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Mouse models of severe asthma: Understanding the mechanisms of steroid resistance, tissue remodelling and disease exacerbation

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ABSTRACT

Severe asthma has significant disease burden and results in high healthcare costs. While existing therapies are effective for the majority of asthma patients, treatments for individuals with severe asthma are often ineffective. Mouse models are useful to identify mechanisms underlying disease pathogenesis and for the preclinical assessment of new therapies. In fact, existing mouse models have contributed significantly to our understanding of allergic/eosinophilic phenotypes of asthma and facilitated the development of novel targeted therapies (e.g. anti-IL-5 and anti-IgE). These therapies are effective in relevant subsets of severe asthma patients. Unfortunately, non-allergic/non-eosinophilic asthma, steroid resistance and disease exacerbation remain areas of unmet clinical need. No mouse model encompasses all features of severe asthma. However, mouse models can provide insight into pathogenic pathways that are relevant to severe asthma. In this review, as examples, we highlight models relevant to understanding steroid resistance, chronic tissue remodelling and disease exacerbation. Although these models highlight the complexity of the immune pathways that may underlie severe asthma, they also provide insight into new potential therapeutic approaches.

Key words: animal models, asthma, cell biology, cytokine, infection and inflammation.

Abbreviations: AHR, airway hyperresponsiveness; AM, alveolar macrophage; AP-1, activator protein 1; BET, bromo- and extra-terminal; CCL, C-C motif chemokine ligand; CD, cluster of differentiation; CFA, complete Freund's adjuvant; CXCL1, keratinocyte chemoattractant (KC); dsRNA, double-stranded

RNA; EGFR, epidermal growth factor receptor; FGF, fibroblast growth factor; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage CSF; GR, glucocorticoid receptor; GRE, glucocorticoid response element; HDAC, histone deacetylase; HDM, house dust mite; HRV1B, human rhinovirus 1B; IFN, interferon; Ig, immunoglobulin; IL, interleukin; iNOS, inducible nitric oxide synthase; LABA, long-acting beta-agonist; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MCP-1, monocyte chemoattractant protein 1; MIF/MMIF, macrophage migration inhibitory factor; miR, microRNA; MMP, matrix metalloproteinase; MyD88, myeloid differentiation primary response gene 88; MΦ, tissue macrophage; NFκB, nuclear factor kappa-B; OVA, ovalbumin; PBMC, peripheral blood mononuclear cell; PDE, phosphodiesterase; PM10, particulate matter <10 µm; PVM, pneumonia virus of mice; RSV, respiratory syncytial virus; RV, rhinovirus; STAT6, signal transducer and activator of transcription; TGF, transforming growth factor; T_H, T helper; TLR, toll-like receptor; TNFα, tumour necrosis factor alpha.

INTRODUCTION

Asthma symptom severity can be intrinsically mild, moderate or severe, in the absence of treatment. However, even very severe symptoms can often be well controlled by standard therapy. Disease severity, in turn, is defined as the amount of treatment required for asthma symptoms to be well controlled. Thus, individuals with mild-to-moderate asthma typically respond well to treatment with inhaled corticosteroids and long-acting beta-agonists (LABAs). However, a subset of individuals has severe treatment-refractory asthma, which fails to respond to these standard therapies. These individuals require high-dose corticosteroids 'to prevent symptoms from becoming "uncontrolled", or have disease which remains "uncontrolled" despite this therapy'.¹ Individuals with severe asthma have increased disease burden and risk of exacerbation, compared to individuals with mild disease.

Reduced response to corticosteroid treatment is termed steroid resistance or steroid insensitivity. Individuals with severe, steroid-resistant asthma continue to experience symptoms despite 'optimized' therapy,

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although they may still gain some benefit from treatment. Clinically, steroid-resistant disease typically manifests with persistent neutrophilic airway inflammation and elevated inflammatory mediators in the lung. Over the long-term, steroid insensitivity and persistent inflammation are likely to lead to exaggerated airway remodelling. Unfortunately, these individuals also continue to experience the negative side effects of high-dose corticosteroid therapy, including increased risk of infections and long-term bone loss. Our understanding of the disease mechanisms underlying severe asthma remains incomplete, and as a result alternative treatment options are limited for this patient population.

Animal models are useful tools for the study of pathological mechanisms underlying disease, and for pre-clinical testing of new therapies. Studies on mouse models have contributed significantly to our understanding of the role of atopy (e.g. IgE) and type-2 immune mechanisms (e.g. cluster of differentiation (CD)⁴⁺ T helper (T_H) 2 cells, IL-5, IL-13 and eosinophils) in the pathogenesis of allergic asthma (recently reviewed in Foster *et al.* 2017).² While a correlation between these factors and asthma was recognized in clinical studies, interventional studies in mouse models provided the first functional evidence of their roles in the immune mechanisms linked to asthma pathogenesis (e.g. IL-5 and IL-13⁴; reviewed in Ref.⁵). Building on the mechanistic insights gained collectively in animal models, targeted therapies are now available for the treatment of severe allergic/eosinophilic asthma (e.g. anti-IgE/omalizumab and anti-IL-5/mepolizumab).^{6–8} Unfortunately, these therapies are only effective for a subset of asthma patients. An improved understanding of the mechanisms underlying non-allergic/non-eosinophilic asthma, severe treatment-refractory asthma and disease exacerbations in particular is now required.

No mouse model encompasses all features of severe asthma and there are obvious limitations to the use of animals (Table 1; reviewed in Refs^{9,10}). Mice live relatively short lifespans, do not spontaneously develop disease that resembles human asthma and have important differences in lung structure. Furthermore, assessment typically focuses on short-term models of acute inflammation, with relatively little insight into long-term tissue remodelling. These factors limit characterization of the 'natural' aetiology of asthma and the processes involved in the progression to long-term chronic disease, as it occurs in humans.

