Utility of Animal Models in the Study of Human Airway Disease*

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The principal forms of human airway disease are bronchial asthma, cigarette smoke-induced chronic bronchitis, and cystic fibrosis. Since these conditions do not occur naturally in animals, investigators have chosen different animal species in an attempt to induce in them a syndrome reminiscent of human airway disease. These efforts have been most rewarding in bronchial asthma and most of the data on airway disease in animals have been generated in “asthma models.”

Bronchial asthma has been defined as episodic airflow obstruction which reverses spontaneously or in response to drugs.1 The hallmark of bronchial asthma is airway hyperresponsiveness, ie, exaggerated physiologic responses of the airways to various stimuli, a phenomenon which is usually disclosed in the laboratory.2 While animal models have fallen short in reproducing the human disease asthma, particularly its important clinical feature of spontaneous, sustained airflow obstruction, bronchospasm has been successfully induced in several animal species. Thus, these animals may be considered models of the laboratory manifestations of human bronchial asthma rather than true asthma models.

The following discussion will be limited to the utility of animal models in the study of human bronchial asthma because of the continued scientific interest in them and the wide spectrum of relevant observations available for review. Furthermore, all of the three principal physiologic expressions of airway disease, ie, airway smooth muscle contraction, epithelial hypersecretion, and microvascular hyperpermeability have been demonstrated in these animal models.

Rationale for the Use of Animal Models

The most convincing reason for employing animal models is to address questions about the pathogenesis of airway hyperresponsiveness that cannot be answered in man. At a recent workshop sponsored by the National Heart, Lung and Blood Institute, the participants identified the major strengths and weaknesses of animal models3 (Table 1). Since airway hyperresponsiveness in animals is not a perfect copy of the airway hyperresponsiveness present in patients with bronchial asthma, both the scientist who employs an animal model and the clinician who tries to assess the relevance of observations made in animals should be aware of the strengths and weaknesses of the existing animal models. If animals with airway hyperresponsiveness are not viewed as complete models of human airway hyperresponsiveness but chosen to test a specific hypothesis concerning the pathogenesis of human bronchial asthma, the models can assume great scientific importance. A working knowledge of the biologic profile of the various animal models is therefore helpful.

Experimental Models

The popular models of human airway hyperresponsiveness include different rodents, the dog, the sheep, the pony, and subhuman primates. Previous reviews have either stressed the advantages and disadvantages of the different species as compared to each other or

Table 1—Animal Models of Human Airway Hyperresponsiveness

<table>
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<th>Strengths</th>
<th>Weaknesses</th>
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| Feasibility of invasive
in *vivo* methods | Natural hyperresponsiveness rare in animals |
| Availability of tissue
for *in vitro* study | Interspecies variability in lung anatomy can influence physiologic endpoints |
| Validation of data obtained
in dispersed systems | Interspecies differences in the pathogenesis of hyperresponsiveness |
| Ability to control and manipulate genetic and environmental influences | Preclinical testing of drugs |

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to humans* or classified the animal models according to the cause of airway hyperresponsiveness. These categories are genetically determined hyperresponsiveness, immunologically induced hyperresponsiveness, and nonimmunologically induced hyperresponsiveness. However, the animal models can also be reviewed by describing separately the characteristic target tissue responses in the airway.

Airway Smooth Muscle

Airway smooth muscle hyperresponsiveness to various pharmacologic agonists, chemical mediators, neurotransmitters, and nonspecific irritants has been produced in vivo in a variety of species by controlled breeding (genetically determined), allergen challenge (immunologically induced) and airway damage resulting from chemical, infections or physical injury (non-immunologically induced).

Brunet et al have reported a Sprague-Dawley rat colony which is characterized by airway hyperresponsiveness to allergen and pharmacologic spasmogens. The authors found, by using a respiratory distress index which presumably reflects bronchoconstriction, that the animals exhibited hyperresponsiveness to serotonin and a majority of other bronchoconstricting agents. Drugs commonly used in clinical practice including a methylxanthine, glucocorticoid, and disodium cromoglycate blunted the airway hyperresponsiveness. The pathogenetic pathways responsible for airway hyperresponsiveness have not been identified, but it is of interest that tracheal smooth muscle removed from these animals was not hyperresponsive to the spasmogens for which in vivo hyperresponsiveness was present. In some offspring of Basenji dogs crossed with Greyhounds, a strong sensitivity to Ascaris suum antigen and marked in vivo airway responsiveness to spasmogens and irritants has been observed.* The specific (antigen) and nonspecific responsiveness was greater in these animals than in Basenji dogs, Greyhounds, or mongrel dogs. The mechanisms of hyperresponsiveness in Basenji-Greyhounds are not known, but propranolol has been shown to increase airflow resistance only in the hyperresponsive Basenji-Greyhound dogs but not in other dogs, in analogy to the difference in propranolol responsiveness between asthmatics and normal subjects.* These findings in rats and dogs suggest that in vivo airway hyperresponsiveness can be an inherited trait and that it should be possible to identify genes which determine hyperresponsiveness to specific spasmogens. By crossing different strains of inbred mice, Levitt and Mitzner† have produced strains which were responsive to either serotonin or acetylcholine and strains which did not respond to either agonist. The hyperresponsiveness was consistent with a recessive genotype. These results raise the possibility for further studies to isolate the genes and gene products responsible for airway hyperresponsiveness to specific spasmogens.

