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REVIEW ARTICLE

# Refractory asthma: mechanisms, targets, and therapy

J. L. Trevor & J. S. Deshane

Division of Pulmonary Allergy and Critical Care Medicine, Department of Medicine, The University of Alabama at Birmingham, Birmingham, AL, USA

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**Correspondence**
Jessy S. Deshane, PhD, Division of Pulmonary, Allergy and Critical Care Medicine, Department of Medicine, 1900 University Boulevard, THT-433, University of Alabama at Birmingham, Birmingham, AL 35294, USA.
Tel.: (205) 996-2041
Fax: (205) 934-1721
E-mail: treena@uab.edu

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**Abstract**

Asthma is a common medical condition affecting 300 million people worldwide. Airway inflammation, smooth muscle bronchoconstriction leading to airflow obstruction, and mucous hypersecretion are clinical hallmarks of asthma. The NHLBI Expert Panel Report 3 recommends inhaled corticosteroids (ICS) for patients with moderate to severe persistent asthma. Inhaled corticosteroids (ICS) target gene transcription through their interactions with the glucocorticoid (GC) receptor (GR) at the glucocorticoid response element (GRE). The GC/GR complex enhances anti-inflammatory but inhibits pro-inflammatory mediator production. Classically, asthma has been described as a Th2-associated eosinophil-predominant disease, but recently alternative models have been described including a Th17-mediated neutrophil-predominant phenotype resulting in patients with more severe disease who may be less responsive to steroids. Additional mechanisms of steroid resistance include increased activity of GR phosphorylating kinases which modify the interactions of GR with transcription factors to inhibit the ability of GR to bind with GRE, leading to an increase in pro-inflammatory gene transcription. Oxidative stress also affects the balance between pro-inflammatory and anti-inflammatory gene transcription through the modification of transcription factors and cofactors (such as PI3K) leading to the inhibition of histone deacetylase 2. Continued investigations into the mechanisms behind glucocorticoid resistance will lead to novel treatments that improve control of severe refractory asthma.

Asthma is one of the most common chronic medical diseases globally, affecting over 25 million people in the United States and 300 million people worldwide, with that number expected to rise (1). Additionally, it is one of the most costly chronic conditions, in terms of dollars and human lives, with an estimated 15 million daily-adjusted life years (DALYs) lost annually, and it is implicated in one of every 250 deaths worldwide (1).

Asthma is described as a chronic inflammatory condition affecting the airways consisting of a cellular component resulting in airway inflammation, and smooth muscle hyperresponsiveness in response to direct or indirect stimuli leading to bronchoconstriction. Indirect stimuli, such as that of exercise, result in the release of inflammatory mediators from cells present in the airway contributing to airflow obstruction (2). The clinical result of this inflammation and bronchoconstriction is airflow obstruction that is typically reversible with the use of glucocorticoids, particularly for individuals with an eosinophil-, rather than neutrophil-predominant phenotype. It has been shown that decreases in FEV1 correlate with increases in eosinophil and neutrophil counts in the airways of asthmatic patients. Interestingly, increases in airway eosinophilia, but not neutrophilia, appear to correlate with an increased bronchial hyper-responsiveness based on methacholine challenge testing, suggesting the possibility that targeting the neutrophil may be a potential means of controlling chronic, less reversible airflow obstruction (3). Although inflammatory cell infiltration in the airway has been broadly correlated with the degree of bronchial hyper-responsiveness, there has not been any definitive evidence proving causation.

The disease is diagnosed by eliciting a history of symptoms consistent with asthma (cough, dyspnea, and wheeze) in combination with evidence of reversible airflow obstruction as demonstrated by spirometry, or a positive methacholine challenge test. Current therapy for asthma is based on the severity and control of symptoms, with the backbone of treatment consisting of inhaled corticosteroids (ICS) for those with moderate persistent disease and beyond (4). Unfortunately,

about 10 percent of asthmatics appear to have refractory disease despite receiving optimal therapy, leading to increased morbidity and cost associated with treatment (5).

