there are many different protocol designs, they all share the similar components of a systemic sensitization followed by a local challenge, leading to the development of desired outcome variables that mimic human disease.

Table 1. Animals that exhibit various features of human asthma

	Mice	Guinea pigs	Rats	Rabbits	Nonhuman primates
Early bronchospasm		X	X	X	X
Late bronchospasm		X	X	X	X
Airway hyperreactivity	X			X	X
Lung inflammation (BAL)	X	X	X	X	X
Eosinophilia	X		X		X
Structural changes (remodeling)	X	X	X		X

BAL—bronchoalveolar lavage.

In addition to the varied protocols available, the mouse model of asthma can be made both genetically and immunologically diverse. There are strains of mice that display enhanced airway hyperreactivity and those that are relatively resistant to methacholine challenge. Takeda *et al.* [15] found that despite higher numbers of eosinophils in the bronchoalveolar lavage (BAL) fluid found in sensitized and challenged C57BL/6 mice, airway responsiveness and allergen-specific IgE responses were greater in BALB/c mice after the same treatment [15]. It was further noted that the distribution of eosinophils was different in the BALB/c animals compared with the C57BL/6 mice. The investigators looked for eosinophils in the lungs of both strains using rabbit anti-MFP fluorescent staining, and found that eosinophils were localized to the peribronchial and peripheral lung tissue in BALB/c mice, and were diffusely distributed in the peripheral lung tissue of the C57BL/6 mice. They further speculated that it may not be the number of eosinophils, but the distribution of those cells that dictate airway responses [15].

Several groups have described differences in airway responses among different murine strains. In 1988, Levitt and Mitzner [16] published a study in which airway measurements were performed in nine strains of mice after challenge with acetylcholine and serotonin. This experiment identified the A/J and AKR/J strains as the most reactive to acetylcholine and the C57BL/6, SJL/J, and C3H/HeJ strains as the least reactive to acetylcholine or serotonin.

Other features besides airway responsiveness have also been analyzed through strain comparison. Bronchiolar injury and repair responses were compared among the Swiss Webster, C57BL/6, 129/TerSv, and the 129/SvEv strains and marked differences were found. The authors observed that following naphthalene-induced tissue damage, there were marked strain-related changes in ciliated airway epithelia and the clara cell population before and during airway tissue repair [17]. There has been valid concern among investigators about mouse strain variations and their effect on the "ideal" asthma model. Therefore, it is critical that one chooses an appropriate strain for the desired outcome measures when designing an experimental protocol.

In addition to the existing variation among mouse strains, the ability to induce changes using knockout and transgenic mice is an important method of immunologic manipulation that has become instrumental in the study of allergic airway disease. Investigators have developed mice that either do not express or overexpress a given gene product to study the role of that protein in murine asthma models. Using these observations, it is possible to examine the role of these proteins in human asthma. An example of the use of knockout and transgenic mice can be seen in a series of recent experiments exploring the role of IL-5.

IL-5 is a pro-inflammatory cytokine whose role has been identified as an eosinophil-stimulating factor that induces production and proliferation, and potentiates further activation of eosinophils. When IL-5–neutralizing antibodies were given to OVA-sensitized BALB/c mice prior to airway challenge, both airway eosinophilia and airway hyperreactivity were inhibited [18]. The IL-5 knockout mouse shows a similar outcome with decreased tissue eosinophilia [19]. Two types of the IL-5 transgenic mice display either increased peripheral eosinophilia (NJ 1638) or enhanced tissue eosinophilia (NJ 1726) at baseline. These transgenic mice show an exaggerated response to Th2 polarizing conditions such as helminth infections [20]. A recent study showed that the administration of humanized anti–IL-5 monoclonal antibody decreased airway eosinophilia in human asthmatics, but did not improve asthmatic bronchospasm [21••].

From these examples, it is clear that genetic and immunologic manipulation are important tools in the study of animal models of human disease. Researchers can target a protein, either amplify or delete it in a murine model, and hypothesize a therapeutic agent derived from these findings. In addition, new discoveries about the complexities of the immune system in asthma can be made as we observe how cytokines may regulate other pathways in the inflammatory cascade.