Immunologically-induced airway hyperresponsiveness has been observed in several species. In dogs, rabbits, and sheep, the presence of natural hypersensitivity to or active sensitization to an allergen has been shown to not only produce a bronchoconstrictor response to allergen challenge, but to also induce airway hyperresponsiveness to nonallergenic stimuli; this "nonspecific" hyperresponsiveness is also seen in patients with allergic asthma. In allergic mongrel dogs, a single inhalation challenge with specific antigen has been shown to increase cholinergic bronchial responsiveness after six hours; the hyperresponsiveness lasted up to 96 hours after challenge. Becker et al found in mongrel dogs neonatally sensitized with ragweed antigen, a progressive increase in airway responsiveness to inhaled acetylcholine during a 15-month observation while litter-mate control dogs showed a decrease in acetylcholine responsiveness over the same period of time. Acetylcholine responsiveness was transiently further increased after repeat antigen challenge. In adult sheep with natural sensitivity to A suum antigen, Lanes et al made the interesting observation that immunologically-induced airway hyperresponsiveness was correlated with the pattern of antigen-induced bronchoconstriction. Sheep that developed both an immediate and late bronchoconstrictive response to inhaled A suum extract also developed cholinergic airway hyperresponsiveness 24 hours after antigen challenge, while sheep which only developed an immediate bronchoconstrictive response to antigen did not (Fig 1). The cholinergic airway hyperresponsiveness was blocked by a cyclooxygenase inhibitor, a sulfidopeptide leukotriene receptor antagonist, and a combined leukotriene-thromboxane synthetase inhibitor. Only the anti-leukotriene agents blocked the late phase bronchoconstriction after antigen. This indicates that different arachidonic acid products were responsible for the late phase response and airway hyperresponsiveness after allergen challenge. A role for thromboxane in immunologically induced airway hyperresponsiveness has also been shown in mongrel dogs. Further studies in dogs and sheep have raised the possibility that the antigen-induced release of anaphylactic mediators (eg, LTB4, PAF) may be a critical step in the development of airway hyperresponsiveness. Whether or not the subsequent influx of granulocytes into the lung (as determined by bronchoalveolar lavage) is a prerequisite for the airway hyperresponsiveness remains controversial. The number of inflammatory cells in the airway may be less important than their level of activation.

Airway hyperresponsiveness can also be induced by
airway injury which does not involve IgE-dependent mediator secretion. Such nonimmunologic stimuli include ozone,22,23 endotoxin,19,24 toluene-diisocyanate,25 and viral agents.26 Short-term exposure to ozone has been shown to increase cholinergic airway responsiveness in some but not all of the mongrel dogs tested.28 Neutrophil infiltration of the bronchial wall was found only in the dogs with airway hyperresponsiveness; in dogs rendered granulocytopenic with hydroxyurea, ozone-induced airway hyperresponsiveness and neutrophil influx into the bronchial wall were both absent. Ozone has also been shown to induce cholinergic hyperresponsiveness and an increased number of inflammatory cells in the airways of guinea pigs, but the hyperresponsiveness preceded the inflammatory changes.23 In the same species, TDI has been found to augment cholinergic airway responsiveness regardless of whether or not granulocyte influx into the airway was prevented by a cytotoxic agent.25 Another difference between dogs and guinea pigs was the presumably putative inflammatory mediator responsible for the airway hyperresponsiveness: thromboxane in dogs and lipooxygenase metabolites of arachidonic acid in guinea pigs.27,28 Thus, the influx of inflammatory cells into the airways seems to play a critical role in nonimmunologically-induced airway hyperresponsiveness in the dog model but not in the guinea pig model, and different chemical mediators seem to be involved in the development of airway hyperresponsiveness in the two species. Possibly, the activation of resident leukocytes in the airway is of greater importance than leukocyte influx. In addition to leukocyte-derived mediators as demonstrated by these examples, other mechanisms have also been identified in nonimmunologic airway hyperresponsiveness in animal models. These mechanisms include disruption of the airway epithelium and altered peptidase regulation. Airway smooth muscle hyperresponsiveness to contractile agonists in vitro has been observed after physical removal of the airway epithelium in different species, but the exact mechanisms involved have not been fully clarified. In vivo, the viral infection-related airway hyperresponsiveness of guinea pigs and possibly dogs has been attributed to airway epithelial damage leading to an enhanced vagal reflex activity.29 Alternatively, viral infection could enhance airway responsiveness by interfering with epithelium-associated neutral endopeptidases, thereby potentiating the bronchoconstrictive effects of tachykinins released from afferent nerves in the stimulated airway. For example, Sendai virus infection recently has been shown to enhance tachykinin me-
diated airway responses in the rat. The TD1-induced airway hyperresponsiveness in guinea pigs may also be due to a decrease in airway neutral endopeptidase. These examples demonstrate that the different animal models of airway smooth muscle hyperresponsiveness involve different pathogenetic pathways. The animal studies therefore cannot answer the question of which of these mechanisms are also operative in humans with airway hyperresponsiveness.

