

Promise and Pitfalls in Animal-Based Asthma Research

Building a Better Mousetrap

David B. Corry¹
Charles G. Irvin²

¹Departments of Medicine and Immunology, Baylor College of Medicine, Houston, TX, 77030, USA

²Departments of Medicine and Molecular Physiology and Biophysics, University of Vermont Medical Center, Burlington, VT, 05405

Abstract

Asthma is one of the leading chronic diseases in the world today. An essential component of the asthma research endeavor is the animal-based experimental disease system, which provides knowledge that is not attainable through study of patients alone. Animal research is especially valuable for elucidating pathophysiology, drug testing, and as an adjunct for interpreting the results of human clinical trials. However, controversies surrounding animal systems data and at the interface between animal and human studies raise questions regarding the true utility of experimental asthma research. We consider here the considerable promise and important limitations of animal-based systems and their prospects for the future study asthma.

Key Words

Asthma
Airway hyperresponsiveness
Animal model
TH₂ cell

Introduction

Asthma is a familiar respiratory affliction that exacts significant morbidity from persons of all ages, but especially children. Although asthma disease prevalence and mortality have recently stabilized in the United States, they both remain at nearly twice their levels of 30 yr ago (1), a pattern that is reflected in the health statistics from numerous countries.

These findings have transformed asthma from a relatively obscure disease into an international health problem of epidemic proportions and have propelled a massive expansion in asthma-related research in recent decades (2).

Among several aspects that include in vitro and clinical epidemiologic studies, the experimental animal disease model, more correctly viewed as an animal system, has formed one of the pillars of asthma research. Today, the

Table 1. Components and Measurements of Asthma Models

Antigens	Animals	Physiologic measurements	Mediators of bronchoconstriction	Immune Measurements
Ovalbumin	Mouse	In Vivo	Acetylcholine	Blood/serum
Extracts of	Rat	Airway resistance	Methacholine	Eosinophils
Fungi	Hamster		Histamine	Serum IgE, IgG
Pollens	Guinea	Air Pressure	Serotonin	Cytokines
Mites	Swine	Air overflow	Antigen	Lung/BALF
Insects	Cat	Penh	Electrical field simulation	Eosinophils,
Infections:	Dog	Ex vivo		Th2 cells, Mast
Fungi	Horse	Tracheal ring	Adenosine	cells; IgE/IgG
Helminths	Cow	constiction	Bradykinin	Chemokines
Viruses	Sheep		Vagal stimulation	Cytokines
	Primate			Glycoproteins
				(many others)

mouse is the dominant animal used experimentally, but the success of the animal asthma system has ensured the participation of many domesticated animals and the development of a plethora of reagents and techniques for assessing relevant endpoints (Table 1).

Despite what may appear to be a robust and well-accepted experimental device, animal-based asthma systems today face increased scrutiny and even open questions regarding their validity (3–11). Dissatisfaction with the use of animal systems to investigate asthma is due to markedly discrepant experimental findings as well as disagreement between related animal and human studies. An additional impediment to accepting asthma systems is the perceived difficulty in modeling the notorious heterogeneity of asthma, which takes expression as the many distinct clinical subsets (Table 2). Here, we consider in detail the controversies involving asthma systems research in light of major new developments and discuss how needed changes in animal systems will help ensure their future contributions.

Human Asthma

Asthma is a respiratory disease that often begins in early childhood and that is marked

Table 2. Common Subsets of Human Asthma

Viral induced
Allergic
Non-allergic
Intrinsic
Extrinsic
Occupational
Persistent
Seasonal
Exercise induced
Nocturnal
Steroid resistant

by dyspnea, or difficulty breathing, and other symptoms including cough, chest tightness, chest pain, and wheezing. Symptoms are present intermittently and are characteristically relieved by agents that relax airway smooth muscle (e.g., β adrenergic receptor agonists) or suppress airway inflammation (e.g., corticosteroids, leukotriene modifiers). Where uncertainty exists, the bronchial provocation test is used to determine the presence of airway hyperresponsiveness, which is useful in both establishing and excluding a diagnosis of asthma (Box 1). Although on clinical grounds alone asthma is immediately recognizable during acute attacks, a uniformly

Box 1. The difficulties of measuring airway hyperresponsiveness.