## Pathophysiology of asthma

### Cells and cytokines

The classic model of allergic airway inflammation is characterized by submucosal infiltration of activated Th2 lymphocytes, eosinophils, macrophages, mast cells, and neutrophils (6). This model of asthma is a complex web of cells and cell signaling molecules interacting to elicit an inflammatory response (Fig. 1). Allergen/antigen presentation by antigen-presenting cells to Th0 cells results in Th2 cell differentiation. This primary switching may be induced by the production of thymic stromal lymphopoietin (TSLP) by airway epithelial cells in response to antigen stimulation. Thymic stromal lymphopoietin then acts upon TSLP receptors (TSLPR) expressed by dendritic cells (DCs) to promote the transcription of OX-40L. OX-40L, a member of the TNF family of cytokines, induces expression of Th2 cytokines by the activated DCs leading to inflammatory Th2 cell differentiation (7). The Th2 lymphocytes further produce IL-4, IL-5, and IL-13 cytokines that stimulate B cells to synthesize and release IgE. According to the classic paradigm of allergic airway disease, IgE is then bound to the surface of mast cells where inhaled allergens bind to these receptors precipitating

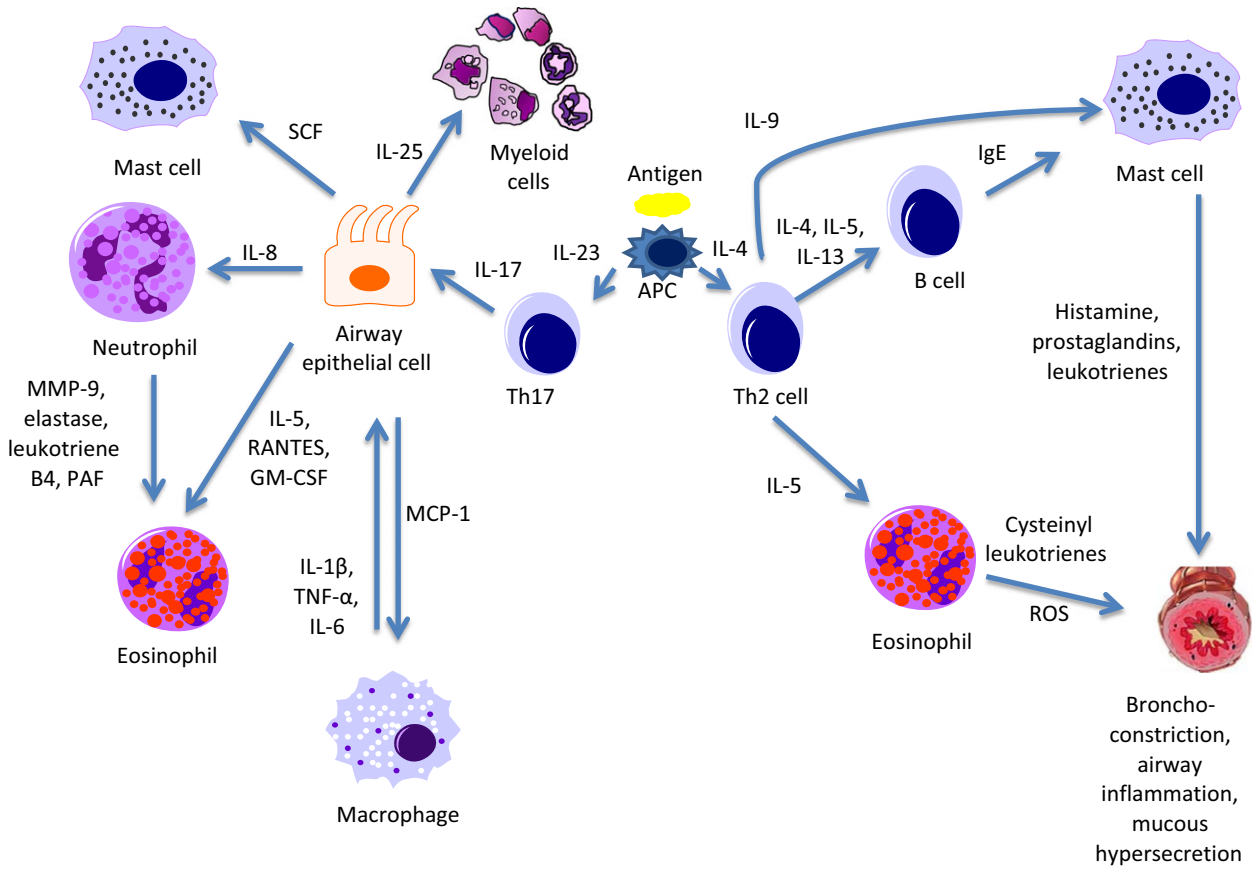


**Figure 1** Th2 and Th17 allergen responses in the asthmatic airway. Upon allergen presentation to Th0 cells by antigen-presenting cells (APC), Th cells differentiate into Th2 cells in the presence of IL-4, and Th17 cells in the presence of IL-23. Th2 cells then go on to produce IL-4-, IL-5-, and IL-13-activating B cells to release IgE which attaches to the surface of mast cells. When stimulated by antigen, mast cells release histamine, prostaglandins, and leukotrienes resulting in smooth muscle bronchoconstriction, airway inflammation, and mucous hypersecretion. Eosinophils activated by IL-5 produce cysteinyl leukotrienes and reactive oxygen species (ROS), which act in a similar manner on the airways, and additionally contribute to oxidative stress. Th17 cells producing IL-17 act on airway epithelial cells to stimulate the release of multiple factors. These factors include macrophage