

REVIEW

Aligning mouse models of asthma to human endotypes of disease

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ABSTRACT

Substantial gains in understanding the pathophysiologic mechanisms underlying asthma have been made using preclinical mouse models. However, because asthma is a complex, heterogeneous syndrome that is rarely due to a single allergen and that often presents in the absence of atopy, few of the promising therapeutics that demonstrated effectiveness in mouse models have translated into new treatments for patients. This has resulted in an urgent need to characterize T helper (Th) 2-low, non-eosinophilic subsets of asthma, to study models that are resistant to conventional treatments such as corticosteroids and to develop therapies targeting patients with severe disease. Classifying asthma based on underlying pathophysiologic mechanisms, known as endotyping, offers a stratified approach for the development of new therapies for asthma. In pre-clinical research, new models of asthma are being utilized that more closely resemble the clinical features of different asthma endotypes, including the presence of interleukin-17 and a Th17 response, a biomarker of severe disease. These models utilize more physiologically relevant sensitizing agents, exacerbating factors and allergens, as well as incorporate time points that better reflect the natural history and chronicity of clinical asthma. Importantly, some models better represent non-classical asthma endotypes that facilitate the study of non-Th2-driven pathology and resemble the complex nature of clinical asthma, including corticosteroid resistance. Placing mouse asthma models into the context of human asthma endotypes will afford a more relevant approach to the understanding of pathophysiological mechanisms of disease that will afford the development of new therapies for those asthmatics that remain difficult to treat.

Key words: asthma, glucocorticoid, hypersensitivity, lung, mouse.

Abbreviations: AHR, airway hyperresponsiveness; alum, aluminium hydroxide; CD, cluster of differentiation; COX, cyclooxygenase; GC, glucocorticoid; HDM, house dust mite; IgE, immunoglobulin E; IL, interleukin; OVA, ovalbumin; Th, T helper.

INTRODUCTION

The specific manifestations of clinical and experimental asthma result from complex interactions between genetic and environmental factors. Similar to clinical endotyping, which classifies asthma based on underlying pathophysiologic mechanisms and which has already been used to select specific types of asthmatics likely to respond well in clinical trials, mouse models can also be characterized and validated according to the contributing pathophysiology. Consequently, asthma endotypes and mouse models can be aligned, resulting in a stratified approach to preclinical asthma research that will facilitate conducting mechanistic studies aimed at better understanding the types of asthma that remain difficult to treat.

ASTHMA OVERVIEW

Asthma is a common chronic disorder of the airways that involves a complex interaction of airway reactivity and inflammation. About 300 million people suffer from asthma worldwide, and the US asthma prevalence is approximately 1 in 12 adults and 1 in 11 children.¹ The two mainstays of asthma treatment are β_2 adrenergic receptor (β_2) agonists, which inhibit airway narrowing and closure, and glucocorticoids (GC), which target the inflammatory component of asthma. Consistent use of GC does not prevent progressive decline in lung function, and the protracted use of anti-inflammatory GC often results in significant side-effects including immunosuppression, cataracts, growth deceleration, osteoporosis and dysphonia.² Conventional second-line treatments for asthma include pharmacologics targeting leukotriene signalling, anticholinergic bronchodilators, methylxanthines and omalizumab. Omalizumab is a humanized anti-immunoglobulin E (IgE) monoclonal

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antibody that is available as a second-line therapy for patients with elevated IgE and allergy to a known aeroallergen. For this subset of patients, omalizumab is highly efficacious, enabling patients to reduce the amount of GC required for asthma control.³ Omalizumab represents a success in matching the pathophysiologic mechanism of disease presenting in a subgroup of patients to a specific therapy.⁴ Additional targeted pharmacologic agents have demonstrated inconsistent results in clinical trials to date, with the most promising results based on studies that have synchronized human disease endotyping with mechanistic insights derived from animal models.^{5,6}

IMPORTANCE OF MOUSE MODELS IN ASTHMA RESEARCH

Thorough investigation of complex diseases, such as asthma, requires that the disease be studied in the context of the entire organism. For example, the three-dimensional structure of the lung is critical when evaluating pulmonary injury, the response to which is abnormal in asthma and re-establishing homeostasis requires the endogenous network of all cell types. Whereas *in vitro* systems such as *in silico* and mathematical models, biomimetic systems and microfluidics help address certain mechanistic pathways, animal models remain the most physiologic replication of both pulmonary cell type diversity and three-dimensional structure.⁷ Despite anatomical, cellular and functional differences between human and rodent lungs, mouse models are an indispensable tool to address the complex interactions of multiple organ systems. Furthermore, the discovery of novel drug targets and the development of safety profiles for novel pharmacologic agents demands a non-human, *in vivo* experimental approach.⁷

The underlying pathology of asthma is complex, heterogeneous and in many instances poorly understood. Both genetic factors predisposing individuals to atopy and airway hyperresponsiveness (AHR; defined as exaggerated airway sensitivity, reactivity and maximum responsiveness to stimuli), as well as environmental factors, are thought to be involved in asthma development. Asthma is further complicated by the rich natural history of the disease, including when antigen sensitization or susceptibility first develops, exposures to sensitizers or triggers that occur throughout a lifespan, and the provision of medications that while they may or may not effectively treat the disease at one point in time can lose efficacy and elicit adverse side-effects. Much of what is known about allergic airway disease and asthma is based on studies using animal models, particularly the laboratory mouse (*Mus musculus*). Conventional mouse models of asthma rely on immunologic consequences of allergic sensitization and challenge.⁷ A traditional mouse model of allergic asthma employs intraperitoneal injections of the adjuvant aluminium hydroxide (alum) in conjunction with the antigen ovalbumin (OVA). Following the sensitization period and a rest period during which the adaptive immune response is generated, mice are re-exposed to antigen

during the antigen challenge phase. Usually a single antigen challenge phase (or a round of challenges) is comprised of multiple consecutive days of antigen exposures.