Of the many different endpoints that can be measured with asthma models, by far the most difficult, and most controversial, is airway hyperresponsiveness (*AHR*). Although the contractile nature of the mammalian airway was realized nearly 100 yr ago (102), a practical test for asthma based on the ability of cholinergic and other neurogenic stimulation to promote airway constriction required many decades of refinement. The modern bronchial challenge test is used in cases where the diagnosis of asthma is equivocal. Patients are asked to inhale an aerosol containing a defined concentration of methacholine or histamine (variant assays may use many other bronchoconstricting agents) after which the forced expiratory volume in 1 s (FEV1) or airway resistance is measured. The reduction in FEV1 or increase in airway resistance are determined with successive doubling doses of bronchoconstricting agent and when a 20% fall, or increase for airway resistance, is reached as compared to baseline values, the test is terminated. Using linear interpolation, the provocative concentration of bronchoconstricting agent that induced a 20% change from baseline values is determined (PC₂₀ values). PC₂₀ values significantly below the normal values established for the laboratory are indicative of the presence of asthma.

AHR as determined in humans requires a cooperative subject, and thus the identical measurements of FEV1 and airway resistance from experimental animals are not feasible. However, several reliable indicators of airway caliber can be obtained from anesthetized and paralyzed animals receiving mechanical ventilator assistance after challenge with methacholine, acetylcholine, and other agents (Table 1). Commonly obtained airway physiological endpoints in animals include airway pressure, resistance, and compliance and closely related derivative measurements (94). Unfortunately, these measurements often require considerable

animal handling and surgical skill together with facility with complex electromechanical equipment and software. Moreover, the extensive surgery demanded usually permits only single, terminal evaluations of physiologic endpoints (94).

To address these limiting factors, a rapid, non-invasive, means of determining AHR repeatedly in the same mouse was recently introduced using the whole body plethysmograph. Using highly sensitive transducers and sophisticated software, this innovative device scrutinizes the flow of gas traversing the plethysmograph, interpreting maximal and minimal changes in gas flow as mouse-dependent inspirations and expirations, respectively. Instead of assessing airway caliber using first-order Newtonian mechanical physical principles (i.e., through measurements of airway resistance or pressure), the whole body plethysmograph combines a time analysis of the different phases of the breathing-altered flow waveform with peak inspiratory and expiratory flow measurements to yield an empiric equation for the calculation of enhanced pause (Penh). The Penh measurement, which has no physical basis, was originally shown to correlate with true measurements of airway resistance in mice undergoing methacholine challenge testing (103). However, a more-detailed analysis of Penh has shown it to be an unreliable marker of airway hyperresponsiveness in multiple mouse strains (82). Among several practical and conceptual problems, many factors with no relation to bronchoconstriction markedly affect the measurement of Penh, only some of which can be controlled by the investigator (104). Although the technical skill required for reliably measuring AHR in animals will probably never be fully circumvented, more rapid techniques based on traditional methods that also permit the measurement of airway resistance repeatedly in the same animals may be possible with further refinements in techniques.

acceptable definition has remained elusive, at least in part because of the non-specific nature of symptoms and a clinical spectrum that blends with many additional disease processes (Fig. 1).

Asthma patients are classified into clinical subtypes according to highly characteristic environmental agents and other exposures that elicit typical disease symptoms (Table 2). Respiratory viruses are the most frequently

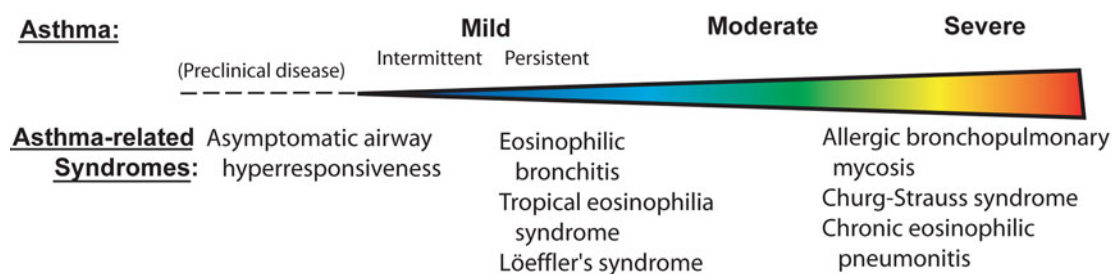


Fig. 1 Relative severity of asthma and asthma-related syndromes. Based on severity of symptoms and medications required, asthma is classified as mild, moderate, or severe. Asthma-related syndromes are classified according to similar criteria as shown. Only asymptomatic airway hyperresponsiveness is a preclinical disease because it is associated with no symptoms.

