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REVIEW

Modeling asthma: Pitfalls, promises, and the road ahead

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Abstract

Asthma is a chronic, heterogeneous, and recurring inflammatory disease of the lower airways, with exacerbations that feature airway inflammation and bronchial hyperresponsiveness. Asthma has been modeled extensively via disease induction in both wild-type and genetically manipulated laboratory mice (*Mus musculus*). Antigen sensitization and challenge strategies have reproduced numerous important features of airway inflammation characteristic of human asthma, notably the critical roles of type 2 T helper cell cytokines. Recent models of disease induction have advanced to include physiologic aeroallergens with prolonged respiratory challenge without systemic sensitization; others incorporate tobacco, respiratory viruses, or bacteria as exacerbants. Nonetheless, differences in lung size, structure, and physiologic responses limit the degree to which airway dynamics measured in mice can be compared to human subjects. Other rodent allergic airways models, including those featuring the guinea pig (*Cavia porcellus*) might be considered for lung function studies. Finally, domestic cats (*Feline catus*) and horses (*Equus caballus*) develop spontaneous obstructive airway disorders with clinical and pathologic features that parallel human asthma. Information on pathogenesis and treatment of these disorders is an important resource.

KEYWORDS
airways, eosinophils, inflammation, leukocytes, veterinary

1 | INTRODUCTION

Asthma is a chronic disease of the lower respiratory tract, characterized by variable and recurring airflow obstruction, bronchial hyperresponsiveness, and airway inflammation.[1] Recent research has led to an understanding of asthma as a heterogeneous disease with multiple etiologies. The factors that initiate asthma and agents that elicit exacerbations are numerous and complex, and include infectious, environmental, allergic, and genetic components (reviewed in[2–6]).

Asthma has been modeled extensively in laboratory mice. Genetically defined mouse strains have been a mainstay of medical research since the 1950s, and as a species, mice have virtually overtaken the field of in vivo modeling of asthma, notably since the development and ready availability of transgenic and gene-deletion technologies.[7–12]

There are researchers who believe that mouse asthma models have limited predictive reliability, and that clinically useful information will emerge only from direct exploration of humans and human disease (e.g. ref. 13). In this review, we will consider the principles of in vivo modeling of human disease, as well as the specific features of mouse models and their ongoing use for the study of asthma as allergic airways disease. A summary of this information can be found in Table 1. We will also highlight some of the literature on asthma and related disorders in other mammalian species.[14,15]

Abbreviations: AHR, airways hyperreactivity; ASM, airway smooth muscle; CCS, corticosteroids; FDA, Food and Drug Administration; IAD, inflammatory airways disease; ILC2, type 2 innate lymphoid cell; LABA, long-acting beta-agonist; RAO, recurrent airways obstruction; Th2, type 2 T helper cell; TSLP, thymic stromal lymphopoietin

2 | ASTHMA: HISTORICAL PERSPECTIVE AND RECENT ADVANCES

2.1 | Early history of asthma

The earliest descriptions of asthma were those of Dr. Henry Hyde Salter (1823–1871) who described a cohort of patients whose respirations were normal at baseline, but experienced intermittent bouts of breathlessness and cough that improved spontaneously. He attributed the condition to abnormal contraction of airway smooth muscle (ASM) caused by perturbed function of the nervous system.[16] Interestingly, Salter prescribed black coffee, a beverage with relatively high levels of the xanthine alkaloid, theobromine. Both theobromine and the related compound, theophylline, which reduce ASM contraction in part

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TABLE 1 Human asthma and mouse allergic airways disease: are we not men?