The pulmonary pathology and AHR following antigen challenge in an alum/OVA model of allergic airway disease results largely from a T helper (Th) 2-driven immune response. The biologic agents that have demonstrated recent success in the treatment of clinical asthma were designed based on the original Th2 paradigm for studying asthma integrated with human data that identified clinically relevant biomarkers (reviewed in⁵). While the Th2 paradigm for studying asthma has advanced our understanding and treatment of patients with atopic asthma, it omits about 50% of patients with clinical asthma that is not characterized phenotypically by Th2-mediated, atopic airway disease.⁸ Research conducted using an alum/OVA model has translated into only a limited number of clinically efficacious treatments.^{4,7,9} Furthermore, individuals who do not respond to inhaled GC, and do not fit criteria for omalizumab therapy, have few therapeutic options.⁴ The urgent need for characterizing Th2-low, non-eosinophilic subsets of asthma,¹⁰ and for therapies for patients with severe asthma,^{11,12} warrant a different approach—in both understanding clinical asthma and studying asthma at the bench.¹³ In line with this goal, a new methodology for classifying patients based on pathophysiologic mechanisms has been proposed that addresses the heterogeneity in clinical asthma, and new mouse models of asthma are being utilized that more closely resemble the diverse clinical features of asthma.

ASTHMA CLASSIFICATION AND THE DEVELOPMENT OF ASTHMA ENDOTYPES

Whereas asthma has traditionally been classified as intermittent, mild persistent, moderate persistent and severe persistent based on subjective parameters and objective assessments, more recent guidelines suggest classification based on asthma severity and control.^{14–16} However, such classifications do not reflect underlying pathophysiology. While different clinical phenotypes of asthma have been recognized,^{17,18} these do not necessarily match underlying pathophysiology with assignment to a particular group.

Consequently, grouping patients based on established pathophysiologic and mechanistic parameters, known as endotyping, has been proposed by experts from the European Academy of Allergy and Clinical Immunology and the American Academy of Allergy, Asthma & Immunology.¹⁵ The PRACTALL report described six asthma endotypes based on clinical characteristics, biomarkers, lung physiology, genetics, histopathology, epidemiology and response to treatment. These endotypes comprised aspirin-sensitive asthma, allergic bronchopulmonary mycosis (a mould-induced hypersensitivity within the airways), allergic asthma, late-onset asthma, hypereosinophilic asthma and asthma in cross-country skiers.¹⁵ While

these endotypes still require validation through longitudinal epidemiologic studies, the PRACTALL report pointed out that by defining phenotypes in terms of the underlying pathophysiologic mechanism, endotypes could improve clinical outcomes by enabling physicians to better cater treatment based on underlying mechanisms, similar to that already in practice for omalizumab.

Building on this approach, Agache *et al.* further refined the concept of asthma endotypes by starting from broadly defined, clinically observed phenotypes (allergic asthma, intrinsic asthma, neutrophilic asthma, aspirin intolerant asthma and extensive remodelling) and aligning each phenotype with molecular biomarkers measurable in the clinic, which also reflect the underlying mechanism.¹⁹ Thus, biological markers including sputum cytology, genetics and single nucleotide polymorphisms, gene expression profiles and proteins provide details describing the underlying pathophysiology.¹⁹ When patients are grouped based on pathological features or biomarkers, distinct subgroups emerge within which a specific biologically targeted therapeutic ameliorates disease activity.⁴ In addition to the aforementioned example of omalizumab, lebrikizumab is a humanized monoclonal interleukin (IL)-13 neutralizing antibody and periostin is a biomarker indicative of IL-13 activity.^{20,21} Only asthmatics with high periostin levels respond to lebrikizumab.^{21,22} Additionally, moderate-to-severe asthmatics with elevated circulating or sputum eosinophil levels who were poorly controlled on corticosteroids and long-acting beta agonists were selected for the evaluation of dupilumab, a monoclonal antibody inhibiting IL-4 receptor alpha to block signalling by the agonist IL-4 and IL-13.⁶ In this population, treatment resulted in an 87% reduction in asthma exacerbations as well as several other benefits to asthma control, indicating that careful selection of subject populations based on endotype can bolster treatment efficacies that might be masked in a more heterogeneous patient population. The process of endotyping represents a step in the direction of personalized medicine and has already enabled better clinical trial design. Furthermore, better characterization of patient endotypes will result in the identification of novel biomarkers and pharmacologic targets, offering the potential to further refine therapeutic approaches.