Airway Epithelium

In patients with bronchial asthma, the airway epithelium secretes liquids at an increased rate and probably of abnormal rheologic properties, and removes the secreted liquids by ciliary activity at a diminished rate. The combination of hypersecretion and impaired mucociliary interaction is felt to be responsible for the accumulation of secretions in the airways. Since inflammation seems to play a critical role in the airway smooth muscle component of airway hyperresponsiveness, the effects of inflammatory products on airway epithelial function have also been studied extensively in animals.

The airway surface liquids consist of a gel (mucus) and a sol (periciliary fluid) produced by mucus cells and by epithelial ion and water transport. Lipoxigenase metabolites of arachidonic acid (15-hydroxyicosatetraenoic acid, sulidopeptide-leukotrienes, thromboxane A2 analogues, prostaglandin E1 and E2), platelet activating factor, adenosine, and substance P have been shown to increase the secretion of mucus substances in different species. In addition, serum exudation has the potential of stimulating mucus secretion in the airway. Inflammatory stimuli can also increase net ion, and hence, water transport across the airway epithelium towards the airway lumen thereby contributing to hypersecretion. Thus, histamine, several arachidonic acid metabolites, bradykinin, and substance P have been reported to increase chloride secretion by the canine tracheal epithelium. Eosinophil major basic protein, another inflammatory mediator, has been found to have similar effects. At least in the dog, prostaglandin E1 and E2 seem to be potent stimulators of chloride secretion and some of the other mediators may exert their action on chloride secretion by the generation of prostaglandin E1 and E2.

Ciliary dysfunction is another possible cause of impaired mucociliary interaction. In contrast to epithelial secretion, ciliary activity has been shown to respond inconsistently to inflammatory stimuli. While serum protein, eosinophil major basic protein, and adenosine have been found to inhibit ciliary activity in rabbits, guinea pigs and lower animals, the leukotrienes C4, D4 and the prostaglandins E1 and E2 appear to stimulate ciliary beat frequency in sheep. Ciloinhibition would be expected to decrease and cilostimulation to increase mucociliary transport.

The interrelationship among the various components of mucociliary interaction has been examined in allergic sheep. In this model, antigen challenge reduced tracheal mucociliary transport rate, an effect which was prevented by pretreatment with cromolyn sodium suggesting the involvement of inflammatory mediators in its pathogenesis. In vitro studies utilizing tracheal cells or tissues showed that the acute antigen-induced slowing of mucociliary transport was associated with mucus and water hypersecretion but not with ciloinhibition; the secretory effects could be blocked or blunted by pretreatment with cromolyn sodium, a glucocorticosteroid and a sulidopeptide-leukotriene antagonist. Exogenous leukotriene D4 stimulated radiolabeled macromolecule and water secretions and decreased mucociliary clearance in the trachea. From these results, it was concluded that in allergic sheep, antigen-induced mucociliary dysfunction is related to the release of inflammatory mediators (eg, sulidopeptide-leukotrienes) and was due primarily to an abnormality in epithelial secretion. It remains to be shown if immunologically-induced airway secretory dysfunction is also present in other animal models and what the nature of the respective inflammatory mediators might be.

