

Animal Models of Asthma

Pro: Allergic Avoidance of Animal (Model[s]) Is Not an Option

Our ability to manipulate the genetic make-up of the mouse provides a most powerful means to perform highly controlled experiments in a mammal (1). That is, overexpressing or deleting individual genes, often at a particular time or in specific cell types, gives scientists the opportunity to determine the function of the protein encoded by the gene *in vivo*. When performed in the context of models of disease, such genetic manipulation enables us to dissect pathogenetic pathways more thoroughly than ever before. We simply cannot mechanistically examine humans like we can animals, particularly the mouse.

However, there is no question that humans and mice differ; how else does one explain the whiskers and tail? Nevertheless, we also share an enormous amount of genetic material and have mastered common solutions to physiologic challenges, including the development of lungs, heart, blood, and the vascular system to acquire oxygen from the atmosphere and deliver it to tissues as a primary source of energy. Furthermore, we both have an innate and adaptive immune system to handle invading pathogens. Although it is true that the structures of the lung, cells, and proteins used to carry out these parallel functions are not entirely conserved, the use of animal models to understand human disease is justified by the fact that the general strategies and the specific molecular pathways used are similar in both species.

Thus, although the issues raised in the accompanying “Con” editorial are elegant, correct, and important, they are not just cause to abandon the use of these models altogether. Rather, they should be taken under advisement as we design our experiments and interpret our results, for the trick to animal modeling lies in examining the most relevant pathways and knowing how far to take the analogy.

LUNG STRUCTURE

To appropriately design and interpret mouse models of disease, we must understand the similarities and differences in lung structure between mouse and man. Mice are obligate nasal breathers that breathe via an extensive olfactory (but poor filtering) epithelium followed by six to eight generations of branching airways before the terminal bronchioles dump into the alveolar ducts and alveoli. In contrast, human airways undergo 20 to 23 branches and the terminal bronchioles open into gas-exchanging respiratory bronchioles, a structure not found in the mouse. Although alveolarization occurs to a greater degree before birth in humans than in mice, airway development, which is most relevant for asthma, precedes birth in both species. Also, contrary to popular belief, mice do have both bronchial and pulmonary circulation. In fact, as reported in this *Journal*, after chronic allergen challenge, there is airway vascular remodeling in mice, as in humans (2).

Mice have few submucosal glands compared with humans. And although their airways are small compared with humans', they are large relative to the size of the organism. Given these large pipes and few branches, it is difficult to model small airway

disease in the mouse. Moreover, the relatively wider airways, coupled with a decline in airway resistance with decreasing body mass in small mammals compared with large ones, result in a large ventilatory dead space, which is compensated for by a much higher breathing frequency (3). Finally, because mice are kept in pathogen-free facilities, we eliminate environmental similarities to humans in an attempt to keep the experiment controlled.

DISEASE MODELING

How does one go about developing an animal model for a human disease? Our ability to model the disease depends on our knowledge of the human phenotype that we are trying to model. In addition, what we call a “disease” may be more appropriately labeled as a “syndrome” because multiple overlapping or even distinct pathogenetic mechanisms may lead to different manifestations of a single disease. Thus, one should use animals to model specific disease phenotypes, not the whole syndrome.

Models are most likely to mimic true pathogenetic pathways when they are based on the known etiology or insult that causes the human disease. For example, with chronic obstructive pulmonary disease, we have an advantage in that we know that cigarette smoking causes it, and that exposure of mice to cigarette smoke leads to inflammation and airspace enlargement similar to that seen in humans (4). However, proper modeling is made difficult by the fact that long-term, low-dose exposure is required. Although this model is better suited for emphysema than for small airway disease for the anatomic reasons stated, modeling in infection could lead to a more pronounced airway phenotype. Now that we have identified specific mutations in protooncogenes (e.g., *kras*) (5) and growth factors (e.g., EGFR) in humans that lead to cancer, we are finding a remarkable ability to replicate the disease and response to therapy in the mouse (6). Obliterative bronchiolitis is a classic example of a disease for which we know the etiology—lung transplantation. Yet, the small size of the mouse precludes this surgical procedure.

Intratracheal bleomycin administration, a model for the fibroproliferative phase of acute respiratory distress syndrome and perhaps pulmonary fibrosis, is another complicated model (7). Working in its favor is the fact that bleomycin is a cause of human fibrosis; however, the acute inflammation-driven model that leads to fibrosis is different from the idiopathic pulmonary fibrosis that might have been initiated by inflammation but on diagnosis has switched to an inflammation-independent fibroproliferative process. This concept of acute inflammation being necessary to initiate but not necessarily sustain established disease is likely relevant to asthma models as well.