chemoattractant protein-1 (MCP-1) which recruits macrophages, IL-5, regulated on activation, normal T cell expressed and secreted (RANTES), and GM-CSF (granulocyte–macrophage colony-stimulating factor) which activate eosinophils, IL-8 which mobilizes neutrophils, stem cell factor (SCF) which works to promote mast cell survival, and IL-25 which induces myeloid cells to release Th2-type cytokines. Neutrophils release matrix metalloproteinase 9 (MMP9), elastase, leukotriene B4, and platelet-activating factor (PAF), which work to enhance the activity of eosinophils. Activated macrophages release IL-1, tumor necrosis factor alpha (TNF-α), and IL-6 which interact with other inflammatory cells and result in a positive feedback loop with airway epithelial cells.

the release of histamine, prostaglandins, and leukotrienes during cellular degranulation. These cell signaling molecules induce smooth muscle bronchoconstriction and further propagate the inflammatory response. Th2 lymphocytes also produce IL-9 which stimulates mast cell proliferation in the airway (8), and IL-5, a cytokine associated with eosinophil survival (6). Eosinophils are believed to participate in the inflammatory response through the release of mediators including cysteinyl leukotrienes and reactive oxygen species resulting in bronchoconstriction, mucous secretion, and structural damage to the airways (9–12).

However, current evidence supports the role of respiratory viruses, as opposed simply to exposure to environmental allergens, on the development of asthma exacerbations. Most likely, exacerbations frequently arise from a complex interaction between the two. Respiratory viruses, in particular human rhinovirus (HRV), have been shown to be present during asthma exacerbations (13, 14) and been demonstrated to induce asthma exacerbations in susceptible individuals following inoculation (15). The mechanism of action for the viral induction of asthma exacerbations appears to be related to insufficient IFN-γ and IL-10 response and augmented IL-4, IL-5, IL-13 response. This suggests either impaired Th1 or heightened Th2 immunity as a mechanism for virus-induced airway inflammation.

