Nonhuman primate models of asthma

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Asthma is a complex human disease that does not have an accurate counterpart in any common model organism. Most of our understanding of the immune mechanisms underlying asthma comes from studies in man and mouse. However, there are fundamental differences between the spontaneous disease in man and the experimentally induced counterparts in mice. We advocate more extensive use of nonhuman primate asthma models to reconcile these differences between man and mouse.

The past decade has seen dramatically increased use of mouse models of allergic asthma to study the fundamental immunologic causes of the disease, as contrasted to the pharmacologic or physiologic consequences, and to identify and test novel therapeutic strategies (1, 2). This increased focus has not been without controversy (3), and there are ample reasons for caution in extrapolating the findings in experimentally induced mouse models to the naturally occurring human disease. The fundamental immunologic abnormality of both human allergic asthma and mouse models is an inappropriate, poorly controlled T helper type 2 (Th2) response to one or more airborne allergens. There are, however, important differences between the two species, including techniques for measuring pulmonary function, chronicity of the disease process, and species differences in expression of, or responses to, key cytokines and mediators (2). Large animal models of asthma have been developed in dogs (4), sheep (5), and monkeys (principally rhesus and cynomolgus macaques) (6–11). All have proven useful for studies of pharmacology and physiology and for preclinical development of drugs that provide symptomatic relief for asthma. We contend, however, that monkey models are the most suitable for studying immune regulation and effector functions in asthma and for evaluating novel immunomodulatory therapies. There are two basic reasons for this assertion: first, the "toolbox" for the analysis of immune responses in primates is far more complete than in dogs or sheep. Second, novel therapeutic agents, especially biologics such as antibodies, cytokines, and nucleic acids that specifically target the human immune system, are much more likely to be active in other primates than in animals less genetically similar to man. The goal of this commentary is to highlight monkey models of allergic asthma and to suggest their use as a bridge between mouse models and human asthma.

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Monkey models of allergic asthma

Nearly 40 years ago researchers began to use rhesus monkeys (*Macaca mulatta*) sensitized to the intestinal helminth parasite *Ascaris suum* to study allergen-induced immediate asthmatic responses in the airways (6). A majority of primates caught in the wild demonstrate skin test sensitivity to soluble extracts of *Ascaris* (12), presumably from prior natural exposure to *Ascaris suum* or a related parasite (13). As with most helminth parasites, *Ascaris* exposure of monkeys evokes a strong Th2-biased T cell memory response and increased levels of IgE (12, 14). Subsequent airway challenge of sensitized monkeys with *Ascaris* extracts results in both immediate and late phase asthmatic reactions (6, 7), followed by airway eosinophilia and hyperresponsiveness (8). These responses are comparable to those of atopic asthmatics to an inhalation challenge with an appropriate allergen (15). The *Ascaris* model is attractive because animals already appropriately sensitized can easily be identified. In addition, the "allergens" are not common environmental antigens, and exposure of the animals can be accurately regulated and documented.

Other models for allergic asthma have been developed in rhesus or cynomolgus monkeys using important human allergens, including house dust mite (9–11) and the birch pollen allergens, Bet V1 and V2 (16). In general, animals need to be deliberately sensitized with these allergens, although spontaneous allergic sensitization to dust mite and cedar pollen occurs in monkeys (17). These induced asthma models have both advantages and disadvantages compared with the *Ascaris* model. The major advantage is control over the sensitization process, permitting greater standardization of the model and allowing research into factors that prevent the onset of allergic asthma. However, current protocols are both time and labor intensive and require repeated aerosol challenges (10, 11). The best characterized of these induced models involves injection, followed by repeated aerosol challenge of house dust mite allergens (10).

The rationale for developing asthma models in monkeys includes the genetic and physiological similarity to humans, the similarity of human and monkey lungs in terms of anatomy, histology, and ultrastructure, and their size, which permits pulmonary function measurements and bronchoscopy with techniques and instruments used in human studies (10, 18, 19). Although none of the primate models represent the process of natural sensitization and frequent allergen exposure that occurs in humans, many key features of human allergic asthma are observed (Table I). The responses to allergen challenge in monkeys closely resemble those in man in terms of both physiological and immunological parameters. Cohorts of sensitized primates are usually maintained and challenged repeatedly over several years, and develop impor-

tant characteristics of chronic human asthma, such as extensive airway remodeling (E.C.M. Martin, personal communication; reference 20) and increased baseline reactivity to methacholine (10).