implicated causes of asthma attacks, especially in children, and tobacco smoke and air pollution are other major inciting agents. Strikingly, the majority of asthma patients are atopic (12,13), a term that reflects the production of immunoglobulin E (IgE) and both the genetic predisposition toward immediate-type immune reactions and exposure to causative environmental agents such as pollens, dust mites, fungi, and many insects. Atopy is largely controlled by T helper type 2 (Th2) cells, CD4+ T cells that secrete a restricted repertoire of cytokines that includes interleukin 4 (IL-4), IL-5, IL-9, and IL-13 and through which are strongly linked to the pathogenesis of allergic asthma (Fig. 2). If evidence for atopy is present, patients are referred to as extrinsic, atopic, or allergic asthmatics, whereas lack of atopy is referred to as intrinsic or non-allergic asthma. In general, symptoms of asthma are provoked when triggering agents are inhaled from the environment. Some agents, such as viruses and pollen, are only intermittently present, whereas patients may be continuously exposed to other agents such as fungi, giving rise to the terms seasonal and persistent (or perennial) asthma, respectively. Occupational asthma refers to asthma acquired in the work place, where dozens of potentially toxic agents have been identified.

The strong link between specific environmental agents and asthma suggests that sensitization, especially Th2 cell-dependent allergic immunity, to inhaled substances is important to the disease process, at least for patients in which potential sensitizing agents are identifiable. However, asthma symptoms are induced in additional patient subsets simply by exercise or rapid breathing, clinical features of disease which alone suggest no particular link to inflammatory mechanisms. A final category of asthma, steroid resistant, refers to patients who are relatively unresponsive to anti-inflammatory corticosteroid therapy, again suggesting that immune mechanisms may be less important in these patients (Table 2).

Heterogeneity in Asthma

Although a large group of asthma patients clearly has an allergic basis for their disease, the diverse clinical subsets of asthma suggests an overall complex disease process with a heterogeneous pathogenesis. However, closer examination of ostensibly nonatopic asthma subsets reveals unexpected evidence of allergic inflammation including eosinophilic airway inflammation and peripheral blood Th2 cells in patients with exercise-induced asthma (14,15) and Th2-related allergic

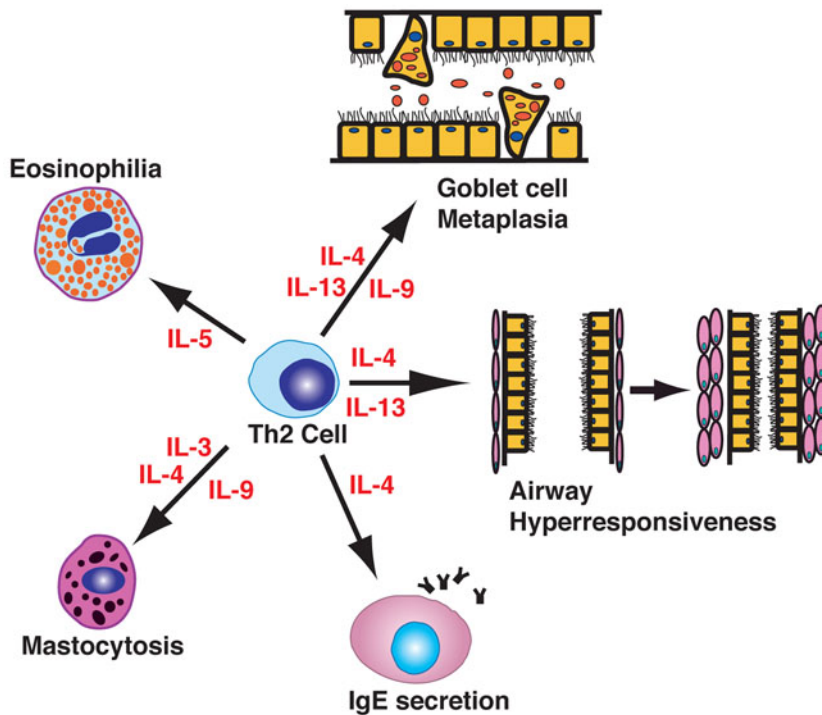


Fig. 2 The central role of the Th2 cell in allergic asthma. Through its secreted cytokines, the Th2 cell controls the growth and differentiation of eosinophils, mast cells, and IgE-secreting B cells. Th2 cytokines also directly affect lung tissues, resulting in goblet cell metaplasia with glycoprotein hypersecretion and airway hyperresponsiveness, the principal causes of airway obstruction in asthma.