Limitations of the Mouse Model of Asthma

Although the mouse model is an excellent tool for determining immunologic factors involved in human asthma, some limitations exist in its usefulness in interpreting the physiologic contributions in the disease process. Many investigators believe that pulmonary involvement in human asthma is restricted to the conducting airways,

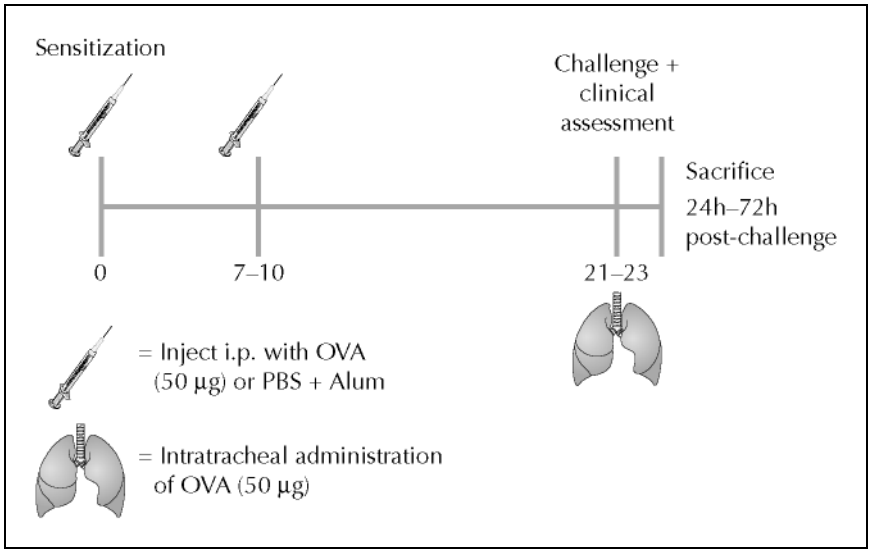


Figure 2. Outline of sensitization and challenge protocol using ovalbumin. Animals are sensitized via intraperitoneal injection with antigen plus adjuvant on days 0 and 7 through 10. They are challenged with intratracheal administration of antigen (or PBS) on days 21, 22, and 23, and are sacrificed on day 24.

whereas allergic airway disease in mice may extend to the lung parenchyma and visceral pleura. Furthermore, the magnitude of AHR in mice does not approach that found in human asthma [22]. In human asthma, eosinophil granule proteins (*eg*, ECP, EBP, EPO) are readily detected in BAL fluid. This is not observed in the mouse model of asthma, as mice deficient in EPO or MBP do not exhibit an altered phenotype in response to allergen challenge, as would be expected from observations in human disease [23]. Another concern arises when choosing an appropriate antigen for sensitization and challenge. Pollens, dust mites, molds, and cockroach proteins are common inciters of human disease. These allergens, however, do not elicit ideal responses in the mouse, and OVA, which is most often used in these models, is not a naturally occurring human allergen. Furthermore, despite the advancements of genetic and immunologic manipulation of mouse models, there is no evidence that mice exhibit intrinsic asthma, which is often found in human disease. However, in spite of the differences between mice and humans, we cannot discount the importance of the murine model of asthma. The use of the mouse in the study of many human disease processes has been a crucial stepping stone in our understanding of human pathology.

Other Rodent Models

Guinea pig models are similar to those of mice, involving sensitizations with OVA on day 0 followed by an inhalational challenge between days 18 and 21. An influx of eosinophils was found in the BAL fluid of these animals 24 hours after OVA challenge, and was sustained up to 72 hours after challenge. The enhanced numbers of eosinophils in guinea pigs at baseline have allowed for a more detailed identification of the role of these cells in allergic asthma. Specifically, guinea pig studies have elucidated that MBP is a core-granule protein of the eosinophil and that its release induces epithelium-dependent contraction of airway smooth muscle [24]. Additionally, eosinophil MBP blocks M2-muscarinic receptors and is thought to be a mediator of AHR via this mechanism [25]. Several papers have described eosinophil release of MBP at the site of muscarinic receptor expression, which may be a key factor in bronchohyperresponsiveness [26]. This can be further supported with the observation that there is a general inhibition of bronchial hyperresponsiveness when OVA-sensitized guinea pigs are given rabbit anti-guinea pig MCP [27]. However, other investigators have seen a correlation between the increase in airway eosinophils after OVA sensitization in the guinea pig model and airway responsiveness to acetylcholine [4], suggesting that the role of eosinophils may not be as critical in allergic airway responses as was previously believed. Finally, guinea pigs have proven to be good models to assess whether a therapeutic agent will impact clinical and histologic endpoints of disease, such as eosinophilia, early and late bronchospasm, or mucus production in an animal predisposed to experimental asthma. Hashimoto *et al*. [28] found that mucus production and airway eosinophils were increased in OVA-sensitized and challenged guinea pigs that were also exposed to diesel exhaust fumes. Experiments such as this, which utilize environmental irritants as sensitizers, are critical as we continue to study the rising incidence of human asthma.