Asthma, in the form of allergic airways disease, has been modeled extensively in inbred mouse strains, a phenomenon that has accelerated since the development of transgenic and gene-deleted technologies.
While asthma was first identified as a primary disorder of airway contractility, the inflammatory components that have been reproduced most successfully in mouse models. Among these, antigen sensitization and challenge models—ovalbumin, and more recently, house dust mite, cockroach, and fungal extracts—have contributed to our understanding of the T1/T2 disease paradigm, the role of Th2 cytokines (IL-4, IL-5, and IL-13), and parallel pathways involving epithelial cytokines (IL-25, IL-33, and TSLP) and innate lymphoid cell responses. Taken together, these observations contributed to the development biologic therapies (e.g. mepolizumab, dupilumab) that block cytokine signaling and limit airway inflammation.
While humans and mice are closely related species from the overall perspective of life on Earth, there are critical distinctions that require careful consideration and attention to experimental design. For example, mouse and human eosinophils differ significantly from one another at molecular level (e.g., expression of specific receptors, granule proteins, and cytokines, ex and in vivo responses). Yet, as a distinct leukocyte lineage, human, and mouse eosinophils are distributed in similar sites throughout the body, they maintain parallel developmental patterns in the bone marrow and they display largely conserved responses to Th2 cytokine provocation.
Yet, despite careful attention to experimental design, differences in lung size, structure, and physiologic responses limit the degree to which airway dynamics measured in mice can be compared directly to those from human subjects.
Among the challenges for the future, chronic allergen exposure models that include physiologic exacerbants (bacteria, tobacco, viruses, ozone) are already under development, together with models directed toward understanding the pathophysiology of unique asthma phenotypes.

by inhibiting phosphodiesterases, thus increasing intracellular cAMP, were first-line therapy for asthma until relatively recently.[17]

Sir William Osler (1849–1919) further defined the pathological and clinical features of asthma, and reported increased mucus in the airways, specialized inflammation of the bronchioles, and a familial predisposition. He was also the first to recognize the association of asthma with allergy (e.g. hay fever) as well as the existence of specific environmental triggers, including dust, cats, stress, and upper respiratory infections.[18] Despite Osler's description of asthma as an inflammatory disease, treatment both then and for the next 100 years remained squarely focused on bronchospasm and bronchodilators, including adrenaline, isoprenaline, and theophylline.

2.2 | Asthma as disease of inflammation

As far back as the 1950s, clinicians began to report on the use of broad-spectrum anti-inflammatory agents for the treatment of acute asthma.[19] Randomized, placebo-controlled trials in the 1970s clearly established the efficacy of inhaled corticosteroids (CCS) to reduce disease exacerbations,[20] prompting broader recognition of the importance of airway inflammation as a critical component of asthma pathogenesis.

More recently, the link between allergic responses and asthma has been more clearly defined. Among these features, there is now clear understanding of the crucial roles of IgE, mast cells, eosinophils, and basophils in the immune response to respiratory allergens.[21,22] Several more recent discoveries have further clarified our understanding of mechanisms generating allergic airways inflammation, including:

- the T1/T2 paradigm of adaptive immunity, in which specialized type 2 T helper cell (Th2) lymphocytes generated during the allergic response secrete a unique cohort of cytokines, including IL-4, IL-5, and IL-13[23]
- respiratory epithelial barrier damage and dysfunction, leading to release of a distinct set of cytokines that can also drive the type 2 response (e.g. IL-25, IL-33, thymic stromal lymphopoietin (TSLP)) and
- characterization of lung resident type 2 innate lymphoid (ILC2) cells activated by the aforementioned epithelial cell-derived mediators, which generate the cytokines IL-5 and IL-13.[24]

Taken together, these studies have identified new molecular targets for the treatment of asthma and have driven the development of biologics, notably, anti-cytokine, and anti-cytokine receptor monoclonal antibodies that disrupt pathways implicated in allergic inflammation.[25,26]

2.3 | Asthma heterogeneity

At current writing, asthma subtypes have been identified and are differentiated primarily by clinical features. However, they may also be distinguished by unique molecular pathways of inflammation, termed "endotypes."[27] At present, neither clinical or molecular classifications of asthma endotypes have been precisely or uniformly defined. In general, most mild-to-moderate or moderate to severe forms of asthma of childhood onset are characterized by atopy (i.e. IgE-mediated sensitization to allergens), CCS responsiveness, and evidence of Th2-type immunity (eosinophilia, high serum periostin, and exhaled nitric oxide (FeNO) levels[26,28]). However, a distinct phenotype, or subset, classified as "severe eosinophilic asthma" has been identified, with onset of moderate to severe disease in early to mid-adulthood, higher levels of blood and lung eosinophils, and high FeNO levels, yet no evidence of atopy; effective treatment of these patients requires systemic CCS or biologic therapy. Another subgroup with adult onset severe asthma is characterized by the absence of atopy, and absence of eosinophils, but rather evidence for non-T2 processes including T17 immunity and lung neutrophils; this form is also associated with female gender and associated obesity. With ongoing utilization of unbiased genomic and transcriptomic studies, more precise molecular classifications of specific asthma subgroups and endotypes will most likely emerge.