Furthermore, most current mouse models induce type-2 inflammation, which is associated with allergic/eosinophilic asthma in humans. These models typically use chicken egg ovalbumin (OVA), the archetypal type 2-inducing antigen. Although OVA does not trigger asthma in humans, it is a useful tool to study immune and pathological features driven by allergic inflammation. Sensitization and subsequent challenge with OVA in the lung induces hallmark features of allergic asthma, including production of antigen-specific IgE, mucous hypersecretion, airway hyperresponsiveness (AHR) and eosinophilic infiltration. In long-term exposure models, OVA challenges also induce features of airway wall remodelling (e.g. thickening of the basement membrane, fibrosis and epithelial cell hyperplasia and

hypertrophy). A growing number of studies are also now employing clinically relevant allergens (e.g. house dust mite (HDM), birch pollen extract, *Aspergillus fumigatus* and ragweed) to induce type-2 inflammation.

Understanding the pathophysiological mechanisms underpinning non-allergic and severe asthma is a major area of unmet clinical need. Mouse models are now being developed to obtain insights into relevant disease pathways that mimic several key aspects of non-allergic and severe asthma in humans. Collectively, in this review, we will highlight data relevant to the induction of steroid resistance, chronic tissue remodelling and disease exacerbations.

STERIOD RESISTANCE

A key feature of severe asthma is that it is, by definition, refractory to standard corticosteroid treatment. While this is a recognized clinical feature of disease, the underlying molecular and cellular mechanisms mediating steroid resistance are varied and complex (reviewed in Refs^{11,12}).

At the molecular level, corticosteroids diffuse across the plasma membrane, where they bind to glucocorticoid receptor alpha (GR α) and direct its translocation into the nucleus. Steroid resistance can occur through impaired glucocorticoid-GR α binding and reduced nuclear translocation, as observed in peripheral blood mononuclear cells (PBMCs) isolated from steroid-resistant asthmatic patients.¹³ In the nucleus, activated GR α binds to glucocorticoid response elements (GREs) on DNA, in the promoter region of steroid-responsive genes. GR α binding recruits co-activator/repressor molecules, increasing expression of anti-inflammatory genes and suppressing pro-inflammatory genes. Altered gene expression occurs through DNA acetylation, which can be altered in severe asthma (e.g. through reduced histone deacetylase (HDAC) activity¹⁴). In severe asthma, excessive activation of inflammatory pathways may also impair GR α -mediated suppression (e.g. through activation of the transcription factor AP-1, which interacts with GR^{15,16}). Furthermore, recruitment of the p65 NF κ B subunit to NF κ B-dependent gene promoters can be resistant to steroid inhibition in severe asthma, as seen in isolated smooth muscle cells.¹⁷ Steroid-mediated gene regulation can be inhibited by GR β , an alternatively spliced form of GR that competes for GRE binding.¹⁸ Increased GR β expression has been described in individuals with steroid-resistant asthma,^{19,20} which may act as a dominant-negative regulator of steroid activation. Corticosteroids can also mediate effects through other mechanisms, including inhibition of mitogen-activated protein kinase (MAPK) signalling²¹ and activation of pathways that destabilize inflammatory cytokine mRNAs,²² which may be altered in severe asthma. Pro-inflammatory responses by the innate immune system, particularly in response to pathogens, may also limit the efficacy of endogenous and exogenous steroids, as this is a powerful natural host defence response. In summary, there are numerous mechanisms that can lead to glucocorticoid resistance at the cellular level, via alterations at numerous stages of glucocorticoid signalling.

Table 1 Some strengths and limitations of mouse models to study asthma

Strength	Limitation
Readily manipulated to induce consistent disease pathology	Do not spontaneously develop asthma or AHR
Allow interventional preclinical functional studies (e.g. gene modification, antibody administration and drug treatment)	Differences in lung structure from humans (e.g. limited airway muscle)
Well-characterized reagents and inbred mouse strains available (e.g. OVA and HDM)	Limited breadth of allergens used to induce atopy
Similar genetic background (reduces variability and allows direct comparison of treatment response)	Limited genetic diversity (fails to model differences in outbred human population)
Well-established models of allergic/eosinophilic disease phenotypes	Fewer models of non-allergic/neutrophilic phenotypes or acute exacerbations
Access to tissues (e.g. total lung samples, lymph nodes and bone marrow)	Relatively short lifespan, limits assessment of long-term pathology
Allows for the spatiotemporal analysis of the pathogenesis of disease initiation and progression	

AHR, airway hyperresponsiveness; HDM, house dust mite; OVA, ovalbumin.

Corticosteroid treatment can affect the type of inflammation present in the lung, while the type of inflammation present can also modulate steroid responsiveness. For example, corticosteroid exposure can skew the T_H1/T_H2 balance,²³ boosting T_H2 responses, which are associated with allergic asthma. Furthermore, corticosteroids typically promote production of the anti-inflammatory cytokine IL-10 by regulatory T cells. However, in individuals with severe asthma, steroid-induced IL-10 production by regulatory T cells is reduced.²⁴ IL-17-producing T_H17 cells can also be increased in severe asthma²⁵ and IL-17 can increase GR β levels,^{26,27} which would inhibit GR signalling. Macrophage migration inhibitory factor (MIF or MMIF) is also increased in individuals with severe asthma²⁸ and appears to promote innate immune responses through suppression of steroid-induced signalling pathways.²⁹ Thus, steroid responsiveness at the disease level depends on inflammatory profiles.