inflammation in the airways of intrinsic (nonatopic) asthma patients (16) that is indistinguishable from that observed in atopic asthma (17). Furthermore, despite the great diversity of inciting agents linked to occupational asthma, many are associated with IgE and other atopic responses. Among the best studied of these agents, including significant correlation from animal systems, are microbial proteinases, used extensively in industry and incorporated into many detergent products (18,19), and diisocyanates, a component of industrial paints (20,21). Although steroid-resistant asthmatics are less responsive to the anti-inflammatory effects of corticosteroids, this appears to be due to defects in glucocorticoid receptor expression and not any fundamental difference in underlying pathogenesis

relative to atopic asthmatics (22–24). These findings indicate that Th2 cell-dependent atopy and allergic inflammation are not restricted to the asthma subset that is allergic, but are, rather, fundamental characteristics of most or all asthma clinical subsets.

It should be noted that, at multiple levels, asthma is independently linked to many additional respiratory conditions. These unusual illnesses overlap in many clinical regards with asthma, but are sufficiently unique to warrant separate diagnostic categories (Fig. 1, Table 3). Expressed in terms of their approximate clinical severity, which is determined by the severity of symptoms and the medications required, mild and moderate asthma occupy the middle portion of an allergic respiratory disease spectrum that also includes at the mildest extreme,

Table 3. Asthma-like Respiratory Syndromes

Disease or syndrome	Asthma-like features	Asthma dissimilar features	Risk factor
Asymptomatic AHR	AHR	No symptoms to suggest disease	
Eosinophilic bronchitis	Cough, Airway eosinophilia	No AHR; few attacks of dyspnea	Asthma
Tropical eosinophilia syndrome	Cough, Wheezing, AHR, Airway eosinophilia, increased IgE levels	Chest roentgenogram abnormalities; transient symptoms; caused by migrating parasite larvae	Exposure to parasites
Loeffler's syndrome	Cough, Wheezing, AHR, Airway eosinophilia, increased IgE levels	Chest roentgenogram abnormalities; transient symptoms; caused by migrating parasite larvae	Exposure to parasites
Allergic bronchopulmonary mycosis	AHR, Airway eosinophilia	Chest roentgenogram abnormalities; fungal growth in airways	Asthma
Churg-Strauss syndrome	Cough, wheezing, AHR, Airway eosinophilia increased IgE levels	Chest roentgenogram abnormalities; autoimmunity; pulmonary and systemic vasculitis; multiorgan dysfunction	Asthma
Chronic eosinophilic pneumonitis	AHR, cough, wheezing, airway eosinophilia increased IgE levels	Chest roentgenogram abnormalities; organizing pneumonitis; excessive eosinophilia	Asthma

AHR: airway hyperresponsiveness

asymptomatic airway hyperresponsiveness and at the opposite extreme, severe asthma, allergic bronchopulmonary mycosis, Churg–Strauss syndrome, and chronic eosinophilic pneumonitis. Mild to moderate asthma is of similar severity to three additional entities, eosinophilic bronchitis, tropical eosinophilia syndrome, and Löeffler’s syndrome.

However, these non-asthmatic respiratory syndromes share more than simply a phenotypic resemblance to asthma; the disease groupings in Fig. 1 reflect a common etiology (Table 3). Asymptomatic airway hyperresponsiveness is a strong risk factor for the subsequent development of both atopy and symptomatic asthma (25,26). Furthermore, asthma is itself the most important risk factor for the later development of allergic bronchopulmonary mycosis, Churg–Strauss syndrome, chronic eosinophilic pneumonitis, and, to a lesser extent, eosinophilic bronchitis (27).

Some of these conditions are also linked to the same environmental triggers that are associated with asthma, especially fungi (Table 3). Finally, the essential features of tropical eosinophilia and Löeffler’s syndromes, which are the result of aggregation in the lungs of the larvae of different helminth parasites, can be reproduced in parasitized mice, producing a disease model that is in most respects indistinguishable from the standard asthma system (28,29). Together, these findings provide abundant evidence that asthma and related clinical syndromes are all linked by a common allergic immunopathologic mechanism that translates, for reasons that are currently obscure, into heterogeneous clinical phenotypes.