Although asthma is typically associated with Th2 cytokines and eosinophilia, a subset of asthmatics have neutrophil-predominant Th17-associated disease (Fig. 1). Patients with mild to moderate asthma typically have disease characterized by Th2 cytokine expression with eosinophilic inflammation and respond well to ICS although a subset of Th2 high eosinophil-predominant asthmatics will have refractory disease despite receiving optimal treatment. These individuals appear to be particularly responsive to treatment with an anti-IL-5 antibody such as mepolizumab (16). Those with more severe, steroid-resistant disease appear to have their cellular milieu defined by Th1/Th2 cytokine expression and neutrophilic airway inflammation with less reversible airflow obstruction, but without increased bronchial hyper-responsiveness based on methacholine challenge testing (3, 17, 18). Among those with neutrophil-predominant disease, Th17 cells and their associated cytokine IL-17 have been noted to play a significant role in airway inflammation. IL-17 expression has been shown to be increased in the bronchoalveolar lavage (BAL) fluid, sputum, and sera of patients with asthma, where the severity of the disease correlates with an incremental increase in the presence of IL-17. IL-17 expression by Th17 cells has been demonstrated to augment *in vitro* glucocorticoid beta (GR-β) expression by airway epithelial cells. Glucocorticoid receptor beta (GR-β), an alternative isoform of glucocorticoid receptor alpha (GR-α), functions to suppress GR-α-mediated anti-inflammatory gene transcription through competitive inhibition of transcription at the glucocorticoid response element (GRE) (19). It has also been shown that IL-17 recruits neutrophils by promoting release of IL-8 from airway epithelial cells, and may be the link between T lymphocytes and granulocytes in the asthmatic airway (20, 21). *In vitro* studies have examined GC responsiveness of human airway epithelial cells following preincubation with IL-17A. Cells exposed to IL-17A were less able to inhibit tumor necrosis factor alpha (TNF-α)-induced IL-8 production after GCs were introduced, suggesting that the presence of IL-17-producing cells may render airway epithelial cells less responsive to GCs (22). Additionally, IL-17 has been shown to be a potent activator of endothelial cells, promoting transmigration of neutrophils to sites of inflammation (23). Induced sputum obtained from severe asthmatics demonstrates relatively high levels of neutrophils and appears to correlate with the severity of disease (24). Airway neutrophils produce proteases and lipid mediators, such as matrix metalloproteinase 9, elastase, leukotriene B4, and platelet-activating factor, that further propagate the inflammatory cascade and also appear to be responsible for the recruitment of eosinophils (20).

Airway epithelial cells are key players in the inflammatory response. They too have been shown to release IL-5 in addition to stem cell factor, a cytokine that supports survival of mast cells within the airway, and macrophage chemoattractant protein-1 (MCP-1). Alveolar macrophages, recruited by MCP-1, may also play an important role in the inflammatory process. It is thought that these macrophages may be a source of IL-1β, TNF-α, and IL-6 which they release following allergen binding to low-affinity IgE receptors. These cytokines might act on epithelial cells to stimulate the release of GM-CSF, IL-8, and regulated on activation, normal T cell expressed and secreted (RANTES). Both RANTES and GM-CSF work to recruit eosinophils to the airway and promote their survival (6, 8). Macrophages have also been shown to secrete elastase and metalloproteinases which are capable of degrading elastin in the airway extracellular matrix (25, 26).

Airway smooth muscle cells (ASMCs) play a role in the pathogenesis of airway inflammation. It is thought that viral infections may precipitate an asthmatic response in the airway through increased production of interferons and tumor necrosis factor alpha (IFNs/TNF-α) as demonstrated through *in vitro* exposure of ASMCs to these cytokines. Following exposure, increased levels of pro-inflammatory molecules were produced by ASMCs, GR-β expression was found to be upregulated, and increased contractility was noted through the production of calcium regulatory protein CD38 (27). Airway smooth muscle cells from patients with severe asthma were also noted to be corticosteroid unresponsive based on measured levels of cytokine expression following pretreatment with dexamethasone and stimulation with TNF-α, compared to those with nonsevere asthma. This may occur through the actions of TNF-α-induced p38 mitogen-activated protein kinase (MAPK) activity inhibiting anti-inflammatory gene transcription (28).

Additionally, myeloid-derived regulatory cells have also recently been implicated as critical regulators of allergic airway inflammation. Oxidative stress during airway inflammation regulates the expansion, activation, recruitment, and function of these immunoregulatory cells. Differential regulation by nitric oxide- or superoxide-producing subsets of these immature myeloid cells contributes to the balance of immune suppression and exacerbation of airway hyper-responsiveness (29).

### Structural changes of the airways

Airway remodeling as a consequence of inflammation is another characteristic of asthma (30–33). Structural changes that occur due to inflammation include thickening of the basement membrane, subepithelial fibrosis, goblet cell metaplasia, neovascularization, and increased airway smooth muscle mass (34). Examination of the relationship between airway remodeling and degree of asthma severity determined that clinical and functional severity scores of asthma were the strongest predictors of increased subepithelial layer thickness, independent of duration of disease, FEV1, or PC20M (31). This suggests that all patients with severe, poorly controlled asthma can experience deleterious effects on the structure and function of their airways, regardless of the duration of disease. While a definitive cause/effect relationship has yet to be conclusively established, airway remodeling has been associated with irreversible decline in FEV1, loss of bronchodilator reversibility, and increased airway hyper-responsiveness (35, 36). Additionally, correlation was found between increases in basement membrane thickness in the cartilaginous airways and cases of severe fatal asthma in a retrospective study (37).