Studies of sputum, blood, bronchial brushings, and airway tissue also delineate pathological and molecular features that contribute to our understanding of clinical phenotypes.[29] Severe asthma has been defined as either $T2^{hi}$ (eosinophilic) and $T2^{lo}$ (noneosinophilic).[28,29] Within the $T2^{hi}$ group, nonallergic asthma may be driven by epithelial damage/activation due to pollutants, infection, or even allergens containing protease activity (e.g. house dust mite, molds), resulting in activation of the IL-25/IL-33 TSLP-ILC2 pathway without a concomitant IgE response.[28] $T2^{lo}$ asthma can be associated with mixed T1/T17 responses and airway neutrophils, which might be due to chronic bacterial colonization; interestingly, treatment with azithromycin

improved symptoms in this subgroup in a randomized, placebo controlled trial.[30] Some patients can be classified into a pauci-granulocytic subgroup, which is associated with fewer exacerbations. Such patients respond less well to anti-inflammatory therapies but may improve with bronchial thermoplasty.[31,32] Finally, it should be mentioned that the true incidence of $T2^{lo}$ asthma is difficult to define as elements of $T2^{hi}$ disease may be masked by chronic CCS usage.[28]

2.4 | Biologic therapies for asthma

Molecular classifications have paralleled the development of biological therapies that target specific molecules involved in the T2 immune response. The first to be approved by the U. S. Food and Drug Administration (FDA) was omalizumab, a mAb that prevents IgE binding to high affinity IgE receptors. Omalizumab improves symptom scores and reduces CCS usage and exacerbations in several large-scale trials, with greatest effects in $T2^{hi}$-type asthma.[33] A mAb targeting IL-5 (mepolizumab) received FDA approval in 2015 after several large trials showed efficacy in severe eosinophilic asthma, with CCS-sparing, reduced blood/sputum eosinophils, reduced numbers of exacerbations, and improved quality of life based on symptom questionnaires.[34,35]

Dupilumab, a mAb that targets the IL-4 receptor alpha subunit shared by both IL-4 and IL-13, has shown promise in improving lung function and reducing exacerbations in moderate to severe eosinophilic asthma even when baseline CCS and long-acting beta-agonist (LABA) therapy was withdrawn.[36] A more recent double-blind placebo controlled study of add-on therapy in patients with persistent/severe asthma despite usage of high dose CCS/LABA demonstrated decreased severe exacerbations and increased FEV1 in all populations regardless of blood eosinophil counts.[37] Finally, a mAb targeting TSLP (tezepelumab) significantly reduced exacerbations compared to placebo in patients with moderate to severe asthma that remained uncontrolled by CCS/LABA independent of blood eosinophil counts.[38]

3 | ANIMAL MODELS OF HUMAN DISEASE: GENERAL CONSIDERATIONS

Before moving forward to the specifics of asthma models, this section will review some of the general concepts underlying animal models of human disease. This discussion is taken largely from an excellent consideration of this subject by Jann Hau entitled "Animal Models for Human Diseases" in Sourcebook of Models for Biomedical Research.[39]

Ideally, an appropriate animal model will provide the researcher with a means to explore novel mechanisms, explain complex interactions and/or predict responses to treatment. Hau considers five categories of disease models, which will help us to conceptualize this subject with respect to asthma, notably:

- Induced disease
- Genetic manipulations
- Spontaneous disease
- Negative disease
- Orphan diseases

Allergen challenge models, with or without systemic sensitization, are all examples of induced disease, in which previously normal, healthy animals are challenged with allergen, and a phenotype resembling asthma develops. Ovalbumin, fungal filtrates, house dust mite, and cockroach extracts are among the popular allergens featured in mouse model studies (reviewed in[7–9,40,41]).