Utility of Animal Systems in Asthma

One of the principal utilities of animal systems is to clarify disease pathogenesis, as animals are uniquely amenable to mechanistic

studies that are difficult or impossible to perform with human subjects. Careful observations of asthma patients provided the first link to an immune-related cell, the blood eosinophil, nearly a century ago and asthma was strongly linked to immunoglobulin E (IgE) soon after the discovery of this antibody isotype (30). Approximately 20 yr ago, investigators reproduced in antigen-challenged animals the airway physiological changes that had long been observed in asthmatic humans (Box 1) and soon thereafter provided the first experimental link between lung eosinophils and altered airway physiology (31,32). Subsequent human studies confirmed the association between lung eosinophils and allergic asthma (33).

The most important insight into the allergic nature of asthma derives from the original identification of Th2 and Th1 cells using mouse T cells (34). Th2 cells were ultimately shown to underlie many of the immunological phenomena linked to allergic asthma; eosinophilia and IgE production, the two immunological phenomena most strongly linked to asthma of all kinds, are controlled largely by Th2 cell-derived IL-5 and IL-4, respectively (35,36) (Fig. 2). Th2 cells very similar to their murine counterparts were later shown to exist in the lungs of asthmatic humans (17). More recently, animal systems have extended the Th2 paradigm in asthma to show the importance of IL-13, and the IL-4/IL-13 signaling pathway that ultimately activates the transcription factor STAT6, to most experimental asthma endpoints (37–39). Th1 cells, that secrete predominantly interferon gamma (IFN- γ) and IL-2, although not required for the expression of allergic disease, may be important modifying factors in asthma and specifically may lead to exacerbated disease (40).

The potential importance of numerous other allergic inflammatory agonists and

antagonists has been demonstrated through asthma systems. This growing list includes complement proteins (41), syndecan-1 (42), inducible costimulator (ICOS) (43), prostaglandins (44), OX-40 (45), and many others.

In addition to pathogenetic insight, animal studies provide preclinical evidence of efficacy for new asthma medications, the most recent examples being the leukotriene modifiers, omalizumab, a humanized anti-IgE antibody and many other pharmaceuticals currently in development (46–52). In a similar regard, animal systems offer an adjunctive means for interpreting the outcomes of human clinical trials. The following example illustrates how the combination of human and animal studies provides complex and often surprising insights not necessarily attainable through human study alone.

B cells and IgE

In addition to the association of IgE with asthma, elucidation of the immunological mechanism of immediate hypersensitivity established IgE as a potentially critical effector molecule in asthma (53–55). Initial studies of the importance of IgE in animal systems tended to confirm an important role for IgE in asthma and supported the nascent efforts of the pharmaceutical industry to develop therapeutics to inhibit this immunoglobulin (56,57). However, subsequent animal studies using mice deficient in IgE and other immunoglobulins established definitively that IgE was not required for allergen-induced airway hyperresponsiveness or any other objective aspect of the asthma phenotype aside from IgE production itself (40,58–60). Nonetheless, omalizumab was developed as a novel agent for use in asthma, at least in part because IgE depletion in mice showed some anti-inflammatory effect (46). On the one hand, clinical trials involving omalizumab in

asthma have vindicated the studies of IgE-deficient and IgE-depleted mice: depletion of IgE in human asthmatics has little or no clinically significant effect on objective measures of airway obstruction, including FEV1 and PC₂₀ values (airway hyperresponsiveness) (61,62), although it does reduce allergic inflammation of the lung (62). Conversely, omalizumab is clearly effective in asthma because it markedly improves symptom and quality of life scores, decreases the need for other potentially toxic asthma medications such as glucocorticosteroids, and decreases the requirement of emergency medical care, among other salutary effects (63–65).

Human and animal studies of IgE in asthma are, of course, not entirely comparable—in several animal studies, IgE was removed genetically, and in humans IgE was depleted pharmacologically, among other differences. Furthermore, animal-based asthma systems cannot as yet model the subjective endpoints that are the focus of many asthma clinical trials (quality of life and symptom scores, rescue medication usage, emergency room visits). Nonetheless, the lack of complete efficacy of omalizumab as predicted by mouse studies suggests that inflammatory mediators, and therefore drug targets, more important than IgE exist in asthma. Comparison of mouse and human studies further raises the striking possibility that the clinical efficacy seen with omalizumab may reflect activities unrelated to IgE inhibition—a possibility that was not excluded in clinical trials—and which suggests novel anti-inflammatory properties of immunoglobulins that may be amenable to further exploration using animal systems (66).