IL-33, a member of the IL-1 family, has been implicated in the airway remodeling process. It is produced by airway epithelial cells and smooth muscle cells to stimulate collagen synthesis by fibroblasts, resulting in increased reticular basement membrane thickness. IL-33 is important in switching from Th1 to Th2 responses *in vivo* (38), producing Th2 cytokines IL-5 and IL-13, as well as inducing cytokine release from mast cells (39) and increasing expression of IL-17 by Th17 cells (40). The action of IL-33 is unaffected by exposure to high-dose steroids, suggesting that this interleukin plays an important role in the pathogenesis of steroid-resistant asthma (41). These various mechanisms lead to structural changes that result in a loss of elastic recoil with increasing lung compliance, most pronounced at the peri-bronchiolar level, resulting in irreversible obstructive small airways disease (42).

### Mechanisms of action of corticosteroids

Corticosteroids comprise the backbone of asthma therapy and act to reduce inflammation through both gene activation and suppression (43). Corticosteroids cross the cell membrane and bind to the glucocorticoid receptor (GR) in the cytoplasm (Fig. 2). Once bound, conformational changes occur that result in the release of nuclear chaperone proteins such as heat shock protein 90 (hsp-90) exposing two nuclear localization sequences (NL1 and NL2) on GR (44), allowing it to

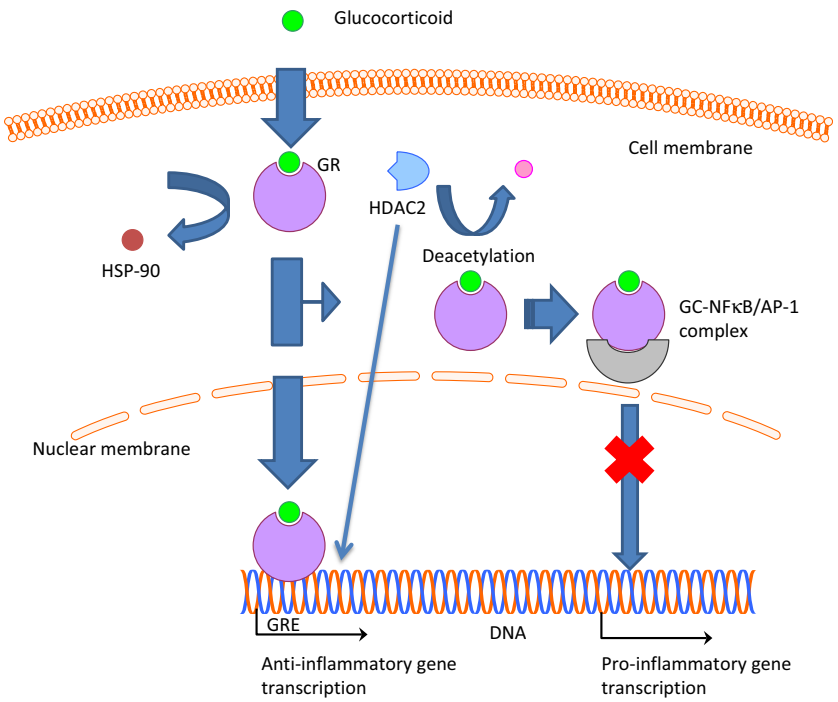


**Figure 2** Mechanisms of action of glucocorticoids (GC). GC diffuse across the cell membrane where they bind with GC receptors (GR) in the cytoplasm. Upon binding of the GC, this causes release of inhibitory proteins such as heat shock protein 90 (hsp-90), allowing the GC-bound GR to diffuse across the nuclear membrane where it binds to the glucocorticoid response element (GRE). The GRE is responsible for transcribing anti-inflammatory proteins. Additionally, binding of GC to GR results in recruitment of histone deacetylase 2 (HDAC2), which is responsible for deacetylating GR, permitting its binding to nuclear factor-kappa B (NF-κB) and activating protein-1 (AP-1). Upon binding, these transcription factors are deactivated, thereby inhibiting the transcription of pro-inflammatory proteins. Additionally, HDAC2 deacetylates the histone permitting transcription of anti-inflammatory genes by GR.