Airway Microcirculation

In patients with severe bronchial asthma, the airway mucosa is morphologically characterized by microvascular congestion and edema of the airway wall. These findings suggest that the airway circulation participates in the asthma-associated airway inflammation. Since the airway circulation is difficult to study in humans because of the requirement of invasive techniques, animals have been used to answer questions about the vascular manifestations of asthma. Airway anaphylaxis in animals has been the best studied model in this regard. In allergic sheep, inhalation challenge with specific antigen has been shown to lead to an increase in total bronchial blood flow which corresponded to the immediate increase in airflow resistance; pharmacologic blockade studies showed that the changes in blood flow were not caused by bronchoconstriction. The allergen-induced increase in blood flow may be accompanied by microvascular hyperpermeability as demonstrated by Persson et al who found increased macromolecular transport from the airway vasculature to the airway lumen in allergic guinea pigs as early as five minutes following local antigen challenge. Similarly, intravenously administered ovalbumin has been shown to cause Evans blue extravasation in the conducting airways of sensitized guinea pigs.
The histologic demonstration of subepithelial edema and capillary engorgement in sensitized rats and guinea pigs during inhalation challenge with antigen or during systemic anaphylaxis is in keeping with the physiologic findings.54-55 Locally released histamine, platelet activating factor, prostacyclin, bradykinin, sulfidopeptide-leukotrienes, tachykinins, and reactive oxygen radicals have all been shown to either increase airway blood flow or increase microvascular permeability or both in different animal species.56

Comparisons Between Animal Models and Human Disease

In evaluating the merit of using animals as models of bronchial asthma, several questions arise. Are the functional characteristics of the human disease present in animals? Can the typical histologic lesions of asthma be reproduced in animals? Does the similarity in the physiologic endpoints between humans and animals imply similar underlying mechanisms?

Since spontaneous bronchoconstriction generally does not occur in animals, a meaningful physiologic comparison between patients with asthma and the animal models is limited to induced airway responses. Immunologically-induced airway responses are of special interest as they tend to encompass more features of asthma than responses to other stimuli. Airway function and mucociliary clearance have been assessed in humans and several animal species (Table 2). The antigen-induced changes are quite similar among them indicating that animals are acceptable models with respect to these physiologic endpoints. However, the predominant sites of airflow obstruction could vary among different species due to difference in lung anatomy. Bronchovascular responses to immunologic or nonimmunologic stimuli have thus far only been determined in animals, and comparisons with humans are therefore not possible.

<table>
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<tr>
<th>Airflow Resistance</th>
<th>FRC</th>
<th>TLC</th>
<th>PaO₂</th>
<th>MC</th>
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<tbody>
<tr>
<td>Asthmatic subjects</td>
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<tr>
<td>Sheep</td>
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<td>Dog</td>
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<td>Guinea pig</td>
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<td>Pony</td>
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*Adapted from Wanner and Abraham2 and Broadstone et al.17 FRC is functional residual capacity; TLC, total lung capacity; PaO₂, arterial oxygen tension; MC, mucociliary clearance. ↑ is increased; ↓ is decreased; ← is no change or inconsistent change; NR, not reported.

The morphologic changes in human bronchial asthma typically consist of submucosal gland hypertrophy and hyperplasia, loss of cilia, increased amounts of mucus in airway lumina, varying degrees of subepithelial vascular congestion and edema, and airway smooth muscle hypertrophy.57-58 In addition, infiltration of the airway wall with leukocytes is frequently present. Most of these lesions have not been observed in animals with genetically determined airway hyperresponsiveness, and immunologically and nonimmunologically induced airway obstruction. If histologic changes have been present, they have differed from or represented only some of those seen in patients with bronchial asthma. Acute subepithelial edema and a transiently increased number of inflammatory cells have been found in some animals after airway challenge.54,55,57,58 In contrast, repeated antigen challenge by inhalation has been shown to produce no bronchial abnormalities (only pulmonary eosinophilia) in sensitized guinea pigs, and in allergic sheep with up to 20 antigen challenges, only a modest decrease in the number of ciliated cells in the tracheobronchial tree has been observed but not other features of human bronchial asthma.59-61

Inflammation appears to play an important role in bronchial asthma and participates in many of the airway responses observed in animal models. As far as they have been examined to date, the physiologic endpoints are also similar in humans with bronchial asthma and animal models, but the pathogenetic pathways vary among different species. Creating a complete model of human bronchial asthma may be an unattainable goal. However, animal models will continue to be useful in addressing selected pathogenetic and therapeutic issues in the study of bronchial asthma and other forms of airway disease.

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