Although the guinea pig model of asthma has many advantages as we study allergic disease, their utility is limited by several factors. The genetic versatility we find in the mouse model does not exist in the guinea pig, and, therefore, it is not an appropriate model for intrinsic components of asthma. There is also a lack of suitable guinea pig species-specific reagents such as cytokines, chemokines, and antibodies. Additionally, guinea pigs exhibit a greater response to histamine challenge than their human counterparts do. This may be attributable to the baseline eosinophilia observed in the guinea pigs as compared with human models [12].

Although rats do not display the same genetic versatility as the mouse model, they have proven useful models in the study of experimental asthma, particularly with the recent availability of rat-specific immunologic agents and inbred strains. Brown-Norway rats, like humans, produce IgE as a primary antibody mediator of anaphylaxis. These rats possess both histologic and immunologic features of allergic airway disease, with an increase of both eosinophils and neutrophils in the BAL fluid and lung tissue as well as increased MBP, EPO, and MPO (myeloperoxidase) levels. The Lewis and Fischer rats did not show these marked changes and have not been useful as comparative control animals in the study of human allergic disease [29]. Disadvantages of the rat model arise when considering bronchoconstriction and induced airway hyperreactivity. Studies have shown inconsistent results and antigen desensitization when using aerosolized agents with rats. Because of this inconsistency, exogenous agents (*eg*, mediators, cholinergics) must be given intravenously to be effective [12].

Although rabbits do not technically belong to the rodent family, they deserve some mention in this section. In the rabbit, the target organ for anaphylaxis is the lung. This creates an ideal situation to focus on the pulmonary effects of allergic disease alone. Both early- and late-phase bronchospasm, as well as enhanced cellular infiltrates and induced AHR, can be demonstrated in the rabbit model. Some shortcomings to this model exist, however, as the availability of immunologic reagents is limited (compared with mouse models). Also, the role of adenosine A1-receptors, which are abundant in rabbit airways, is uncertain in human disease. This may create some ambiguity when considering mediators of allergic asthma [12].

Although smaller animal models have the obvious advantage of easier handling and lower cost, their differences from humans may counteract some of the benefits. It is important to understand, however, that the primary purpose of these animal studies is not to exactly reproduce human asthma. Rather, we use these models to help us understand the immunologic and cellular mechanisms that develop in allergic disease, and to examine the effects of exogenous agents on these factors.

Nonhuman Primate Models

Development of nonhuman primate (monkey) models of asthma and AHR have been based on the identification of animals exhibiting natural allergic sensitivity and induction of allergic sensitivity. Patterson *et al*. first described a subpopulation of rhesus monkeys that exhibited natural sensitivity to *Ascaris suum* antigens. Since that time, several investigators [30–32] have published reports using these animals to study allergen-induced airway responsiveness. The model was refined by inducing sensitivity in previously nonsensitive monkeys by infection with *Ascaris suum* eggs [33]. It has subsequently been established that periodic subcutaneous injection of dust mite allergen in rhesus monkeys elevates serum levels of allergen-specific immunoglobulins, and induces allergic responses [34]. An alternative method for sensitizing young adult rhesus monkeys to dust mite is to administer a series of three injections of allergen plus adjuvant followed by repeated intranasal and aerosol exposures to the allergen [35•]. These studies demonstrate an extensive submucosal thickening, airway smooth muscle reorganization, and dense neuronal innervation of the airways from primary afferent neurons originating from the solitary tract nucleus, following repeated allergen exposure [36•].