ASTHMA

When it comes to modeling, the major problem with asthma is that we know it when we see it, but we do not fully understand the etiologic basis for the disease. Hence, the best that we can do is model specific phenotypic aspects. Using ovalbumin and a variety of other antigens, investigators have been very successful in developing models with acute allergic inflammation and hyperresponsiveness as the readouts. In susceptible strains, these models are characterized by elevated IgE, eosinophilia, and a

Th2-like T-cell response with increased levels of interleukin (IL)-4, IL-5, IL-13, eotaxin, and RANTES. Reversible airway obstruction is also an important feature of this model. These findings have solidified the concept that asthma is a Th2-mediated disease. But these concepts are too simple, and the recent addition of viral infections to allergen models has begun to delineate a more complicated interaction whereby a viral Th1 response via dendritic cells augments the classic asthma Th2 pathway (8, 9). Chronic antigen challenge models are in their infancy, but so is our understanding as to the importance of airway remodeling in human asthma. The combination of animal models and human investigation will be required to resolve the importance of and mechanisms involved in airway remodeling.

Anti-IL-5 therapy represents one of the few attempts to translate findings in mice to humans—and it has failed. IL-5^{-/-} mice were protected both from acute allergic inflammation, airway hyperresponsiveness (10), and chronic airway remodeling (11). However, clinical trials using IL-5 antagonists in both mild asthma (12) and chronic severe asthma (13) failed to provide a clinical benefit. These findings put both eosinophil lovers and animal researchers on the defensive. (Note: human researchers claim the Th2 hypothesis as theirs, but they are more than happy to blame the failure of the trials on the mice.) The eosinophil community has been quick to point out that the number of patients in these studies was few, and that approximately 50% of tissue eosinophils persisted. A recent study using eosinophil lineage ablation, achieved by deleting a GATA -1 promoter site, showed that eosinophils are only important in aspects of chronic remodeling (collagen deposition, smooth muscle mass) (14). Given these findings, perhaps anti-IL-5 therapy could be effective in preventing but not reversing established asthma. Although this interpretation may not be clinically useful, it is consistent with the animal findings.

INTERPRETATION OF DATA

A complicating factor in the history of asthma animal research has been the scientific community's willingness to measure PenH as a surrogate for airway responsiveness. This method, now known to be flawed (15), was easily applied and hence readily accepted in this era when our physiologic expertise is poor and expediency required. We now must reevaluate many published and accepted concepts that were based on this technique. Accurate, invasive physiologic testing in mice is essential and achievable. However, it will require our reinvestment in physiology education and research.

Another difficulty with animal research is that it is now easy to take gene-targeted and transgenic mice "off the shelf," but few have sufficient understanding of the methods entailed in genetic engineering and their inherent limitations. Does the investigator know whether insertion of the phosphoglycerol kinase promoter used to drive the neomycin resistance gene has silenced neighboring genes as well? Does the investigator know whether the transgenic mouse that he or she acquired was replicated in multiple founder lines, and hence that the phenotype is not dependent on the site of insertion into the genome? Are the mice in known, pure-strain backgrounds? Have the strains been inbred so much that additional mutations are accumulating?

Strain differences are often used as an argument against mouse models. In fact, this is a great strength. Just as "outbred" humans may respond differently to similar stimuli, so too may different inbred strains of mice. Responses within an inbred strain are extremely uniform. Therefore, we can use intrastrain similarities to perform controlled experiments that cannot be done in humans. And, conversely, we can use interstrain genetic and phenotypic differences to determine the genetic basis for disease.

Finally, we must remember to interpret results from transgenic and gene-targeted mice cautiously. If overexpression of a protein in a transgenic mouse results in a disease phenotype, we can conclude that the protein has the capacity to cause the phenotype. However, we cannot without further examination conclude that this protein is in any way related to the human disease. If the protein is known to be associated with the human disease, then the case is getting stronger. On the other hand, if we delete a gene (and the deletion does not cause a developmental defect), and apply the knockout to a disease model, then differences in the response of wild-type and knockout mice can prove that the protein is directly involved in the disease model pathway.

CONCLUSIONS

As stated by Paigen, the editor of *Nature Medicine*, over a decade ago, "One invariable lesson of biological research has been the difficulty, virtual impossibility, of reliably predicting the properties of intact organisms from the properties of their constituent tissues, cells and molecules" (16). I would argue that no single method in isolation is sufficient. Human studies are essential to identify the relevant genes and proteins altered in disease states. Animal models can prove causality. But one must then go to cell culture and to the test tube to dissect the mechanism, and then back to the animal model to confirm that mechanism.

This is an iterative process that builds on the existing literature from all disciplines. And, there are areas for improvement in all disciplines. We must fine tune our human phenotyping, know what we are modeling in animals, interpret cautiously, and devise stepwise plans in humans before jumping into a large, expensive trial. The ultimate tests are human clinical trials. The bet here is that using the multidisciplinary approach outlined here, with a heavy dose of animal models, we are on the verge of improving the health of patients with asthma and other devastating diseases.