A number of mouse models exhibit steroid resistance, following administration of recombinant cytokines, transfer of immune cells or sensitization and allergen challenge. Infection-induced exacerbation of disease often also induces steroid resistance, as discussed later in this review.

Our research provides evidence that transfer of allergen (OVA)-specific polarized T_H1 cells and subsequent challenge with both OVA and LPS were sufficient to induce neutrophilia, macrophage activation and steroid-resistant AHR.³⁰ Notably, increased LPS exposure levels are associated with asthma severity.^{31,32} However, transfer of T_H2 polarized cells under the same circumstances induced a steroid-sensitive eosinophilic phenotype.³⁰ Through interventional studies, we provided evidence that development of steroid-resistant AHR required pulmonary macrophages, the LPS receptor toll-like receptor-4 (TLR4) and the downstream innate signalling molecule myeloid differentiation primary response gene 88 (MyD88).³⁰ Similarly, adoptive transfer of OVA-specific T_H17 cells and subsequent OVA challenge induced neutrophil accumulation in the lung and AHR, which was resistant to dexamethasone treatment.³³ Further, co-transfer of T_H2 and T_H17 cells together induced steroid-resistant disease

with mixed eosinophilic/neutrophilic inflammation, AHR and mucus hypersecretion.³⁴ These findings in mouse models demonstrate the key importance of T_H cell polarization in regulating the type of inflammation in the lung, and provide evidence that T_H1/T_H17 -skewed responses can induce steroid-resistant inflammatory and pathogenic pathways. T cell polarization secondary to lung infection may also explain steroid resistance observed in infection-induced exacerbation models (described below). Thus, T cell responses and the type of T cell-mediated inflammation in the lung affect steroid responsiveness.

Further studies have highlighted key roles for innate inflammatory pathways in steroid resistance. Interferon γ (IFN γ) and LPS are critically involved in regulating innate and adaptive non-allergic immune pathways and increased levels of both molecules are linked to severity of asthma. In a reductionist model to initially identify potential pathophysiological roles of these molecules, we demonstrated that administration of recombinant IFN γ and LPS (concurrently but not alone) into the lungs of mice induces steroid-resistant AHR.³⁵ This occurred through cooperative signalling between IFN γ and LPS, which increased IL-27 production from pulmonary macrophages.³⁵ Administration of recombinant IL-27 and IFN γ inhibited glucocorticoid-dependent GR translocation in pulmonary macrophages,³⁵ a potential mechanism underlying clinical steroid resistance. Thus, activation of pulmonary macrophages by IFN γ and LPS results in the production of IL-27, which together with IFN γ inhibits GR translocation to the nucleus and results in steroid-resistant airway inflammation and AHR.

In subsequent studies using the same model, we demonstrated that microRNA-9 (miR-9) levels were synergistically increased following IFN γ /LPS co-exposure in isolated macrophages and lung tissue *in vivo*.³⁶ miRs regulate cellular function by inhibiting mRNA translation and are important regulators of pulmonary inflammatory responses.³⁷ Of clinical relevance, miR-9 expression levels were also increased in sputum samples from neutrophilic steroid-resistant asthmatic patients.³⁶ Antagomir-mediated (antisense oligonucleotide) inhibition of miR-9 function restored

GR nuclear translocation and thus steroid sensitivity in isolated macrophages, and in several models of steroid-resistant AHR.³⁶

In further studies, we also directly targeted transcriptional activation of pro-inflammatory genes involved in the innate immune response. Administration of an anti-inflammatory molecule (I-BET-762), which inhibits transcriptional activation of inflammatory genes by bromo- and extra-terminal (BET) proteins also protected from steroid-resistant AHR in this LPS/IFN γ -induced model.³⁸ Treatment with andrographolide, a bioactive molecule with anti-inflammatory and antioxidative properties, also restored dexamethasone sensitivity in the same model.³⁹ Andrographolide treatment increased HDAC2 activity,³⁹ which typically acts to suppress gene transcription. A key role for the inflammatory cytokine TNF α was also recently demonstrated in a model of steroid-resistant neutrophilic airway inflammation induced by sensitization with complete Freund's adjuvant (CFA), where inhibition of TNF α could restore steroid sensitivity.⁴⁰ These findings provide support for key roles of innate immune inflammation in the development of steroid resistance.

Taken together, these studies provide evidence that steroid resistance at the disease levels results from the interplay between steroid resistance at the cellular level and the local immune response (Fig. 1). Steroid resistance is associated with increased T_H1/T_H17 responses and activation of innate inflammatory pathways. Activation of pulmonary macrophages by inflammatory cytokines serves as one mechanism that promotes steroid-resistant inflammation and AHR. Furthermore, pharmacological interventions that suppress transcription or translation of pro-inflammatory cytokines may be a novel therapeutic strategy to reduce steroid-resistant inflammation or restore steroid responsiveness.

CHRONIC INFLAMMATION AND TISSUE REMODELLING

A key feature of asthma is the chronic nature of disease and long-term tissue remodelling of the lung, including fibrosis, goblet cell hyperplasia, smooth muscle thickening and increased vascularization.⁴¹ Individuals with severe asthma have increased tissue remodelling, compared to individuals with mild-to-moderate disease.^{42–44} However, most animal models only assess acute lung inflammation and tissue responses over a relatively short time period. To determine the importance of chronic inflammation and mechanisms underlying long-term lung tissue remodelling, a number of chronic challenge models have been developed (reviewed in Refs^{45,46}).