As mentioned earlier, there are several shortcomings to the nonhuman primate model of asthma. The cost and convenience of feeding, housing, and upkeep of these animals create limitations on the absolute number of subjects required for each experiment. Furthermore, primate studies are chronologically extensive (months to years), as opposed to rodent models that last several weeks. It is also unclear as to whether or not experimental asthma in the nonhuman primate is close enough to human asthma that the costs of time, maintenance, and experiments are justified. As we learn more about the immune system in animal models of human disease, this may become more apparent.

Future Directions in Animal Models of Asthma

The two fundamental characteristics of asthma—AHR and inflammation—are influenced by intrinsic factors in both rodents and primates. These differences may manifest as wide variations in the in vivo response to allergen provocation observed within a single species, between species, and between the two genuses. Identifying the fundamental genetic and biochemical mechanisms that underlie these differences will enhance our understanding of the disease process and potentially aid in determining appropriate therapeutic targets. The recent advent of microarray gene expression analysis has allowed investigators to answer these questions with reasonable accuracy. It has also made it possible to identify large sets of target genes that may yield more detailed and descriptive analyses of genetic differences through the subsequent application of more conventional approaches (*eg*, Northern Blot analysis or quantitative reverse transcriptase-polymerase-chain reaction). Using this technology, Karp *et al*. [37] has recently identified complement factor 5 (C5) as a susceptibility locus for allergen-induced AHR in experimental allergic asthma in mice. Specifically, the AHR-resistant strain C3H/HeJ and the AHR-susceptible strain A/J exhibit allelic variation in the C5 gene and expression of the A/J C5 allele in C3H/HeJ X A/J backcrosses also induces susceptibility to allergen induced AHR, suggesting the involvement of C5 gene expression with AHR development [37].

Since the development of species-specific microarray systems, the effect of asthma-inducing substances on gene expression by isolated cell types from rodents (*eg*, alveolar macrophages, T cells, and airway epithelial cells) and humans (*eg*, blood macrophages and eosinophils) can be assessed. For example, Kioke *et al*. [38] has demonstrated that diesel exhaust particles induce the expression of several antioxidative genes demonstrating that alveolar macrophages are indeed a pulmonary defense system against oxidative stress. A similar approach was utilized by Wills-Karp and Ewart [39] to explain the wide variation in airway responses to intravenous methacholine exposure among inbred strains of mice. In this study, quantitative trail locus (QTL) linkage analysis was used to map the genes of the various strains of mice in an attempt to identify AHR-related genes. Genetic analysis of different strains of animals has led to an increased appreciation of the complexities involved in choosing the appropriate model to use in any given study of allergic airways disease. However, this should not discourage the use of animal models. Rather, it should encourage their use, as we will undoubtedly identify strains of animals that exhibit genetic alterations akin to those observed in subpopulations of humans.

The use of rodents and nonhuman primates in preclinical evaluations for candidate drug development in asthma is critical for the Food and Drug Administration's approval to proceed to phase I clinical trials. In the preclinical stage, animal models of disease ensure both the efficacy and safety of candidate drug compounds. Antibodies and antisense-oligonucleotides (ODNs) are two new classes of therapeutic agents that have emerged in recent years and are aimed at the specific inhibition of theoretical asthma-inducing proteins and messenger RNA sequences, respectively. Anti–IL-5 treatment was one of the first cytokine-neutralization approaches utilized. One group used the OVA model of asthma in mice to demonstrate that anti–IL-5 treatment abolishes airway eosinophilia and development of AHR [18]. This study was followed by the independent development of two humanized monoclonal antibodies directed against IL-5 (Sch 55700, Schering Plough and SB-240563, SmithKline Beecham). The efficacy of Sch 55700 was further tested in mice, rabbits, guinea pigs, and cynomolgus monkeys and was reported to potently inhibit airway eosinophilia in allergic models [40••]. SB-240563 was shown to decrease peripheral eosinophils by greater than 98% in cynomolgus monkeys [41].