Controversies with Asthma Systems

The preceding discussion suggests a robust experimental system and a productive collaboration between human and animal asthma

investigators. However, the following example reveals how confusion, and not insight, may predominate when comparing roughly comparable animal and human studies.

IL-5 and Eosinophils

In addition to the long-standing association between the eosinophil and asthma, the contents of eosinophil granules were shown to be highly toxic when administered to the rodent airway, thus implicating eosinophils as potentially important mediators of diseases such as asthma (67,68). Additional animal studies unambiguously revealed the crucial role of IL-5 as an eosinophil growth and differentiation factor, thus suggesting that neutralization of this Th2 cytokine would be effective in eliminating eosinophils and for treating asthma (69). However, initial studies of rodents with eosinophilic lung inflammation, whether induced by recombinant IL-5 or antigen, indicated that neither IL-5 nor eosinophils were required for airway hyperresponsiveness and other manifestations of asthma (70,71). Although later studies have confirmed these findings (72,73), many additional animal studies have indicated a requisite role for IL-5 and eosinophils in mediating the experimental asthma phenotype (74–77). This comparison is remarkable for the especially large number of conflicting studies, and, for this reason, the role of IL-5 and eosinophils in experimental asthma technically remains undecided.

Because of the potential benefit of IL-5 blockade, clinical trials that explored the utility of inhibiting IL-5 in asthma proceeded. Although humanized anti-IL-5 antibodies given to asthma patients clearly depleted the peripheral blood of eosinophils, the results of two clinical trials failed to show clinically significant benefit from this intervention, although the primary intent of these studies was safety (78,79). An additional surprise revealed in a subsequent clinical study was

that anti-IL-5 therapy failed to completely remove eosinophils from the lung (80)—a result that animal studies completely failed to predict.

The results of the anti-IL-5 studies exemplify the serious conflicts between animal studies that are possible and the inability of animal studies to guide clinical trials—critical issues that fuel the debate over the relevance of animal systems in asthma (7). Nonetheless, careful consideration reveals the nature of the controversies. Unlike mice in which IL-5 was typically inhibited at the time of first exposure to allergen, that is, during the initial induction of lung inflammation, in humans anti-IL-5 therapy was administered at a point long after disease had been established, i.e., during chronic disease. In fact, although chronic asthma models have been described (81), the vast majority of animal studies model only the effects of acute inflammation—a practice that may be inadequate for understanding the effects of therapeutic interventions at chronic stages of disease that are more representative of asthma.

Additional technical issues likely contributed to the discrepancies in the animal studies of IL-5 and eosinophils. There is no dispute that inhibition of IL-5 strongly inhibits eosinophilia during acute allergen challenge in asthma systems. Rather, disagreement principally centers on the effect of eosinophil depletion on a single parameter of the asthma phenotype: airway hyperresponsiveness (Box 1). In general, studies in which airway pressure, resistance, and related measurements were used tended to support a less than crucial effect of eosinophil depletion, whereas studies that used alternative measurements of airway hyperresponsiveness (e.g., Penh) predominantly indicated a beneficial effect. The wide use of Penh measurements in recent years has resulted in additional controversies in experimental asthma research

that may be understood based on the use of this non-invasive, but unreliable, method for assessing airway hyperresponsiveness (82).

Numerous other technical challenges posed by asthma systems are accommodated in different laboratories through the use of very distinct and possibly incompatible methods. In addition to the duration of antigen challenge and protocols for assessing changes in airway physiology, the nature of the antigen used to induce inflammation can strongly influence both immunological and physiological endpoints of interest (Box 2). Other essential factors that should be considered in the design of asthma systems include the most appropriate, experimental animal; the age, sex, genetics, and immune status of that animal; the method of administering antigen to the lung; the experience of the investigators; the techniques used for measuring secondary endpoints (e.g., serum IgE levels); and the presence of comorbid conditions, such as pneumonia, that might influence the lung immune response to antigen challenge. Many of these issues are, of course, not unique to asthma systems. Nonetheless, differences in how such factors were managed likely provide insight into additional unresolved issues in asthma systems such as the relative importance of CD8+ T cells (83,84), matrix metalloproteinases (85,86), $\gamma\delta$ T cells (87,88), NK+CD4+ T cells (84,89), and other controversial issues in asthma systems research.