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The Mouse Trap It Still Yields Few Answers in Asthma

The discovery and “re-discovery” that asthma is an inflammatory disease, arguably the most important advance in the understanding of this disease, came about through human investigations (1, 2). Although details have improved, large gaps remain in our understanding of the onset, persistence, consequences, and treatment of this inflammation. Since 1966, there have been over 8,000 papers published using human subjects, tissue, or cells to study asthma immunology and pathophysiology. In that period, over 2,300 papers were published using murine models to study allergic airway pathobiology. The majority (60%) of murine studies have been published within the last 5 years, indicating an enormous escalation in their use. In fact, most prominent basic laboratories use some variation of this murine system. In contrast, only 22% of human-based asthma studies have been published within the last 5 years. The best murine studies are published in the highest impact journals, such as *Nature* and *Science*, yet only the most extraordinary human-based studies receive such recognition. Although murine models have provided insights into immunologic processes, have they really improved the understanding of human asthma, or, more importantly for patients, led to the discovery of new therapies? Generally speaking, the answer has to be “no.” Furthermore, there are several instances where animal “models” of asthma may have even misdirected resources and thinking.

Despite their attractiveness from a technologic, transgenic, and drug-screening perspective, murine models have a range of shortcomings, many of which were outlined several years ago in *Trends in Pharmacological Sciences* (3). Those “original” defects remain unresolved and more are now appreciated. Until these shortcomings are addressed, murine models will continue to limit the understanding and treatment of human asthma.

CHRONIC ASTHMA IS A DISEASE UNIQUE TO HUMANS

Mice do not have asthma. Even the most “hyperresponsive” strains of mice do not exhibit spontaneous “symptoms” consistent with asthma, nor is their bronchial hyperresponsiveness (BHR) “severe” or understood mechanistically, as most mouse airways do not contain smooth muscle bundles. Although mice can be manipulated to develop allergic/Th2-type immune responses, this usually requires highly artificial peritoneal allergen sensitization procedures and adjunctive (alum) stimulation. The murine allergen challenge system does not involve either immediate or late airway obstruction, which can be physiologically measured without an additional (methacholine) challenge. Although immunization methods have been developed to invoke long-term allergen sensitivity, as opposed to tolerance, we are not aware of any murine model that develops spontaneous acute bronchoconstriction which characterizes human asthma (4).

Almost total dependency on allergen-driven murine models also diminishes the importance of other environmental factors to

the development of asthma, such as oxidant stress, viral infection, obesity and other aspects of diet, and exposure to tobacco smoke and occupational and other pollutants. In human asthma, the population-attributable risk (PAR) of atopy on asthma is less than 50% (5), and for Der p1 and Fel d1 exposure, the PAR is only 4 and 11%, respectively (6), raising questions whether atopy/allergy has been overinterpreted as a risk factor for the origins of asthma. Studies describing IgE and Th2-type lymphocytic responses in mice should not refer to them as models of “asthma,” where the disease focus is the conducting airway, but as models of systemic allergic sensitization, which are frequently self-limiting (7).

ASTHMA COMMONLY ORIGINATES IN EARLY LIFE

It is likely that differences between mouse and human disease begin early in the development of the species. Mouse lungs are fully developed at birth, whereas both nonhuman and human primate lungs continue to develop from months to years after birth. In asthma, which often begins in the first few months of life, this difference has considerable implications. In humans, a very different, immature lung is exposed to the environment during times of initial exposures to antigens or agents that interfere with lung maturation (e.g., pollutants and diet), as compared with the mature lung present in the mouse. This immature human lung, both at a structural and immunologic level, responds much differently from that of a mouse lung. Examples of this are the effect of maternal smoking in pregnancy to impair lung growth (8) and increase asthma and BHR in the offspring (9).

CHRONIC ASTHMA IS A DISORDER THAT INVOLVES CHANGES IN THE EPITHELIAL-MESENCHYMAL UNIT SPECIFIC TO HUMAN LUNGS

Even in adulthood, the structure of mouse airways bears little resemblance to that in humans (Figure 1). There are many fewer airway generations in mice (3, 4), as compared with the 27 to 29 generations in humans, suggesting much greater variety in airway structure in the latter, as well as differences in flow dynamics. Mouse lungs lack submucosal glands in every airway except the trachea, whereas human airways have an abundance of these glands throughout the large and medium-sized airways. Furthermore, in the large airways of the mouse, the bronchial epithelium is not fully stratified as it is in the larger airways in humans. This may have an impact on the way the airway epithelium responds to different inhaled stimuli (10), such as respiratory viruses and pollutants (11).

In humans, the smooth muscle is arranged in spiral bundles, which in asthma increase markedly in size due to muscle cell hypertrophy, hyperplasia, or both (12). The “smooth muscle” in mouse airways beyond the trachea and first-generation bronchi