Genetic manipulations (i.e. gene-deleted or transgenic mice) can generate disease phenotypes de novo or may alter the phenotype of induced disease. Among the most familiar of these are mice that overexpress cytokines, including interleukin-5, eotaxins, and/or interleukin-13, that display airway inflammation, hyperresponsiveness, and collagen deposition without allergen provocation. Similarly, cytokine, cytokine-receptor, and related gene-deleted mice that do not respond to allergen provocation as do wild-type mice are examples of genetic manipulations that generate a negative disease phenotype.[42–44]

Orphan diseases are disorders of nonhuman species for which no human correlate is currently known. This category is not directly relevant to the current discussion.

Spontaneous diseases are natural conditions that arise in nonhuman species that can reveal parallel mechanisms of disease. In healthy vivarium conditions, laboratory mice do not develop allergic airways disease spontaneously, nor has spontaneous disease been reported among mice kept as household pets or among out-bred strains in their natural habitats. By contrast, asthma is a common disorder among household cats of all breeds, with clinical features that clearly resemble human disease (reviewed in[45]). Similarly, horses display a variety of spontaneous phenotypes; recurrent airway obstruction (RAO; heaves) includes airway inflammation with neutrophil predominance, while inflammatory airway disease (IAD), is a related disorder with a mixed neutrophil/eosinophil phenotype (reviewed in[46]). Domestic dogs typically develop dermatologic, rather than respiratory allergies, although respiratory inflammation, termed allergic bronchitis, has can develop in response to environmental allergens[15,47]; this spontaneous condition is not as common nor is it as clearly defined as similar disorders in cats and horses. In the 1980s–1990s, the Basenji-Greyhound breed was used for exploratory studies of induced respiratory responses. The focus of these studies was primarily bronchoconstriction, although the presence of eosinophils in the blood and airways and their potential contributions to this disorder were noted.[48]

We will consider both induced and spontaneous allergic diseases in mice and in other mammalian species in the Sections to follow.

3.1 | Modeling airway disease in mice

In 2016, Banfield et al.[49] published a ground-breaking manuscript in Nature Microbiology titled simply "A new view of the tree of life." Utilizing cutting edge sequencing technology, the authors reconstructed genomes of previously underrepresented life forms. One notices that, put in this perspective, larger organisms, notably those of the Animal Kingdom, are far from the dominant life forms on the planet.

3.2 | Humans and mice are closely related species

Within the Animal Kingdom, and specifically within the Class Mammalia, anatomically modern humans (*Homo sapiens sapiens*; Order Primata) and likewise, modern mice (*Mus musculus*; Order Rodentia), are closely related large organisms with complex brains, neural circuitry, instincts, and behavioral patterns. Current estimates from the fossil record together with genome sequencing result in estimates of divergence of the primates and rodents to have taken place approximately 80 million years ago.[50] At the molecular level, the genomes of humans and mice are remarkably conserved. Although the mouse genome is somewhat smaller than the human, eighty percent (80%) of mouse coding sequences have direct (1:1) orthologs in the human genome, with most of the remaining divergence related to species-specific gene clusters.[51] At the same time, it is critical to recognize that the immune system evolves under distinct, likely pathogen-directed external constraints,[52] which may differ even between closely related species given distinct environments and habitats.

3.3 | Where do laboratory mice come from?