Several models have demonstrated links between specific inflammatory molecules and tissue remodelling by using transgenic overexpression. For example, transgenic overexpression of both IL-5 and the eosinophil chemokine eotaxin-2 induced spontaneous airway remodelling, associated with eosinophil degranulation within the lung.⁴⁷ In specific cases, acute challenge can also cause severe disease with features of tissue remodelling. In a recent report,

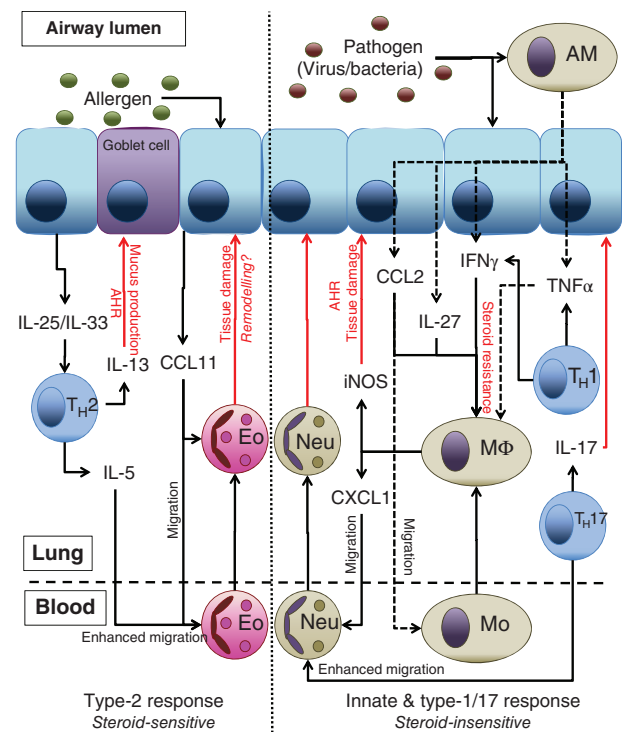


Figure 1 Type-2 airway inflammation, innate immune and type-1/17 activation and steroid-responsiveness. In established allergic airway disease, T_H2 cells expand and play a pivotal role directing the local allergic inflammatory response, producing IL-5 and IL-13. IL-5 promotes proliferation and differentiation of Eos, while also enhancing CCL11-mediated Eo recruitment. Bronchial epithelial cells produce cytokines, including IL-25 and IL-33, at differing stages of disease. These processes are typically responsive to steroid treatment. Exposure of the airway epithelium and lung-resident AMs to pathogens results in innate immune activation. This includes local pro-inflammatory cytokine production (e.g. CCL2 and TNF α), which promote the recruitment and activation of M Φ and induce steroid-resistant inflammation and AHR. Production of chemokines also recruits neutrophils into lung tissue and the airways. Innate immune activation skews T_H responses to a T_H1 or T_H17 phenotype, with associated cytokine production (e.g. IFN γ or IL-17 production, respectively) and steroid-resistant disease. Disease outcomes are indicated in red. AHR, airway hyperresponsiveness; AM, alveolar macrophage; CCL, C-C motif chemokine ligand (CCL2, monocyte chemoattractant protein 1 (MCP-1); CCL11; eotaxin); CXCL1, keratinocyte chemoattractant (KC); Eo, eosinophil; IFN γ , interferon γ ; iNOS, inducible nitric oxide synthase; M Φ , tissue macrophage; Mo, monocyte; Neu, neutrophil; T_H, T helper.

transfer of T_H2 cells and lung stimulation with *Blomia* mite protein allergen caused severe lung inflammation, through increased type-2 inflammation (IL-4 and IL-13).⁴⁸ In this model, increased mucus hypersecretion, tissue remodelling and severe inflammation were observed.⁴⁸

Most models use repeated chronic allergen challenges to induce tissue remodelling, which are characterized by allergen-specific T_H2 responses. Chronic asthma models typically consist of an initial allergic sensitization step, followed by repeated low-level allergen exposure/challenge over an extended period of time (up to 12 weeks) to induce chronic allergic

inflammation. For example, we assessed the effects of chronic allergen (OVA) exposure for 8 weeks on lung inflammation, tissue remodelling and AHR.⁴⁹ Repeated low-dose allergen exposure caused low-grade eosinophilic airway inflammation and AHR and key features of airway remodelling including goblet cell hyperplasia, epithelial thickening and subepithelial fibrosis.⁴⁹ In this model, CD4⁺ T cells were critical for disease, as depletion of CD4⁺ T cells reduced AHR and features of airway remodelling.⁵⁰ CD4⁺ T cell depletion also reduced chronic inflammation, including reduced accumulation of Ig-producing plasma cells.⁵⁰ Notably, eosinophil accumulation and eotaxin expression were unaffected by CD4 depletion, indicating eosinophils are not required in this model.⁵⁰ The timing of CD4 depletion may be critical, as depletion after allergen sensitization protected from eosinophilic inflammation, but had no effect on tissue remodelling.⁵¹ In other models, eosinophils appear to be required for airway remodelling, with reduced matrix and collagen deposition observed in eosinophil-deficient mice after an 8-week OVA-challenge chronic model.⁵² The infiltration and activation of T_H17 cells, which express IL-17A, can also promote tissue remodelling in chronic models, independent of T_H2 cells.⁵³ Key roles have also been demonstrated for mast cells (which bind IgE and mediate downstream responses following allergen recognition)⁵⁴ and macrophages (via bFGF and TGFβ1 expression)⁵⁵ in chronic allergen-induced tissue remodelling. These findings highlight roles for T_H cells and innate immune cells (including macrophages, mast cells and eosinophils) in tissue remodelling.