Technical misadventures are, furthermore, neither the sole domain of animal systems nor related exclusively to the choice of analytical method. Perhaps most frequently, asthma clinical trials appear to go awry simply due to lack of pathophysiologic insight (Table 4). As discussed above, only after completion of three clinical and innumerable animal studies was it appreciated that neutralization of IL-5 only incompletely removes eosinophils from asthmatic lung. Similar lack of insight affected

Box 2. Induction of allergic lung inflammation in experimental animals

For most if not all patients with allergic asthma, allergic lung inflammation probably results from immunological reactions to substances inhaled from the environment. Sensitivity testing suggests that such allergenic material includes shed or secreted products from dust mites, cockroaches and other insects, fungi, and many pollens. Many other organic and chemical substances have been implicated as causes of asthma in occupational settings. A similar strategy of sensitization through the airway is typically used to induce allergic inflammation in various experimental animals, although several genetic mutations in mice also lead to Th2 cell activation and allergic lung inflammation in the absence of allergen challenge (96–101).

The most frequently used allergen in animal systems is ovalbumin derived from chicken egg. Ovalbumin readily induces robust allergic lung inflammation in virtually all animals tested, is inexpensive, is non-toxic (except to those sensitized to it), and has the unique advantage that it can be combined with several very useful immunological reagents, including mice with T cells that respond only to ovalbumin (105). Not surprisingly given these advantages, ovalbumin probably accounts for the vast majority of insights gained through experimental asthma systems and will continue to be an important part of experimental asthma research in the future.

However, ovalbumin is only rarely implicated in human asthma and recent studies have revealed several disadvantages of using exclusively ovalbumin as an experimental allergen. Ovalbumin does not induce inflammation in most mouse strains if given strictly through the inhalation route, which is

presumably the physiologically relevant route of exposure in asthma. Instead, animals must be immunized through a route remote from the lung before subsequent inhalation results in significant inflammation. This two-step protocol is sufficient for inducing robust allergic disease that persists for at least 1–2 wk, but in some mouse strains, continued exposure to ovalbumin leads to diminishing immunologic and physiologic responses and eventual loss of the asthma phenotype after approx 6 wk of continuous exposure (106,107). These limitations can be avoided by giving ovalbumin discontinuously or by using allergens with adjuvant properties such as proteinase activity, which are lacking in ovalbumin (18). Such alternative allergens are derived from fungi (108), pollens (18), helminths (28), and other sources with clear relevance to human asthma (Table 1) and may be combined with ovalbumin to take advantage of its unique reagents.

A distinct approach to inducing asthma-like disease is to administer extracts of helminths to the airways of animals that are naturally parasitized by organisms such as *Ascaris suum*. This protocol avoids what can be a lengthy immunization period (often lasting 2–3 wk), but results in non-standardized sensitization. A more predictable benefit of protocols relying on natural sensitization against helminths is that they are suitable for large animals such as sheep and primates in which, relative to rodents, lung anatomy, physiology, and immunological reactivity may more closely parallel human parameters (47,109). Unfortunately, the expense and difficulty of working with large animals puts them out of reach of most investigators.

studies of IL-4 in asthma. Based on positive studies of mice that suggested IL-4 as an important drug target in asthma, clinical studies with Nuvance, a solubilized IL-4 receptor, were launched (Table 4). Although well tolerated, Nuvance produced only modest benefit in asthmatic patients, insufficient to support further development of this expensive pharmaceutical (90). Additional insight from animal studies suggests why Nuvance likely

failed: to be most effective, a Th2 cytokine neutralization strategy must take into account both IL-4 and IL-13, each of which activates STAT6, yet controls distinct aspects of the disease process (38). Recombinant IL-12 given subcutaneously also failed in asthma clinical trials because of the marked toxicity of this potent cytokine and the need for direct administration to the airways for maximal effectiveness (91,92) (Table 4).

Table 4. Discrepant Human and Animal Asthma Studies

Pharmacologic Target	Targeting strategy		Effect in asthma system	Effect in asthma	Comments
	Mouse	Human			
IL-4	Ab (59,71), gene KO	Soluble receptor (110)	Highly effective	Minimally effective	Effectiveness depends on IL-4 neutralization prior to antigen challenge
IL-5	Ab (71), gene KO (59)	Ab (78)	Ineffective/effective	Ineffective	(See text)
Th2 cells, eosinophils	rIL-12 (91)	rIL-12 (92)	Effective	Ineffective	IL-12 must be given directly to airway for maximal effect; toxic

Ab: antibody; KO: knockout; rIL-12: recombinant IL-12.