Emerging data suggest that distinct cytokine networks are involved in the pathogenesis of autoimmune diseases, that each disease results from a unique cytokine network or combination of networks and that targeting 'nodes' within these cytokine networks is critical to treat disease.²³ Preclinical models should reflect the role these networks play in human disease, as surprising results sometimes ensue following individual cytokine neutralization, such as the increase in adverse reactions produced by an IL-17A blocking antibody to treat Crohn's disease.²⁴ The recent success of anti-IL-13 therapy in Th2^{high} asthma may indicate that IL-13 is a 'node' within the Th2^{high} inflammatory network.^{5,23} Because multiple underlying aetiologies and pathophysiologic mechanisms seem to be responsible for the diversity observed in

clinical asthma, the cytokine networks involved must also be included in the characterization of asthma endotypes.²⁵ Identifying analogous nodes may provide the targets for efficacious treatment in Th2^{low} asthma subtypes as well.^{10,23}

DIVERSITY OF MOUSE ASTHMA MODELS

Study of asthma using mouse models has changed significantly since the 1990s. Alum/OVA models and the traditional sensitization/challenge time course are being supplemented by alternative models utilizing diverse and physiologically relevant sensitizing agents, allergens and triggers; chronic time courses that better represent the natural history of asthma; and measures of lung remodelling that embody the diverse array of clinically observed signs of asthma (reviewed in^{26–28}). Newer models test the role of relevant risk factors, investigate potential pharmacologic targets at clinically relevant time points and study the functional relevance of multiple cell types and tissues in promoting pulmonary inflammation, including the complex interplay between the immune system, structural components and neural networks.

There currently exists a range of mouse asthma models, with diverse underlying pathologic mechanisms ranging from non-atopic to models based solely on the antigen-specific response as the instigating trigger. Models of AHR in response to acute or subacute ozone exposure reside at one end of this spectrum, while models involving the adoptive transfer of antigen-specific effector T-cells reside at the other (Fig. 1). The adoptive transfer of *in vitro* polarized, antigen-specific T-cells provides a method with which to study the contribution of the antigen-specific adaptive immune response in isolation, and *in vitro* polarization permits the investigation of multiple subsets of cluster of differentiation (CD)4⁺ T-cells, including Th1, Th2 and Th17 responses.^{29–31} Transgenic models involving the overexpression of the Th2-specific transcription factor GATA3 or the Th17 transcription factor RORγt also provide methods with which to study the role of the Th2 and Th17 adaptive immune responses in allergic airway disease, respectively, following allergic sensitization *in vivo*.^{29,32} In addition to the Th2 response, the Th17 response and the production of IL-17A are biomarkers of asthma has been reviewed by Alcorn *et al.*,³³ notably of severe, GC-resistant asthma.^{19,29,34–37} Since the functional relevance of the association between the Th17 response or IL-17A production and severe disease remains unresolved and IL-17A is not the only cytokine product of Th17 cells,^{29,38,39} the ability to study components of the adaptive immune response in isolation is clinically relevant to provide better understanding of their contribution to asthma.

Variations on the conventional models of antigen sensitization and challenge that reflect the chronic nature of asthma often result in features of airway remodelling, including smooth muscle hypertrophy, subepithelial collagen deposition and mucus

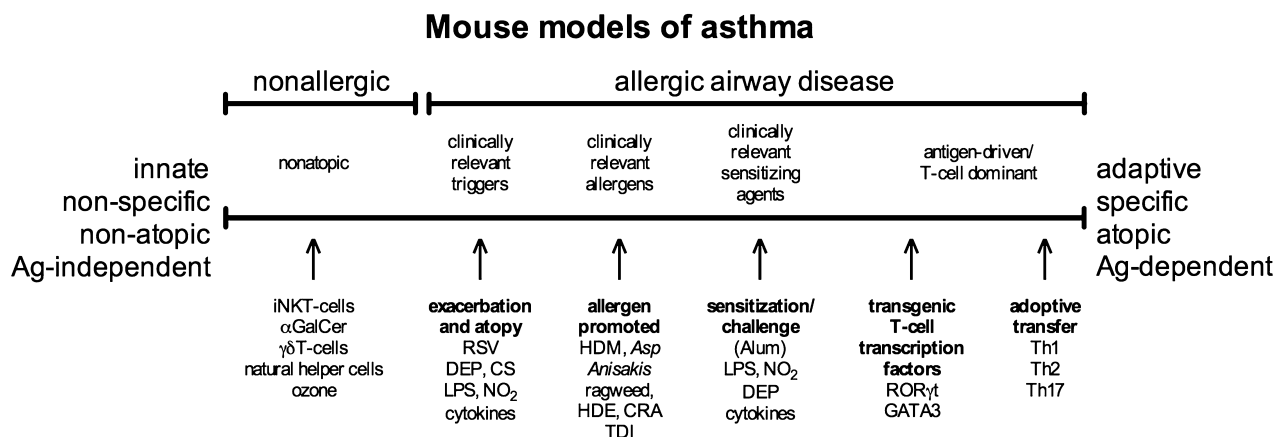


Figure 1 The spectrum of mouse asthma models. Mouse models of asthma can be arranged along a spectrum based on underlying pathologic mechanisms ranging from non-atopic to antigen-driven adaptive immunity. Ag, antigen; Asp, *Aspergillus*; α GalCer, α -galactosylceramide; CRA, cockroach antigen; CS, cigarette smoke; DEP, diesel exhaust particles; HDM, house dust mite; iNKT-cell, invariant natural killer T-cell; LPS, lipopolysaccharide; NO₂, nitrogen dioxide; RSV, respiratory syncytial virus; TDI, toluene diisocyanate.