In his 1995 book, "Mouse Genetics" L.M. Silver[53] details the complete history of *Mus musculus*, including a full background of the evolution of the species and of the development of the inbred strains as models for the study of human disease. Among the key points, the best known of the inbred mouse strains, BALB/c, was initiated from a set of albino mouse progeny in ~1913 by graduate student, Halsey Bagg,[54] who utilized this strain to examine the complex patterns of learning and behavior. Now, 100-plus years later, there are four major branches of BALB/c mice, characterized and described in detail in reference.[55] The C57BL/6 mouse strain, originated by Clarence Little[56] was established as an independent line at the Jackson Laboratory in 1948, and transferred to the National Institutes of Health in 1951, thus differentiating the two strains into C57BL/6J and C57BL/6N, the latter now maintained by Taconic Labs (C57BL/6NTac). A recent comprehensive comparison of these two substrains revealed 34 SNPs, 2 indels and 15 structural variants. Relevant to the subject of this manuscript, the C57BL/6J and C57BL/6NTac substrains display different degrees of hypersensitivity to dinitrofluoro-benzene-derivatized proteins and distinct responses to bacterial pathogen challenge.[57]

3.4 | Do rodents experience spontaneous respiratory allergies?

There is little to no information on spontaneous allergies that may develop in wild-type mice, and it is presumed that this does not occur. Among the few comments relevant to this point, Potter[55] noted that the BALB/cJ strain (in contrast to the BALB/cAn) have a marked tendency to develop facial dermatitis of unknown etiology. In contrast, there are many reports of spontaneous inflammation developing in guinea pigs (*Cavia porcellus*). Guinea pigs kept as household pets can experience spontaneous respiratory inflammation, typically in response to pathogens, but also in response to dust from inappropriate bedding materials [58, 59; also http://www.merckvetmanual.com/exotic-and-laboratory-animals/rodents/guinea-pigs]. Interestingly, guinea pigs were formerly an in vivo model of choice for experimental studies of induced respiratory allergy, notably responses to cigarette smoke.[60] As discussed by Canning and Chou,[61] guinea pigs may be a superior reflection of the human condition, notably with respect to airway mechanics and pharmacologic responses to soluble inflammatory mediators. Guinea pigs have clearly defined eosinophils, with granules containing major basic protein and eosinophil peroxidase.[62] While there is a substantial literature on experimentally induced respiratory allergies in guinea pigs (reviewed in[61]), observations on spontaneous respiratory inflammation have not been fully explored.

3.5 | Induction of allergic airways disease and generation of Th2 inflammatory responses in mice

As there is little to no evidence for spontaneous respiratory allergy in wild-type mice, allergic airways disease has been studied in response to various methods of disease induction. As noted earlier, this subject is quite extensive and has been summarized in numerous excellent recent reviews[7–12]; the reader is referred to these resources for specific details. Allergic airways disease is typically induced via an alum-adjuvanted sensitization and intranasal challenge protocol, historically with the inert antigen, the chicken egg white protein, ovalbumin. Ovalbumin sensitization and challenge results in a profound Th2 response in the respiratory tract, including upregulation of cytokines IL-4, IL-5, and IL-13 associated with dramatic eosinophil recruitment to the lung parenchyma and airways. Cytokine overexpression and gene-deletion studies have clearly documented a role for interleukin-5 in generation, recruitment, activation, and prolonged survival of eosinophils.[63,64] Likewise, IL-13 has profound importance in promoting airway hyperresponsiveness, IgE synthesis, goblet cell hyperplasia and mucus hypersecretion, fibrosis, and collagen deposition.[65–67]

For some time, a substantial debate prevailed regarding the role of eosinophils as primary effectors cells. Initial reports documenting airway responses from two unique eosinophil-deficient mouse strains seemed to contradict one another,[68,69] and fueled questions as to whether mouse eosinophils, with distinct components and limited propensity to undergo degranulation in vivo were adequate replicas of their human counterparts.[70–72] Initial failure of eosinophil-directed therapies on unstratified patient cohorts added further to this perception.[73] Ultimately, our understanding of asthma heterogeneity and the existence of the severe eosinophilic asthma phenotype, the target of two subsequent, successful therapeutic trials[74,75] ultimately vindicated the eosinophil, confirming its role as a critical mediator in at least one specific disease phenotype and pointing even more clearly to the need for precision modeling of human disorders.