Future Prospects for Asthma Systems Research

Controversies regarding asthma systems research have centered on the complex and heterogeneous nature of asthma as suggested by the many distinct disease variants (Table 2). The concept of heterogeneity extends to additional respiratory conditions with some resemblance to asthma, yet are sufficiently distinct to warrant separate disease classifications (Fig. 1, Table 3). The diversity that exists within asthma and asthma-related diseases is perhaps the main reason that a uniformly acceptable definition of asthma does not exist and suggests, to many investigators, that simplistic animal systems as currently used may be inadequate for the study of asthma.

However, the relevance of asthma systems lies not in terms of their ability to finely mimic the more diverse clinical manifestations of allergic lung disease, but rather to reproduce in detail the underlying allergic immune mechanisms responsible for most forms of asthma and asthma-like diseases

and correlate them with a limited set of clinically relevant disease variables. Drug testing will remain an important aspect of asthma systems research, but the greater impact of asthma systems will continue to be in elucidating immunopathogenetic mechanisms.

Controversies in asthma systems, as reflected in widely divergent findings despite similar study designs, are a growing problem that appears to be most closely linked to technical aspects of complex experimental protocols. The contribution of specific technical issues (e.g., the use of Penh) to conflicting experimental findings often cannot be determined with certainty, but such unresolved issues are irrelevant to the ability of asthma systems to adequately model important aspects of asthma and contribute to drug testing and understanding disease pathophysiology. Clearly, however, improvements are needed in asthma systems to make them less controversial and thus more credible.

A variety of approaches is necessary when initially attempting to solve the technical difficulties posed by animal-based asthma systems

(Table 1), but continued use of new methods is appropriate only if subsequent studies provide adequate validation. Airway physiologic methods that are not based on first-order Newtonian physical principles (e.g., P_{enh}) have been shown to be unreliable and their use, even as screening methods, should be abandoned in favor of well-established methods that are based on sounder principles (Box 1) (93,94). Ovalbumin will continue to be an important agent for induction of acute allergic lung disease, but its serious limitations suggest the need to use agents that more closely resemble allergens implicated in human disease. This issue is particularly relevant to the study of chronic asthma, for which ovalbumin is particularly ill suited (Box 2). Adoption of more reliable airway physiologic techniques and more appropriate antigens, and the use of more chronic allergen challenge protocols will alone solve many of the discrepancies with experimental asthma research by ensuring more realistic asthma systems. It is conceivable that with the widespread adoption of chronic models, more diverse disease phenotypes, perhaps similar to the many asthma-related respiratory syndromes (Table 3), will eventually emerge.

In addition to chronic aspects of disease, animal-based asthma systems have almost completely ignored the earliest factors that lead to the initiation of allergic inflammation. Specifically, no mechanism adequately explains why Th2-biased allergic inflammation (as opposed to other forms of inflammation) is the typical result of exposure to the many known allergens. Another important and unresolved controversy in asthma that could be addressed with animal asthma systems is the nature of “spontaneous” disease. Specifically, it remains unclear whether some forms of asthma (e.g., intrinsic asthma) are entirely endogenous in origin, possibly even having an autoimmune component (95), or if asthma virtually always originates through an environmental exposure. Mice with a variety of

genetic manipulations that confer spontaneous (i.e., without the need for allergen challenge) allergic lung inflammation have been produced (96–101) and may be used to dissect this important issue.

Current animal systems also do not effectively model asthma exacerbations, which account for most asthma-related emergency room visits. In part, this reflects a lack of insight into the etiology, and even definition, of asthma exacerbations—whether related to allergen exposure, intercurrent viral infections, emotional state, exposure to other environmental contaminants such as air pollutants, or multiple factors. Rather than an intractable problem, we believe that recent advances in animal-based asthma systems permit this issue now to be rigorously addressed.

In hindsight, it is tempting to view unsuccessful asthma clinical trials as simply folly, but the studies reviewed here reflect appropriate pathophysiologic insight based on information available at the time such studies were designed. As the pathophysiology of asthma becomes increasingly clear, more sophisticated clinical trials, with commensurately more powerful discoveries, will become possible. Enhanced animal systems will accelerate our understanding of allergic disease pathogenesis and increasingly determine which therapeutic targets are most suitable for drug development. The inevitable result of the collaboration between clinical and experimental investigators will be an expanded product pipeline and increasingly effective means for both the prevention and treatment of asthma.

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