Measuring lung function in monkeys

The ability to measure lung function in similar ways in human and monkey studies is a particular advantage. Although spirometry is most often used in humans, this technique is not suitable for primates, as it requires subject cooperation. In primates the noninvasive forced oscillation technique (7, 8, 21) is the preferred techniques for measuring changes in pulmonary function. This technique superimposes forced oscillations onto normal spontaneous breathing either at the airway opening or at the body surface, and measures respectively input or transfer impedance, from which airway resistance and compliance are calculated (21, 22). In humans, the forced oscillation technique is used with subjects unable to cooperate, such as very young children, and for specific research purposes (23). Madwed and colleagues established that forced oscillation in primates provides a consistent method of quantifying airway responses under baseline and bronchial challenge conditions, and the responses observed are similar to those in man, both in healthy subjects and asthmatics (21). This contrasts with the more indirect measurements done in the mouse, such as "enhanced pause" (Penh), which assess functional parameters that are difficult to correlate with parameters affected by human asthma, such as airway resistance or forced expiratory volume (3). Other techniques requiring anesthesia and/or mechanical ventilation have also been used in monkey models (11, 13, 24), and these also measure indices of lung function similar to those in man. Thus, the effects of immunomodulatory agents on clinically relevant lung functions may be easier to establish in monkey than in mouse studies.

Immunological studies with monkey asthma models

The *Ascaris suum* model has been used extensively for the preclinical evaluation of asthma therapies, primarily drugs for symptomatic intervention. The published literature is incomplete, as much of this work has been done by pharmaceutical companies and remains unpublished. Early work established the effects of well-known antiallergy and antiasthma medications in primates and found them very similar to their known effects in man (8), which supports the clinical relevance and predictive value of testing pharmacological agents in the primate asthma model.

Monkey asthma models have been used in only a few cases to test interventions aimed at modifying the underlying Th2-biased immune response. Although these studies were designed to provide preclinical support for clinical trials in man, they serve to demonstrate the functional similarity of pulmonary immune responses between monkey and mouse models. In a series of pioneering studies, Wegner, Gundel, and colleagues used monoclonal antibody treatment of the *Ascaris* monkey model to demonstrate that intercellular adhesion molecule (ICAM)-1 (25) and endothelial leukocyte adhesion molecule (ELAM)-1 (26) were important for allergen-induced cellular infiltration of the airways. More recently, monoclonal antibodies to human IL-5 (24) and IL-13 (Dr. P.D. Monk, personal communication) have been shown to inhibit airway responses to allergen challenge in the *Ascaris* model, confirming earlier demonstrations in mouse of the importance of these two cytokines. A different type of immune modulator, a synthetic CpG-containing oligonucleotide, has also been shown to inhibit allergen challenge responses and to slow or reverse the process of airway remodeling in a house dust mite model (20). Indeed, such preclinical development studies provide most of our understanding of the immune mechanisms in

Table I. Comparison of principal features of human asthma and monkey asthma models

	Human atopic asthma	Monkey asthma models
Features of chronic disease		
Allergen-specific IgE and skin test positivity	+	+
Eosinophils and IgE^+ cells in airway	+	+
Th2 cells in airway	+	?
Mucous cell hyperplasia	+	+
Subepithelial fibrosis	+	+
Basement membrane thickening	+	+
Persistent baseline hyperreactivity to histamine or methacholine	+	+
Episodic wheezing and airflow obstruction	+	−
Bronchoconstriction triggered by nonatopic stimuli: e.g., cold air, exercise, viral infection	+	?
Response to inhaled allergen challenge		
Th2-associated cytokine and chemokine induction	+	+
Migration of eosinophils, lymphocytes, and dendritic cells into airways	+	+
Early and late bronchoconstriction responses	+	+
Enhanced reactivity to histamine challenge	+	+

For monkey: +, demonstrated in one or more monkey models; ?, not reported.

monkey asthma models, as very few studies have been done to characterize the model, per se.

Reagents for primate immunology studies

One advantage of using primate versus other large animal models for immunology and asthma research is the extensive set of antibody-based reagents available. Virtually all of these antibodies were originally developed for detection of human molecules but have significant cross-reaction with the monkey orthologs. The NIH Nonhuman Primate Reagent Resource (http://NHPreagents.bidmc.harvard.edu) has been developed to facilitate access to existing reagents used in nonhuman primate models and to develop new reagents. It is clear from this website and catalogs of several major antibody vendors that a large subset of anti–human antibodies react efficiently and specifically with the corresponding monkey antigens. As a consequence of this reagent availability and the immunological characterization done in monkey studies for simian immunodeficiency virus infection and vaccination (27, 28), the cellular components of the rhesus immune system have been quite well defined and are known to be similar to subsets in man. Protocols have been developed to isolate various subsets of dendritic cells (28) and T cells (27), and the first characterization of these important subsets in the airways has been reported (29).