metaplasia.⁴⁰ Other models that display small airways remodelling reminiscent of more severe and fatal asthma^{26,41} typically combine inhalational exposure to the innocuous antigen OVA with physiologically relevant sensitizing agents, such as nitrogen dioxide, diesel exhaust particles, lipopolysaccharide or endogenously derived cytokines.^{42–46} Similarly, schemed models utilize multiple exposures to allergenic extracts, such as house dust mite (HDM), ragweed, cockroach, *Alternaria* or *Aspergillus*. These allergens have intrinsic adjuvant activity to stimulate innate immunologic mechanisms and also possess antigenic properties capable of initiating the adaptive immune response and inducing immunologic memory.^{47–52}

Models of asthma exacerbation investigate the resulting airway dysfunction and inflammation from innate immune activation in the context of pre-existing allergic airway disease, usually resulting from a known or suspected trigger of asthma exacerbations, such as a viral infection or inhalational exposure to an environmental trigger.^{45,46,53–57} A small number of studies have been performed that involve non-antigen- or unconventional antigen-driven innate-like T-cell responses in non-allergic airway disease.^{58–62}

With the diversification of mouse models, the resulting phenotypes of asthma in these models has also become appreciated to be more complex, with mixed granulocytic infiltrate into the airway, mixed Th2/Th17 adaptive immune responses, variable sensitivity to GC treatment and the elucidation of multiple underlying mechanisms that are model specific.^{29,63,64} When examined critically, the heterogeneity in underlying mechanisms, resulting inflammatory phenotypes and biomarkers, and differential responsiveness to GC resemble the proposed classification of clinical asthma based on endotypes.¹⁹ Importantly, many of the measurements used in assessing inflammatory responses *in vivo* are also utilized in assessment of clinical asthma. For example,

biomarkers and cells are quantified from bronchoalveolar lavage and sputum, peripheral blood mononuclear cells are used to study immune function, and additional indicators of inflammation and airway structure are assessed from samples obtained by biopsy. Furthermore, the recent success of the anti-IL-13 antibody lebrikizumab in asthma was associated with similar biomarkers in both mice and humans.⁵ Thus, as endotypes are further characterized, it will be possible to directly compare underlying pathophysiologic features of clinical asthma with those of mouse models and allow researchers to refine asthma models to more closely resemble specific endotypes.

RECOGNIZING AND CONTROLLING VARIABILITY IN MOUSE ASTHMA MODELS

A 2009 workshop conducted between the National Centre for the Replacement, Refinement and Reduction of Animals in Research of the UK and the UK Medical Research Council acknowledged that variability between mouse models of asthma, including numerous parameters that can impact the outcome of a study (Fig. 2), imparts difficulty in interpreting results and deducing clinical relevance.⁷ If a given protocol does not provide similar results across strains, or even substrains, the underlying variables resulting in these differences warrant further investigation, as they can provide insight as to why so many seemingly different underlying pathologies can manifest in similar clinical symptoms.^{4,7,19} These variables include the mouse substrain, route of sensitization, experiment duration and environmental factors. To better characterize mouse models of asthma, it is critical that these potential variables are recognized and reported.

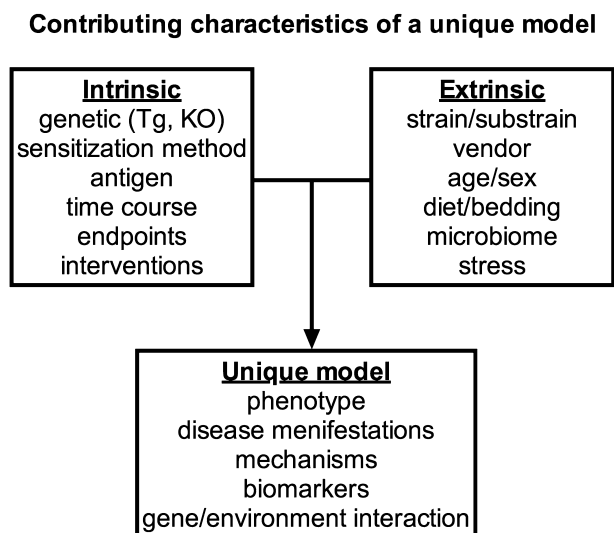


Figure 2 Defining characteristics that make a model unique. Many variables combine to create a unique model. Some of these variables are intrinsic to the experimental protocol and are chosen specifically to address the hypothesis, such as (genetically altered) mouse strains, as well as the method and route of sensitization, the antigen or allergen, time course, endpoint measurements and pharmacologic or behavioural interventions. Variables outside those chosen to specifically address the hypothesis also impact the outcome of a study, including species, substrain, vendor, age, gender, bedding, diet, and other environmental and experimental parameters, which combine to create a model with a unique phenotype, underlying mechanism(s) and associated biomarkers. KO, knockout; Tg, transgenic.