Key roles for individual T_H2-associated cytokines in tissue remodelling have also been reported. Using gene-deficient animals, we demonstrated that airway remodelling did not require IL-5 (or eosinophilia), but did require IL-4.⁵⁶ In contrast, anti-IL-5 antibody treatment dampened inflammation and remodelling, but had no effect on AHR.⁵⁷ Using gene-deficient animals, we also demonstrated a key role for IL-13 in both eosinophil accumulation and airway remodelling, in our chronic OVA challenge model.⁵⁸ IL-13-deficient (*Il13*^{-/-}) mice had decreased eosinophilia and inflammatory cell infiltrate into the lungs and reduced airway remodelling, compared with wild-type mice, although AHR was still induced.⁵⁸ *Il4Ra*^{-/-} mice had normal inflammatory responses and AHR, although some features of airway remodelling (including epithelial hypertrophy and mucus cell hyperplasia) were reduced.⁵⁸ Signal transducer and activator of transcription 6 (STAT6) is a signalling molecule involved in T_H2-associated cytokine responses. *STAT6*^{-/-} mice developed comparable airway lesions to wild-type mice and exaggerated AHR, while in contrast *Il4*^{-/-}*Il13*^{-/-} mice had reduced eosinophil accumulation, airway remodelling and absent AHR.⁵⁹ Anti-IL-13 antibody treatment suppressed eosinophil accumulation and partially suppressed airway remodelling, but had limited effects on AHR.⁵⁷ Similarly, anti-IL-13 treatment reduced inflammation, collagen deposition and goblet cell upregulation when given therapeutically in an 8-week HDM-induced chronic model.⁶⁰ These findings indicate a key role for type-2 inflammation (particularly IL-4 and IL-13) in long-term

allergen-induced tissue remodelling. The relevance of IL-5 (and eosinophils) appears to depend on the specific disease context.

A number of therapeutic interventions have been assessed in chronic asthma models (Table 2). We initially demonstrated that both roflumilast (a selective phosphodiesterase 4 (PDE4) inhibitor) and dexamethasone reduced eosinophil infiltrates, inflammation, subepithelial collagen deposition and epithelial thickening, but only had minor effects on AHR.⁶¹ Dexamethasone also reduced goblet cell hyperplasia, transforming growth factor (TGF)β1 expression and activation of NFκB in the epithelium.⁶¹ Similarly, budesonide treatment reduced AHR, inflammation, collagen deposition and mucus production, with an associated reduction in TGFβ signalling in a prolonged challenge model.⁶³ Pentoxifylline (a non-selective PDE inhibitor) inhibited eosinophil accumulation and epithelial thickening and significantly reduced AHR.⁶¹ In a more recent follow-up study, we demonstrated that chronic allergen exposure significantly induced T_H2 cytokines and IFNγ (typically associated with T_H1 responses) in airway wall cells, and that cytokine expression was suppressed by dexamethasone treatment.⁶² Treatment with roflumilast suppressed IL-17A, TNFα, granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-6, but not other cytokines.⁶² Treatment with both drugs also suppressed TGFβ1 and FGF-2.⁶² Anti-IFNγ antibody administration suppressed AHR and accumulation of inflammatory cells, but had no effect on eosinophils or tissue remodelling.⁵⁷ Administration of the cysteinyl leukotriene receptor-1 antagonist, montelukast, reduced eosinophil infiltration, mucus plug formation, smooth muscle hyperplasia and fibrosis in a 10-week chronic OVA model.⁶⁴ Further, montelukast treatment reversed increases in smooth muscle mass and subepithelial collagen deposition, when administered after allergen cessation.⁶⁵ Treatment with erlotinib, which blocks epidermal growth factor receptor (EGFR) function, reduced AHR, goblet cell metaplasia and airway smooth muscle thickening, in a 9-week HDM challenge model.⁶⁶ Reduced peribronchial fibrosis and total lung collagen, but not mucus production, smooth muscle thickness or AHR have also been reported in matrix metalloproteinase (MMP)9-deficient animals, following chronic OVA challenge.⁶⁷

These studies highlight the diverse interplay between immune cells and inflammatory cytokines in the regulation of inflammation, tissue remodelling and AHR. While individual studies demonstrate reduced tissue remodelling by targeting individual molecules, there is significant redundancy in the mechanism driving pathogenesis. Combinations of treatments or broad-spectrum anti-inflammatory approaches will likely be required to reduce tissue remodelling in chronic disease in the clinic. This may be accomplished by targeting inflammatory gene activation (e.g. BET protein inhibition or blockade of key regulatory miRs) as discussed earlier; however, these approaches have not yet been assessed in chronic disease models.

Several studies have also assessed the persistence of airway remodelling after cessation of allergen exposure.

Table 2 Effects of therapeutic intervention in chronic asthma models. Key findings from highlighted studies are provided

Intervention	Disease outcomes
Roflumilast ^{61,62}	Reduced eosinophilic infiltrate Reduced inflammation Reduced subepithelial collagen deposition and epithelial thickening Dampened IL-17A, TNF α , GM-CSF and IL-6 expression Dampened TGF β 1 and FGF-2 expression Minor effects on AHR
Pentoxifylline ⁶¹	Reduced eosinophilic infiltrate Reduced epithelial thickening Reduced AHR
Dexamethasone ^{61,62}	Reduced eosinophilic infiltrate Reduced inflammation Reduced subepithelial collagen deposition, epithelial thickening and goblet cell hyperplasia Reduced TGF β 1 expression and NF κ B activation Dampened T $_H$ 2 cytokine and IFN γ expression Dampened TGF β 1 and FGF-2 expression Minor effects on AHR
Budesonide ⁶¹	Reduced inflammation Reduced subepithelial collagen deposition, epithelial thickening, mucus production Reduced TGF β signalling Reduced AHR
Anti-IFN γ ⁵⁷	Reduced inflammatory cell infiltrate Reduced AHR
Montelukast ^{62,63}	No effect on eosinophils or tissue remodelling Reduced eosinophilic infiltrate Reduced mucus plug formation Reduced smooth muscle hyperplasia and fibrosis Reversed smooth muscle mass increases and subepithelial collagen deposition (after allergen cessation)
Erlotinib ⁶⁶	Reduced goblet cell metaplasia and airway smooth muscle thickening Reduced AHR