As such, in efforts to parallel the human condition, researchers have recently developed new mouse models of allergic airways disease that utilize physiologic aeroallergens, including extracts of house dust mite, cockroach antigen, and airborne fungi including *Aspergillus* and *Alternaria* species. Such protocols often include prolonged and repeated respiratory challenge without systemic sensitization or adjuvants (e.g.[76–78]). There are also new mouse asthma models that

incorporate pathogen-induced exacerbations including tobacco, respiratory viruses, and bacteria.[79–81]

3.6 | Architecture of and measurements within the mouse airway

Despite the advances that have been made regarding our understanding the inflammatory response, clear differences in anatomy and physiology between mouse and human lungs and airways have the potential to limit extrapolation of findings from rodent models to human asthma. Aside from the obvious differences in size (total lung capacity of an average human is 6000 times that of a mouse), mouse airways have fewer generations and smaller bronchioles—the presumed site of bronchoconstriction in asthma—than human counterparts.[82] While alveoli are smaller in mice than in humans, they make up a bigger fraction of lung tissue, which may have implications for tissue stiffness and elastic recoil. Unlike the human trachea, which is encircled by cartilage, the mouse trachea contains only a few complete rings, a point that could be of importance in studies analyzing airways hyperreactivity (AHR) in which isometric contraction of trachea is measured ex vivo.[82] These and other nuances, including the high respiratory rate of mice (250–350 breaths/minute), distribution of smooth muscle and sensory neurons, distinct cough responses, and the observation that mice are obligatory nasal breathers, all have implications for precise measurements of airway mechanics.

In humans, spirometry, although somewhat effort dependent, can effectively determine airway resistance from air volumes expelled during forced expiration.[83] The difficulty in obtaining comparable measurements in mice has led to a trade-off between noninvasiveness and precision.[83] Previously, AHR was evaluated by "enhanced pause" (Penh), an arbitrary, dimensionless value derived from measurements of pressure changes within a closed chamber containing an unrestrained animal. Current consensus is that Penh values reflect respiration patterns rather than true lung mechanics, and they do not correlate well with invasive measurements of lung resistance.[82,84] At the other end of the spectrum are direct measurements of lung resistance by invasive plethysmography of tracheostomized, ventilated, and paralyzed animals. Animals are administered forced oscillations of air at various superimposed frequencies, and impedance is recorded in the cannula connected to a pressure transducer. This technique is currently considered to be the most precise way to capture both central airway resistance and peripheral tissue compliance. Of note, however, most studies using either technique have failed to demonstrate increased baseline airway resistance in allergen sensitized and challenged mice; instead AHR must be provoked by bronchoconstrictors such as methacholine.[84,85]

3.7 | General conclusions

While certainly not perfect replicas of human asthma, mouse modeling studies have defined the inflammatory response to antigens and aeroallergens and have provided the groundwork for the novel biologics now currently in use. New mouse models will need to target distinct asthma endotypes with greater specificity and precision.

4 | ASTHMA/OBSTRUCTIVE AIRWAY DISEASE IN OTHER MAMMALIAN SPECIES

Asthma, defined broadly, is not unique to humans. Domestic cats and horses develop spontaneous obstructive airway disorders with clinical and pathologic features that resemble the human disease.[14,15]

4.1 | Asthma in cats

Asthma is a common diagnosis among domestic cats (*Feline catus*) in the United States, affecting approximately 1–5%, with a median age at diagnosis of 4–5 years. Primary symptoms include cough, wheeze, and respiratory distress. Lung pathology in asthmatic cats includes Th2 cytokine induction, eosinophil recruitment (a feature that differentiates this condition from chronic bronchitis) and airway remodeling primarily in response to aeroallergens.[45,47,86,87] Experimental induction studies have been carried out in cats, notably those by Norris Reinero et al.,[88] who documented airways hyper-reactivity and eosinophil recruitment in response to house dust mite and Bermuda grass allergens, results suggesting the cats and humans can respond to similar aeroallergens.