ALIGNING MOUSE MODELS AND ENDOTYPES

A recent report addresses the critical role of interdisciplinary research involving mouse models of asthma in elucidating the efficacy of lebrikizumab,⁵ an IL-13 neutralizing antibody.^{65–67} Whereas some studies demonstrated that the IL-13/IL-4R α pathway was required at antigen challenge,^{68,69} others revealed that IL-13 was a target only during challenge, whereas IL-4 was critical during sensitization and thus not a potential pharmacologic target.^{5,70} As it turns out, the biomarkers for IL-13-targeted intervention are the same in mice and humans, suggesting that a proper asthma model can be utilized to better understand a specific, clearly defined subset of asthma.⁵ Thus, studying disease characteristics of specific asthma subpopulations becomes more accessible by correlating a particular endotype with a well-characterized mouse model, resulting in a stratified method of studying asthma at the bench. This starts with understanding how current mouse models fit into the endotyping context.

Because the characterization of asthma endotypes is an evolving process, specific endotypes for the purpose of linking preclinical research with clinical asthma do not yet exist. Nonetheless, mouse models do fit into our current understanding of asthma endotypes. For example, Holgate endorses starting

broadly by describing pathophysiologic subtype as either a Th2^{low} or Th2^{high} subtype,⁴ whereas both PRACTALL and Agache *et al.* propose multiple endotypes.^{15,19} Conventional alum/OVA models that induce Th2-driven disease could be categorized as Th2^{high}, allergic asthma or hypereosinophilic asthma.^{4,7,15,19} A developing understanding of innate lymphocytes as sources of Th2-associated cytokines in human asthmatics and in mouse asthma models reviewed by Monticelli *et al.*⁷¹ demonstrates that previously unappreciated cellular sources of pathogenic mediators may be present in allergic asthma. Elucidating the mechanistic importance of recently identified cells may require further refining of existing asthma models.

Intrinsic asthma—one of Agache *et al.*'s phenotypes for which multiple endotypes were proposed and that is defined as having increased airway reactivity that can occur without obvious airway inflammation—has been modelled using the spontaneously methacholine-hyperresponsive A/J strain of mice, which have provided insight into the genetic,^{72–74} cellular⁷⁵ and physiological⁷⁶ mechanisms of AHR.

Mouse models of asthma that afford the study of disease involving extensive remodelling (oftentimes requiring protracted antigen exposure regimens that do not promote the development of antigen tolerance or exposure to profibrotic agents) have recently been reviewed.^{27,28}

Aspirin-sensitive asthma has been studied using genetic models of cyclooxygenase (COX)-1 and COX-2 deficiency,⁷⁷ as well as leukotriene C4 (LTC4) synthase overexpression,⁷⁸ which have contributed to understanding pathogenic mechanisms and the potential for the use of cysteinyl leukotriene receptor antagonists for the treatment of patients.

Obesity has recently emerged as a major risk factor for asthma. While the study of endotypes of asthma in obesity is at an early stage, at least two phenotypes of asthma have been identified in obese humans that have parallels with mouse models (reviewed in⁷⁹). Obese mice have increased airway reactivity compared with lean mice, which likely models the late onset non-allergic asthma that occurs in obesity; this has been modelled in an open-chested situation to avoid potential confounding of adipose distribution affecting mechanics of the respiratory system.⁸⁰ A variety of obese mouse models have been used to model this increased airway reactivity: these include high-fat diet, leptin deficient (*ob/ob*), leptin receptor deficient (*db/db*) and CPE^{fat} mice.⁸⁰ These same types of obese mice have been used to investigate the response to non-allergic challenge using ozone; this likely models late-onset non-allergic asthma and the increased reactivity and symptoms related to ozone and particulate matter exposure that have been reported in obese humans.⁷⁹ Obese OVA-challenged mice have been used to investigate the effects of obesity on allergic asthma, which models early onset allergic asthma complicated by obesity. Future advances will depend on the accurate extension of clinical endotypes of asthma in obesity being matched to appropriate mouse models of obesity induction, whether they can be genetic or through diet.

The spectrum of the Th17 response in asthma models

Model	Immune response	Spectrum of Th17 response														Th17 dominant
		Th2	Th2>Th17	Th2/Th17	Th2/Th17	Th2/Th17	Th2/Th17	Th2/Th17	Th2/Th17	Th2/Th17	Th2/Th17	Th2/Th17	Th2/Th17	Th2/Th17	Th17	
Th17/IL-17A source	Endogenous	?		endogenous											exogenous	Exogenous
Th17/IL-17A function	Inhibits AHR	?	?	prot.	?	?	protective									Promotes AHR
Glucocorticoid-responsiveness	Th17 response	S	S	S	S	?	S	?	?	?	?	?	?	?	?	Th17 response
	AHR	S	S	S	S	S	?	R	?	?	slightly S	?	R	R	R	AHR