AHR, airway hyperresponsiveness; erlotinib, epidermal growth factor receptor (EGFR) inhibitor; FGF, fibroblast growth factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; montelukast, cysteinyl leukotriene receptor-1 antagonist; NF κ B, nuclear factor kappa-b; PDE, phosphodiesterase; pentoxifylline, a non-selective PDE inhibitor; roflumilast, selective PDE4 inhibitor; TGF, transforming growth factor.

When resolution was assessed for 4 weeks after 8 weeks of chronic allergen challenge, eosinophilic inflammation and mucus cell hyperplasia rapidly resolved.⁶⁸ In contrast, resolution of subepithelial fibrosis and epithelial hypertrophy were delayed.⁶⁸ Similarly, after 7 weeks of repeated HDM challenge, inflammation rapidly resolved but features of tissue remodelling remained.⁶⁹ In a model that assessed differences between acute and chronic (55 days) OVA challenge, chronic inflammation increased matrix accumulation in subepithelial and perivascular regions and increased collagen deposition.⁷⁰ This was associated with increased IL-4, TGF β and IFN γ during the chronic disease phase, while IL-13 levels subsided.⁷⁰ In this model, AHR subsided following cessation of allergen exposure.⁷⁰ These studies provide evidence that when antigen exposure is withdrawn and allergic inflammation resolves, some features of airway remodelling are reversible. However, other features remain and alternative approaches will be required to restore lung structure to a healthy state. Chronic mouse models may be useful for the assessment of novel therapeutic strategies to accomplish this goal.

EXACERBATIONS OF ALLERGIC AIRWAY DISEASE

Exacerbations are a significant contributor to disease burden and healthcare costs for individuals with severe asthma (reviewed in Ref.⁷¹). Exposure to high levels of sensitizing allergen can trigger exacerbations. However, this appears to be relatively uncommon cause of severe exacerbations.⁷² Bacterial infections including *Haemophilus influenzae*, *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* are associated with disease exacerbations (reviewed in Refs^{73–75}). In particular, *H. influenzae* infection has been linked to exacerbations and disease severity in asthmatic patients.⁷⁶ Respiratory viral infections are also associated with exacerbations (reviewed in Refs^{77–80}). Rhinovirus (RV) is commonly detected during acute exacerbations.⁸¹ In adult asthmatic patients, respiratory syncytial virus (RSV) is detectable in a subset of patients and associated with severe asthma exacerbations.⁸² Furthermore, exposure to environmental pollutants can trigger disease exacerbations.^{83,84} Early-life respiratory infections (by bacteria

and viruses) are also associated with the later development of asthma.

Allergen-induced exacerbation

Most mouse models assess responses after allergen sensitization and a varying number of allergen challenges to model allergic inflammation and subsequent airway disease. These studies assess the effects of acute allergen challenge, rather than allergen-induced exacerbations of disease *per se*. Furthermore, models using repeated low-level allergen challenge are typically described as models of chronic asthma (as described above), rather than allergen-induced exacerbation.

We provided the first report on the effects of a single high dose of allergen in our chronic low-dose OVA model described above. This 'acute-on-chronic' exposure induces disease exacerbation, with increased tissue inflammation and AHR.⁸⁵ Importantly, features of disease were steroid resistant and associated with neutrophilic inflammation.⁸⁶ Disease exacerbation was associated with decreased HDAC2 activity and increased NF κ B activity, which were not altered by corticosteroid treatment.⁸⁶ Furthermore, innate inflammatory molecules including TNF α , GM-CSF, IL-8 and p21waf were also increased and not suppressed by corticosteroid treatment.⁸⁶ Interestingly, AHR was undiminished in eosinophil-deficient *dbl-GATA1* mice in the same model, demonstrating that allergen-induced exacerbations of AHR do not require eosinophils.⁸⁵ In contrast, alveolar macrophages played a key role in disease exacerbation, by enhancing CD4⁺ T cell cytokine production.⁸⁷ Thus, allergen exposure can induce disease exacerbations associated with increased innate immune activation (including alveolar macrophages and neutrophils) and increased inflammatory cytokine production.

Pathogen-induced exacerbation

Viral

Several groups have used double-stranded RNA (dsRNA) administration as a surrogate for virus infection. After chronic HDM challenge, dsRNA administration increased neutrophil and T-cell recruitment and induced steroid-resistant inflammatory cytokine production.⁸⁸

Early-life infection with pneumonia virus of mice (PVM) (a mouse model of RSV infection) in conjunction with allergen sensitization/challenge promoted eosinophilic inflammation, allergen-specific IgE and IgG and production of T_H2 cytokines (IL-4, IL-5 and IL-13).⁸⁹ Furthermore, neonatal PVM infection induced AHR and mucus cell hyperplasia, regardless of allergen sensitization/challenge.⁸⁹ Blocking either IL-4 or IL-25 suppressed features of T_H2 inflammation, although treatment had differing effects on disease outcomes.⁹⁰ Anti-IL-4 prevented allergic inflammation and mucus hypersecretion, without affecting tissue remodelling.⁹⁰ Conversely, anti-IL-25 reduced remodelling, but had no effect on tissue inflammation or mucus hypersecretion.⁹⁰