The crossover to macaques of human nucleic acid–based reagents is even more extensive than those of protein and antibody-based reagents. Thus, widely used probes such as PCR primers, microarrays, and small interfering RNAs designed from human gene sequence data can be applied to monkey studies. This is predictable from the >95% sequence identity of human and rhesus genes. Our experience with quantitative PCR measurements of mRNA levels suggests that >90% of primer pairs designed from human sequences work with the same efficiency and specificity in both rhesus (29) and cynomolgus (unpublished data) monkeys. Similarly, microarrays of human sequences, human oligonucleotides, or cDNAs are quite useful (30, 31), although data for a small number genes will be inaccurate or absent. The ongoing rhesus monkey genome project (the first draft was released in January 2005; http://www.hgsc.bcm.tmc.edu/projects/rmacaque) promises to fill the remaining gaps in our set of gene-based monkey reagents.

Why use monkeys instead of mice or humans?

The response of atopic individuals to inhaled allergens is a complex, ordered interplay of mediators, cytokines, and cell migrations throughout the respiratory tract and draining lymph nodes and blood. The primary experimental design used in both human and animal studies of this response is acute challenge with an antigen solution that is either nebulized or delivered by intratracheal or intranasal administration. Allergen-induced changes in lung function are assessed by a variety of noninvasive techniques; however, changes in immune functions require invasive methods of sampling. In man and larger animals this is usually done by bronchoscopy with bronchial lavage or biopsy, whereas mice are virtually always killed for lung and lymph node harvest. Peripheral blood has little utility for measuring immune responses to pulmonary challenge in man (32), and minimally invasive techniques for sampling the respiratory tract, such as induced sputum (33) and exhaled cytokine measurements (34), are technically challenging and limited in scope. This imposes significant limitations for mechanistic studies in man. Few patients will agree to repeated cycles of allergen challenge and bronchoscopy, and there are growing ethical and regulatory constraints on the use of these techniques. Furthermore, treatments in vivo can be done only with approved drugs or experimental drugs under an Investigational New Drug application.

Nonhuman primate asthma models permit many of the experimental manipulations performed in mice but in a model much closer to chronic human asthma in terms of immunology, physiology, and histopathology. The limited data available show that changes in immune parameters in response to inhaled allergen challenge in the monkey *Ascaris* model (7, 8, 35) are largely indistinguishable from those observed when human asthmatics are similarly challenged (Table I) (15). The patterns of cellular infiltration into the bronchoalveolar lavage and the increases in Th2 cell–associated cytokines and chemokines are similar in both models and are quite comparable to responses in the mouse (1). We have recently found a striking similarity between human and monkey allergen challenges in terms of infiltration of dendritic cell subsets and increases in expression of a panel of over 50 allergy-related genes (unpublished data). Thus, at many levels of comparison there is substantial similarity between atopic humans and monkeys in terms of the immune responses and physiological consequences of acute allergen challenge.

Several other types of monkey asthma studies have the potential to fill significant gaps in our understanding of human asthma. For example, the harvest of organs from experimental animals for detailed study of pathology, histology, immune function, and gene expression has been used to great advantage in recent work from Plopper, Hyde, and colleagues (29, 31, 36). Such studies provide insights largely unobtainable from human studies, which rely on limited biopsy samples or on autopsy materials. Captive breeding colonies of rhesus and cynomolgus monkeys also offer opportunities to study the development and possible prevention of atopy and asthma during childhood—research that cannot be readily done in mouse or man. It is now clear that a wide range of environmental factors, superimposed on genetic background, determine the occurrence and severity of allergy and asthma (37). Allergen exposures and infections during the first few years of life, when both the respiratory tract and the immune system are developing rapidly,

are particularly important (38). Most of our understanding of the development of allergy and asthma in children comes from large epidemiological studies; however, few of these have provided correlations of clinical phenotype with sophisticated measures of immune functions (39, 40). Possibilities for experimental intervention to confirm such correlations are very limited for obvious reasons of safety and ethics. The recent demonstration that allergen-specific immunotherapy can halt the progression of allergic rhinitis to asthma has shown the promise of early intervention (41); however, this study was only possible because this form of immunotherapy is an accepted practice in children with allergies. Mouse models are of very limited use; the physiological state of both the immune system and the lung at birth are very different in mice and primates, and development to an adult state occurs in a matter of weeks in mice, not years as in humans. Mechanistic studies in cohorts of neonatal monkeys would not be inexpensive, but they would offer a unique opportunity to study the mechanisms of key risk factors and to evaluate new approaches to the prevention rather than the treatment of asthma.

In summary, we regard monkey asthma models as having substantial untapped potential to help us understand the mechanisms of chronic immune dysfunction in asthma and to explore new therapeutic approaches based on that understanding. For many reasons, not the least of which is cost, they cannot replace mouse studies, but monkey models have developed to the point that they can allow researchers to confirm their findings in a species much more like man, before entering even more costly human studies.

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