Figure 3 The spectrum of the Th17 response and IL-17A in mouse models of allergic airway disease. The underlying adaptive immune response in models of allergic airway disease can typically be placed along a spectrum, ranging from a pure Th2 response to a pure Th17 response, as in adoptive transfer models. In between, models vary in the Th17/Th2 ratio. Importantly, the Th17 response may be endogenously or exogenously generated and introduced experimentally, as in the case of adoptive transfer or the administration of IL-17A in the setting of pre-existing allergic airway disease. In addition, the function of IL-17A and the Th17 response varies with the model used. Usually, the IL-17A promotes neutrophil recruitment, yet its role in promoting AHR varies substantially depending on the model, ranging from pathogenic to protective. The resistance of both IL-17A production and AHR to inhibition by GC is dependent on the model. In general, exogenously derived Th17 cells promote AHR and are resistant to glucocorticoids, whereas in more Th2-driven models, the Th17 response contributes less to disease pathology and AHR development. Furthermore, in many of the mixed Th2/Th17 and predominately Th2 models, the Th17 response or IL-17A negatively regulate(s) the Th2 response, resulting in a protective Th17 phenotype and also inhibiting AHR development. The range of function of the Th17 response argues against its action as a single entity, instead favouring a functional role that is determined by the local inflammatory environment or possibly the magnitude of the response itself. adopt tx, adoptive transfer; Ag, antigen; AHR, airway hyperresponsiveness; Al/O, aluminium hydroxide/ovalbumin; ch, challenge; Dex, dexamethasone; EC, epicutaneous; GC, glucocorticoid; HDM, house dust mite; prot, protective; R, resistant; S, sensitive.

Phenotypes reminiscent of those described for the endotype termed the 'asthma in cross-country skiers'¹⁵ that manifest following hyperventilation of cold, dry air, has been modelled or observed in larger animal models (recently reviewed in⁸¹). This phenotype is not unique to the act of cross-country skiing; instead, it is exercise-induced asthma, which is often more pronounced when exercising in cold air. Although airway reactivity was not measured, a model of endurance exercise in mice was shown to exert airway epithelial damage.⁸²

It is important to recognize that while pathological manifestations and potential biomarkers of asthma may appear similar between a particular endotype of disease and an animal model, the models are typically designed with an accelerated timeline to disease development relative to what occurs in human asthmatics. Consequently, very rarely do mouse models of asthma successfully recreate the rich natural history of these endotypes, potentially making the underlying pathogenesis different from the human condition. Although it will require the implementation of more difficult models, investigators using mice to study asthma should attempt to recreate the natural history of disease to more faithfully model the endotype as it developed in asthma patients. The benefit to translational research would be better models, but the cost would be fewer standardized models accompanied by the potential for vastly different interpretations of mechanisms and mediators.

THE Th17 RESPONSE IN MOUSE MODELS OF ALLERGIC ASTHMA: MODEL CHOICE INFLUENCES INTERPRETATION

The underlying adaptive immune response in models of allergic airway disease can typically be placed along a spectrum ranging from a Th2 response to a Th17-dominant response (Fig. 3). Granted, this is a simplified interpretation of the actual adaptive immune response in these models, and the response likely changes over time.^{83,84} While IL-17A is not the sole product of Th17 cells, this cytokine is the most intensely studied product of Th17 cells and is the cytokine for which the lineage was originally named.³³ Nevertheless, since Th17 cells secrete additional cytokines, including IL-17F and IL-22, it is inappropriate to consider Th17 responses and the activities of IL-17A to be one and the same. According to the comprehensive list of biomarker and associated mechanisms of asthma or allergic airway disease, the Th17 response or IL-17 production involve neutrophilic inflammation, susceptibility to exacerbations, severe asthma, steroid resistance and remodelling, particularly in extended models.¹⁹ A bipartite cluster analysis (a method for assessing relationships between two variables within a complex system; in this case, a relationship between asthma phenotype and cytokine levels was explored) recently identified IL-17 as a

member of an innate immunologic and NF- κ B-upregulated gene cluster (one of three upregulated cytokine gene clusters observed in patients with asthma), suggesting the upregulation of IL-17 in the context of innate immunity.²⁵ The other well-defined cytokine cluster in this analysis was a Th2-dominant cluster, supporting a role for IL-17 production in the context of asthma with a Th2^{low} inflammatory profile.^{4,25} The Th17 response is known to play a critical role in anti-fungal immunity (reviewed in⁸⁵). A study of asthmatic subjects found that patients allergic to fungus had higher percentages of IL-17⁺ neutrophils and CD8⁺ lymphocytes in peripheral blood compared with asthmatic subjects with non-fungal allergies.⁸⁶ Aberrant IL-17 production or Th17 responses may result from sensitization in the context of fungal-derived antigens, adjuvants or anti-fungal innate signalling pathways, indicating a potential role for the Th17 response in allergic bronchopulmonary mycosis and neutrophilic asthma.^{15,19} Thus, studies performed in mouse models that exhibit IL-17 production or a Th17 response are likely relevant to multiple asthma endotypes.