Exacerbations induced by RSV have also been assessed in mouse models (although RSV replication in mice is minimal). We recently reported that RSV infection after allergen (OVA) challenge exacerbates prolonged steroid-resistant AHR, but had no effect on T_H2 responses.⁹¹ Instead, exacerbation required pulmonary macrophage activation and expression of the pro-inflammatory cytokine TNF α and the monocyte/macrophage chemokine CCL2.⁹¹ Anti-inflammatory treatment with I-BET-762, which reduced inflammatory cytokine production (including CCL2, keratinocyte chemoattractant (KC)/CXCL1, IFN γ and IL-27), also protected from exacerbation in this model.⁹¹ Other groups have provided evidence that RSV infection can also exacerbate disease through increased IL-13 expression.⁹² Our collaborators also recently demonstrated that RSV infection-induced exacerbations increased miR-21 expression.⁹³ Blocking miR-21 function restored steroid sensitivity in this model and reduced AHR in the presence or absence of steroid treatment.⁹³

A number of studies have also assessed RV-induced exacerbations in mouse models. It is important to note that due to species differences between human and mouse, 'major group' RV virus cannot infect mouse cells. Thus, most studies have used the minor group human rhinovirus 1B (HRV1B). Interestingly, HRV1B infection after allergen challenge augmented airway inflammation, characterized by increased neutrophilic, eosinophilic and lymphocytic airway inflammation and T_H1 and T_H2 cytokine production.^{94,95} Further, HRV1B infection in a preexisting allergic environment also exacerbated AHR and mucus production.^{94,95} The mechanisms that underpin RV-induced exacerbations are not well understood. Nevertheless, some studies have also revealed roles for $\gamma\delta$ T cells, IL-25 and midline 1 in RV-induced exacerbation models.⁹⁶⁻⁹⁸

A recent publication also demonstrated that influenza (A/X31 H3N2) infection of HDM-sensitized mice impaired lung function and increased mucus production and innate immune lung inflammation.⁹⁹ Exacerbation in this model was resistant to inhaled corticosteroid treatment, but responded to either systemic corticosteroid treatment or anti-IL-5 treatment.⁹⁹ Interestingly, blocking miR-21 also suppressed influenza-induced disease exacerbations, suggesting an overlapping mechanism with RSV infection-induced exacerbations.⁹³

Together, these studies demonstrate that virus infection can exacerbate disease through potentiation of preexisting type-2 immune responses or through activation of innate and/or type-1 inflammatory pathways. Thus, the best intervention strategy to reduce virus infection-induced exacerbation severity will likely depend on the infection context and the preexisting disease phenotype.

Bacterial

A number of models have assessed outcomes after administration of the bacterial ligand LPS to mimic bacterial lung colonization/infection. These studies have revealed the importance of LPS dose and timing on disease outcomes. Low-level inhaled LPS exposure (0.1 μ g) induced T_H2 responses, while higher doses

(100 µg) stimulated T_H1 immune responses, respectively.¹⁰⁰ Repeated LPS administration alone induced neutrophilic airway inflammation and AHR in the absence of allergic sensitization.¹⁰¹ While LPS administration during initial allergic sensitization was protective and inhibited allergen-induced type-2 airway inflammation, LPS administration during allergen challenge increased T_H1 cytokine responses and exacerbated disease.¹⁰² LPS administration during allergen challenge also promoted mast cell activation and T_H2 immune responses.¹⁰³

A number of studies have demonstrated that bacterial infection can induce disease exacerbation. Our colleagues demonstrated that early-life (but not adult) *Chlamydia muridarum* infection exacerbated subsequent allergic airway responses and induced a mixed T_H1/T_H2 response.^{104,105} When animals were allergen-challenged after resolution of *Chlamydia* infection, increased neutrophil accumulation and T_H1/T_H17-associated gene expression were observed, along with steroid-resistant AHR.⁹³ This occurred despite reduced eosinophil accumulation and reduced T_H2 responses.⁹³ Similar to observations in virus infection-induced exacerbation, miR-21 expression was also increased in this model and blocking miR-21 reduced AHR.⁹³ *Chlamydia* lung infection during OVA sensitization and challenge also increased neutrophilic inflammation and IFNγ release,¹⁰⁶ reminiscent of neutrophilic asthma.

Mycoplasma pneumoniae infection in the absence of allergic sensitization also induced airway resistance and neutrophilic lung inflammation.¹⁰⁷ While *M. pneumoniae* infection prior to allergen sensitization protected from disease induction, infection after sensitization induced IL-4 expression and exacerbated allergic disease.¹⁰⁸

Haemophilus influenzae infection prior to allergen sensitization resulted in chronic bacterial infection and steroid resistant, neutrophilic airway disease.¹⁰⁹ In an exacerbation model, *H. influenzae* infection also induced IL-17-dependent neutrophil accumulation in the lung.¹¹⁰ Again, miR-21 was increased by *H. influenzae* infection-induced exacerbation and blocking miR-21 function reduced AHR in the presence or absence of corticosteroid treatment.⁹³

Similar to the findings for virus infection-induced exacerbations, these studies demonstrate that bacterial infection can exacerbate disease through potentiation of type-2 inflammation or activation of innate and/or type-1 pathways. Bacterial infection prior to allergic sensitization often inhibits type-2 responses and can protect from allergic disease. However, when preexisting type-2 inflammation is already established, bacterial infection leads to mixed T_H1/T_H2/T_H17 activation, increased neutrophilic inflammation and features of steroid-resistant disease.