The effectiveness of antisense-ODNs in allergic asthma was first demonstrated, using a rabbit model of asthma, by Metzger *et al.* [42]. In this report, inhaled ODNs directed against the adenosine A(1) receptor (EPI-2010; Epigenesis Pharmaceuticals, Cranberry, NJ) were shown to abrogate airway sensitivity and inhibit histamine-induced responsiveness in allergic rabbits. Further studies in rabbits demonstrated that respirable antisense-ODNs are uniformly deposited throughout the airways and do not exhibit systemic effects.

The use of animal models for the development of novel classes of therapeutic compounds is an invaluable resource, as our society demands more efficient and cost effective drugs to combat diseases such as asthma. The fact that anti–IL-5 treatment was equally effective in reducing eosinophil influx in both human and nonhuman studies is a testimony to the development of rodent and nonhuman primate models of asthma, and should underscore the need for continued basic and applied research in these species to provide a better understanding of the mechanisms of this uniquely human disease.

Another particular advantage of murine models of asthma is the ease of genetic manipulation. Single-gene knockout or transgenic models work well when addressing the simplified, yet unattainable, scenario of single gene–single effect biology. However, many pathways in the immune system demonstrate redundancy and counter-regulation. Backcrossing cytokine-deficient mice and cytokine transgenic mice is one method by which cell-specific production of a cytokine can be addressed. For example, a cross between a gene-specific knockout-mouse with a cell-specific transgenic mouse for that same cytokine allows for the observation of the effects of cell-specific production of that gene. Backcrossing between two individual cytokine-deficient strains may demonstrate any counter-regulatory effects of one cytokine on others, as in the case of IL-10 deficiency and the upregulation of both Th1 and Th2 cytokines.

Recently, investigators have used IL-$10^{(-/-)}$/IL-$4^{(-/-)}$ and IL-$10^{(-/-)}$/IL-$12^{(-/-)}$ mice to establish the mechanism of Th1 skewing versus Th2 skewing, respectively. For example, Hoffman *et al*. [43,44] have shown that the response of Th1 skewed mice (IL-$10^{(-/-)}$/IL-$4^{(-/-)}$) to *Schistosoma mansoni* infection is characterized by very high mortality rates that are linked to overexpression of IL-12. Conversely, death was delayed after schistosomal infection in Th2-skewed mice (IL-$10^{(-/-)}$/IL-$12^{(-/-)}$), but resulted in extensive eosinophilic inflammation of the liver that was induced by a 10-fold increase in IL-4 and IL-13. These discoveries demonstrate the extent to which cytokines can display both redundant and counter-regulatory effector functions. This concept can be easily applied to allergic disease as well as parasitic disease. Our group has recently been using these "double knockout" mice to investigate the role of T-cell polarization in experimental allergic asthma. Similar to the schistosomiasis model, we find extensive Th2 esoinophilic inflammation in Th2-skewed mice. However, the magnitude of Th2-inflammation and cytokine production does not correlate well with the degree of AHR to methacholine. We find these results to be intriguing and are currently pursuing the mechanisms to explain the phenomenon.

Conclusions

There will always be a certain degree of uncertainty when attempting to extrapolate discoveries made with animal

models to human disease. One major contributing factor of this shortcoming is the mere fact that it is impossible to reproduce a disease when we do not fully understand its etiology. This is especially the case in understanding the development of asthma, because there are many subcategories of the disease and obviously many factors that precipitate the symptoms of the disease. Nonetheless, great headway has been made in our understanding of basic immunologic and physiologic principles through the perseverance of investigators who have rigorously pursued the use of various animal models of this disease. Although the magnitude of data garnered from animal models of asthma throughout the late 1980s and the 1990s has led to some disappointing clinical and pharmacologic outcomes, we must remain optimistic and appreciate the complexity of the entire disease process. Furthermore, it must be realized that animal models have led to the identification of numerous targets (*eg,* chemokines, cytokines, and transcription factors) that are currently used in the clinical study of human asthma. Continued collaborations between clinical and basic research will undoubtedly lead to the development of better animal models of the disease and allow a greater understanding of whole-body systems interactions.

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Papers of particular interest, published recently, have been highlighted as:

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- •• Of major importance

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