The variability in the function of Th17 responses and IL-17 in allergic asthma poses a challenge in terms of deciphering their physiologic role in mouse models and in clinical asthma. Consequently, controversies currently exist regarding the role of IL-17A and the Th17 response in promoting airway inflammation and AHR in allergic airway disease.^{29,38,45} The Th17 response may be endogenously generated or the source of the IL-17A or the Th17 response may be exogenous, as in the case of adoptive transfer and administration of IL-17A at challenge.^{29,46,87} Similarly, the combination of exogenous IL-17A with the development of an endogenous adaptive immune response is also an experimental possibility.^{45,46,87} Often, but not always, the IL-17A/Th17 response is involved in promoting neutrophil recruitment, yet its role in promoting AHR varies depending on the model, ranging from pathogenic to protective.^{32,38,39,45,88,89} In general, exogenously derived Th17 cells promote AHR and the development of GC-resistant disease, whereas in more Th2-driven models, the Th17 response contributes less to disease pathology and AHR development.^{29,30,38,90,91} In some models, the endogenously generated Th17 response negatively regulates the Th2 response and is protective against AHR development,^{38,39} whereas in a model of epicutaneous sensitization to OVA antigen, the Th17 response is pathogenic, recruiting neutrophils to the airway and promoting AHR development.^{88,92}

Similar to the variability of the Th17 response function, the sensitivity of the Th17 response to inhibition by GC treatment also varies depending on the model. Even though both the IL-17 response and neutrophils are associated with GC resistance,^{29,32} the sensitivity of the *in vivo* Th17 response may be uncoupled from the sensitivity of the neutrophil response to GC.^{46,90} While GC do have anti-apoptotic effects on neutrophils,⁹³ this discrepancy warrants further investigation. Similarly, GC sensitivity of the inflammatory response may be uncoupled from AHR development.⁹¹ In HDM-promoted allergic airway disease in A/J mice, AHR was minimally sensitive to GC, whereas neutrophil recruit-

ment was inhibited.⁹¹ Furthermore, while a pathologic role for the Th17 response is correlated with GC resistance in some models,^{29,32} IL-17A is protective in respiratory syncytial virus-exacerbated allergic airway disease,^{39,94} a model in which AHR is resistant to GC treatment. Thus, depending on the experimental protocol, the Th17 response contributes differently to the development of AHR and GC resistance, two important measures of clinical severity. In other words, the pathogenicity of the Th17 response fluctuates depending on the environment (i.e. the model) in which it was generated.^{95,96} Considering the heterogeneity of the asthmatic population, the same might be true for the Th17 response in humans, in which underlying mechanisms determine the functional contribution of the Th17 response in the context of the individual's asthma.⁴⁶ In addition, the functional relevance of IL-17A and the Th17 response may also change over time within an individual.^{83,84} For example, IL-17-producing innate-like T-cell populations⁶¹ may contribute to AHR acutely, while IL-17 production inhibits the Th2 response at an intermediate time point³⁸ and promotes airway remodelling in the setting of chronic antigen exposure.^{46,87}

It is also clear that the underlying pathophysiologic contribution of the Th17 response in promoting allergic airway disease varies depending on the source of the IL-17 or the Th17 response. Specifically, a pattern emerges in which administration of exogenous IL-17, Th17 adoptive transfer or forced overexpression of *Roryt* results in a more severe asthma phenotype, whereas the contribution of the endogenous Th17 response is more variable.^{45,89} If IL-17 is a marker of GC resistance in human asthma, then the adoptive transfer model may better replicate that which is observed clinically. The clinical implications of the variable Th17 response in mouse models of asthma await further investigation and characterization.

STERIOD DOSE IN MODELLING CORTICOSTEROID-RESISTANT ASTHMA

Severe asthma associated with a resistance to the therapeutic effects of corticosteroids remains a major clinical hurdle, resulting in disproportionately increased morbidity and mortality relative to mild or moderate disease.^{18,97} The use of mouse asthma models to study corticosteroid resistance are complicated by the dose, route and timing of administration of GC. Doses administered to mice are considerably higher than would be given to a human patient and are oftentimes administered intraperitoneally,²⁹ neither of which adequately simulate the clinical situation. In acute asthma exacerbations, patients are commonly treated with oral GC such as prednisolone or dexamethasone.⁹⁸ GC are traditionally compared on the basis of their anti-inflammatory potency relative to the endogenous active corticosteroid, hydrocortisone (cortisol), with prednisolone and dexamethasone having a potency 4 and 30 times that of cortisol, respectively.⁹⁹ Multiple clinical studies have found dexamethasone and prednisolone equally effective in

treating acute exacerbations of asthma, with dosing of prednisolone at 1–2 mg/kg and of dexamethasone at 0.3–0.6 mg/kg. The duration of anti-inflammatory action differs between these molecules. Prednisolone is relatively short acting, with a half-life of 12–36 h, thereby requiring daily dosing, whereas dexamethasone is longer acting, with a half-life of 36–72 h.¹⁰⁰

Mouse models exploring the mechanisms of steroid-resistant asthma have utilized doses of dexamethasone at 2.5 mg/kg in order to appropriately confirm steroid resistance.²⁹ However, anti-inflammatory doses of prednisolone range from 1 to 2 mg/kg, while doses at 2–4 mg/kg result in immunosuppression⁹⁹ and therefore are not commonly administered to asthma patients. The anti-inflammatory potency of dexamethasone is five to seven times that of prednisolone, such that a comparable dose to a human receiving 1 mg/kg of oral prednisolone would result in 0.17 mg/kg dexamethasone.⁹⁹ With doses of dexamethasone at 2.5 mg/kg, an equivalent prednisolone dose would reach 15 mg/kg, far above a clinically relevant level. Therefore, the use of lower GC doses in mice, along the lines of those used therapeutically in humans, along with an appropriate route of delivery (oral or subcutaneous), could reveal that many asthma models are GC resistant than is currently appreciated. Research focused on developing an understanding of the mechanisms of GC resistance is essential in the development of new therapeutics that is imminently needed for patients with disease complicated by GC resistance. As the scientific community continues to pursue the development of models that adequately address the complexity of the asthma phenotype, con-

sideration must be given to test doses pertinent to human therapy. These models will allow for the research pertaining to understanding essential mediators in steroid resistance and the development of interventions for patients burdened with steroid-resistant asthma.