Fungal

Relatively few studies have assessed the effects of fungal infection or exposure on exacerbation using mouse models. *Alternaria* extract administration to chronically challenged HDM-sensitized mice exacerbated disease with increased AHR, immune lung infiltrate and a mix of inflammatory and T_H2 cytokine induction.¹¹¹ Administration of *A. fumigatus* conidia to HDM-sensitized

mice also increased immune cell recruitment and inflammatory cytokine levels in the lung.¹¹²

Environmental pollutant-induced exacerbation

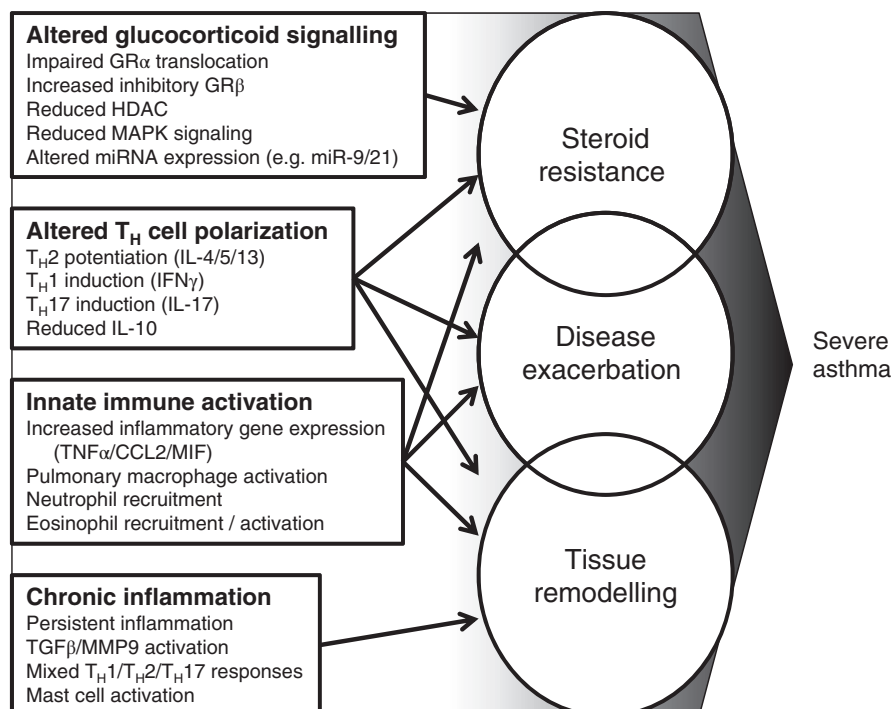
Several studies have demonstrated that environmental pollutants can contribute to disease exacerbation in mouse models. After OVA sensitization and chronic challenge, concentrated ambient particle administration and ozone exposure increased AHR, through increased arginase 1 activity,¹¹³ which is typically associated with type-2 inflammation. Ozone exposure in conjunction with fungal *A. fumigatus* antigen exposure also increased inflammation and AHR, with increased IL-5, GM-CSF and granulocyte CSF (G-CSF) protein levels.¹¹⁴ Exposure to particulate matter <10 µm (PM₁₀) after chronic OVA challenge also increased allergic type-2 lung inflammation, via an IL-33-dependent mechanism.¹¹⁵ Furthermore, diesel exhaust particulate exposure increased innate airway inflammation, mucus secretion and AHR via a KC/CXCL1 and macrophage inflammatory protein (MIP)-2/CXCL2-dependent mechanism, in a cockroach antigen model.¹¹⁶ These studies indicate that lung exposure to components of environmental pollution can also modulate the local inflammatory responses by potentiating type-2 inflammation or increasing innate inflammatory responses, which exacerbate preexisting allergic inflammation.

CONCLUSIONS

No single mouse model provides a comprehensive model of severe asthma in the human. However, existing models can provide mechanistic insights into a number of features of human disease. In this review, we have provided an overview of findings relevant to steroid resistance, tissue remodelling and disease exacerbations. These studies demonstrate the complexity of immune responses involved and the overlapping/redundant mechanisms underlying different features of disease (e.g. pulmonary macrophage activation in both steroid resistance and exacerbation) (Fig. 2). Recurring features include potentiation of pre-existing type-2 immune responses (e.g. increased T_H2 activation, and IL-4, IL-5 and IL-13 expression), altered T cell polarization (T_H1/T_H2/T_H17), activation of innate immune pathways (e.g. macrophage/neutrophil activation) and chronic lung inflammation.

Activation of allergic inflammatory pathways and type-2 immunity has been well characterized in asthma pathogenesis and have led to current targeted therapies. These interventions provide clinical benefit for the subset of severe asthma patients with allergic/T_H2-driven disease. Mouse models are now providing evidence for other pathways, which may be relevant for the treatment of other severe asthma phenotypes. Furthermore, mouse models provide evidence for why current interventions (e.g. corticosteroids) are not effective under certain conditions. Interventions targeting these disease pathways may improve treatment responses to steroid therapy, limit or reverse tissue remodelling and reduce disease exacerbations.

Figure 2 Pathological mechanisms underlying features of severe asthma. A number of mechanisms have been identified in mouse models, including altered glucocorticoid signalling, altered T_H cell polarization, innate immune activation and chronic inflammation, which may contribute to features of severe asthma. Examples are provided for each mechanism. These mechanisms have overlapping effects on the development of steroid resistance, disease exacerbations and tissue remodelling. CCL, C-C motif chemokine ligand; GR, glucocorticoid receptor; HDAC, histone deacetylase; IFN, interferon; MAPK, mitogen-activated protein kinase; MIF, macrophage migration inhibitory factor; miR, microRNA; MMP, matrix metalloproteinase; TGF, transforming growth factor; T_H , T helper.



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