Asthmatic cats respond to steroids with or without bronchodilators. As in humans, these treatments are not curative. Among the experimental treatments currently under exploration for feline asthma, allergen-specific immunotherapy, and tyrosine kinase inhibitors (reviewed in[45]). Interestingly, tyrosine kinase inhibitors were recently utilized as part of a successful human clinical trial for patients with poorly controlled, severe asthma.[89]

4.2 | Asthma in horses

Asthma also occurs spontaneously in horses (*Equus caballus*). A severe form of asthma RAO (also known colloquially as "heaves") has been attributed to transient, reversible bronchoconstriction similar to the pathophysiology of human asthma.[14,46] RAO manifests as increased expiratory effort, poor performance, and cough, and has been associated with aeroallergen exposure. Notably, RAO is more prevalent in stabled horses that have been exposed to high concentrations of environmental allergens, including dust, wood shavings, and moldy hay, but it has also been identified in pastured horses in response to grass pollen. Pathological examination of lung tissue, cells, and mediators suggests that T2 immunity and IgE contribute to airway inflammation in RAO. Interestingly, bronchoalveolar lavage (BAL) fluid from horses with either chronic RAO and/or acute exacerbations contain predominantly neutrophils, although Th2 cytokines have been identified.[90] A milder form of RAO, identified as "IAD" is characterized by nasal discharge and decreased performance, but horses with IAD display no increased respiratory effort at rest. In IAD, mixed/neutrophilic and eosinophilic inflammation may be present.[91] Similar to human asthma, RAO and IAD respond to beta-adrenergic agonists in combination with inhaled or systemic corticosteroids.[92,93]

Pathologically, RAO is associated with airway remodeling, increased airway mucus and epithelial mucous metaplasia, ASM hypertrophy, and subepithelial collagen deposition. Recent studies suggest that endotypes exist in equine RAO as there is evidence for Th17[94] or

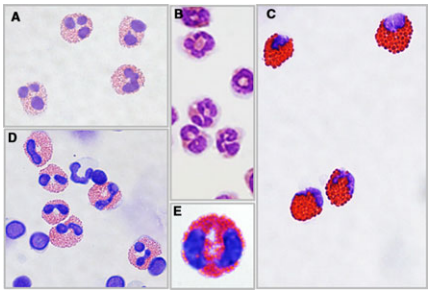

FIGURE 1 Morphologic diversity of mammalian eosinophils. Shown here are eosinophils from (A) human (*Homo sapiens sapiens*), (B) mouse (*Mus musculus*), (C) Horse (*Equus caballus*), (D) cat (*Feline catus*), and (E) guinea pig (*Cavia porcellus*). Eosinophils from human, mouse, cat, and horse are cytospins preparations stained with modified Giemsa (Diff Quik) and photographed on a Leica DMI4000 microscope at original magnification of 40×. The eosinophil from guinea pig was stained with hematoxylin and eosin and imaged with a Nikon E400 microscope, Plan Fluor 100X oil objective, 1.3 N.A

mixed Th1/2 immune responses.[95,96] Of specific interest, several compounds effective in rodent models of allergic airways disease (e.g. PDE4 inhibitors, cysteinyl leukotriene antagonists) were found to be ineffective as therapy for both RAO and human asthma, suggesting that horses might be an appropriate model to inform selection of therapies for trials in humans.[97,98]

The study of asthma in horses has several advantages including the ability to perform serial bronchoscopies and tissue biopsies in sedated, standing animals and determination of lung volumes by spirometry, both measurements that are impractical in rodents. Endobronchial ultrasound is another technique that can provide a relatively noninvasive measurement of ASM mass in horses with RAO.[99] Finally, the equine genome has been sequenced, and an equine tissue bank has been developed for lung research (http://www.ertb.ca), which provides researchers interested in equine asthma studies access to materials even if lacking appropriate facilities to house larger animals.

Images that provide a visual comparison of human, mouse cat, guinea pig, and horse eosinophils are shown in Fig. 1.

5 | CONCLUSIONS

Wild-type and genetically manipulated mice will undoubtedly continue to be a major choice for in vivo asthma models. As such, future studies might move forward with efforts toward modeling asthma as a multifaceted chronic condition with a focus on physiologic aeroallergens and exacerbants. The field in general might benefit from a larger appreciation of the complementary veterinary literature and the opportunity to study spontaneous disease. Overall, it is important to recognize not only the conclusions, but also limitations of each model, as there is no one experiment or trial that can fully reproduce the complexity of the human experience.

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DISCLOSURES

The authors declare no conflicts of interest.

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