CONCLUSIONS

Despite ample progress in understanding the pathophysiologic mechanisms underlying asthma through biomedical research, the mainstays of asthma treatment have not evolved substantially in 20 years, indicating a need for a change in the approach to asthma research and clinical management. The scientific community has responded with an ever-growing number of mouse models in multiple species, strains and environments, with and without antigens, increasing the number of endpoints and *ex vivo* studies, resulting in increased richness in the diversity and complexity of the asthma model. In conjunction with other available techniques for studying human disease, mouse models provide a critical component of multidisciplinary research for studying pathologic mechanisms, drug safety and gene involvement in an *in vivo* system. The recently proposed scheme of endotyping clinical asthma offers the possibility of stratifying asthma treatment in the future.⁴

The heterogeneity intrinsic to clinical asthma has already dictated the inadequacy of a single asthma model.⁹ Thus, limiting mouse models to a select few

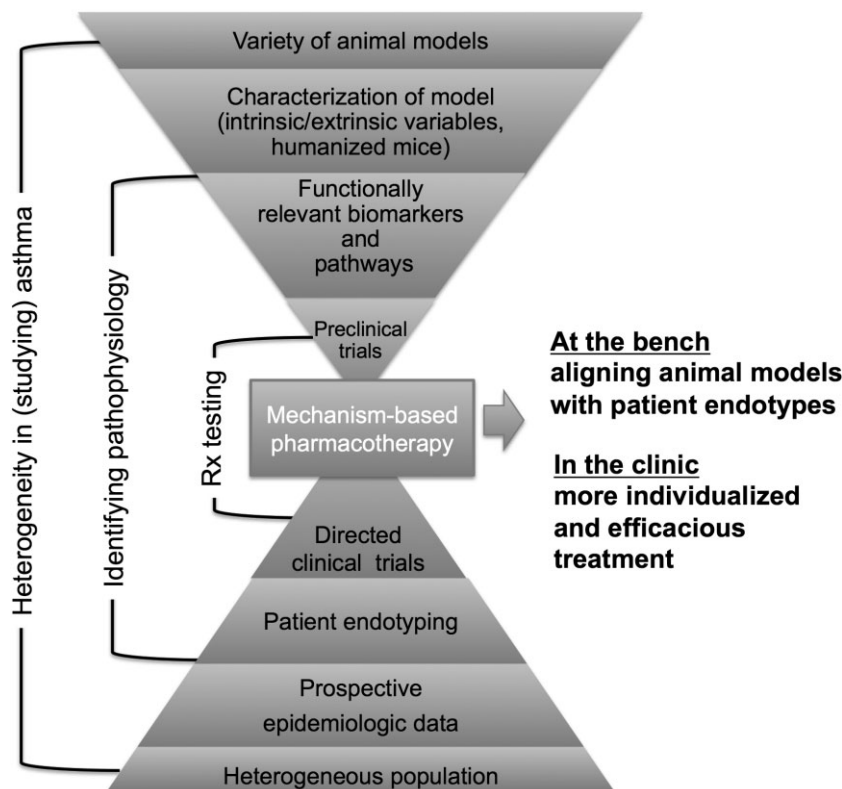


Figure 4 The integration of mouse models and endpoints. The heterogeneity of mouse asthma models parallels the diversity of phenotypes observed in clinical asthma. The evolving classification of clinical asthma based on endotyping warrants that mouse models of asthma continue evolving in order to address emerging hypotheses concerning asthma aetiology, pathophysiology and treatment. Models are dictated by variables both intrinsic and extrinsic to the experimental protocol and can influence the phenotype of that model, including the pathophysiologic mechanisms driving each inflammatory, structural and physiologic endpoint. Thus, examining disease characteristics, mechanisms and pharmacologic targets of specific asthma subpopulations becomes more accessible by correlating a particular endpoint with a well-characterized mouse model.

risks bottlenecking the biomedical research community.⁷ Furthermore, choosing a select few 'representative' models would likely prove difficult, since clinical asthma is currently not sufficiently well characterized.¹⁹ As both models and endotypes become better characterized, the two can be aligned based on appropriate biomarkers and pathophysiology. Aligning and developing mouse models of asthma in the context of clinical asthma, based on pathophysiologic mechanisms, will no doubt be a more effective approach to studying asthma and expanding on available options for clinical treatment (Fig. 4). As mouse models of asthma are better characterized, endotypes and models can be aligned, resulting in a similarly stratified approach to preclinical asthma research.

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