cross the nuclear membrane and interact with the GRE on DNA. The GC/GR complex interacts in a complex and dynamic manner with various transcriptional factors and kinases to alter the transcription of genes (6). One of these interactions involves the transcription of genes such as β-adrenergic receptor, glucocorticoid-induced leucine zipper, and mitogen-activated protein kinase phosphatase-1 (MKP-1). The latter two work as anti-inflammatory proteins with MKP-1 functioning as an inhibitor of MAPK pathways which promote the transcription of pro-inflammatory genes (43, 45). Glucocorticoids also act to suppress the transcription of genes associated with inflammation through their interactions with GR. GR attenuates the activity of transcription factors responsible for transcribing genes of pro-inflammatory proteins, such as nuclear factor-kappa B (NF-κB) and AP-1, by inhibiting histone acetylase through the recruitment of histone deacetylase 2 (HDAC2). HDAC2 deacetylates the GR allowing it to form a complex with NF-κB and AP-1, thereby inhibiting their ability to transcribe pro-inflammatory genes (43, 46).

## Steroid resistance in asthma

Severe/refractory asthma is poorly controlled disease despite optimal therapy and requires that one major criterion and two minor criteria be satisfied in order to meet the clinical definition of disease (see Table 1) (5, 47, 48). Steroid-resistant asthma is a subset of severe asthma defined as a failure to increase FEV1 by 15% following a 7-day course of oral steroid therapy at a dose of 20 mg of prednisolone daily or equivalent (49). Approximately 10 percent of asthmatic patients meet this definition of steroid-resistant/refractory asthma, consuming a disproportionate amount of healthcare dollars due to increased utilization of resources from outpatient clinic visits, emergency room care, and hospitalizations (50, 51). Multiple pathways have been implicated in the pathogenesis of refractory asthma. Some of these pathways include immune-mediated dysregulation of cytokines, defects in the ability of GR to bind drug and translocate into the nucleus, increased glucocorticosteroid receptor β (GRβ) expression, excessive activation of activating peptide-1 (AP-1) and NF-κB, and abnormal histone acetylation (43).

**Table 1** American Thoracic Society Criteria for Severe/Refractory Asthma*

| |
|---|
| Major criteria |
| Treatment with continuous or near continuous (>50% of the year) oral corticosteroids |
| Need for treatment with high-dose inhaled corticosteroids |
| Minor criteria |
| Need for additional daily treatment with a controller medication (long-acting β-agonist, leukotriene receptor antagonist, theophylline) |
| Asthma symptoms needing short-acting β-agonist use on a daily or near daily basis |
| Persistent airway obstruction (FEV1 < 80% predicted, diurnal peak expiratory flow variability >20% predicted) |
| One of more urgent care visits for asthma per year |
| Three or more oral steroid bursts per year |
| Prompt deterioration with <25% reduction in oral or intravenous corticosteroid use |
| Near fatal asthma event in the past |

*One major criterion plus two minor criteria required for diagnosis; other diseases have been excluded, exacerbating factors treated, and patient is generally adherent.

### Cells and signaling pathways contributing to steroid resistance

Th17 lymphocytes appear to play a key role in immune-mediated diseases including steroid-resistant asthma. While its causative role remains uncertain, accumulating evidence suggests a correlation between high levels of IL-17 production by Th17 cells and steroid-resistant disease (52). CD4+ lymphocytes differentiate into Th17 cells in the presence of IL-1β and IL-23, produced by antigen-presenting cells. IL-23 in particular drives the regulation, proliferation, and cytokine production by Th17 cells (19). As discussed, Th17 cells produce IL-17 which is a pro-inflammatory cytokine and appears to drive neutrophil-predominant steroid-resistant asthma (53, 54). Neutrophils have been shown to release TNF-α which induces bronchial hyper-responsiveness in a murine model. Additionally, neutrophils have been found to produce reactive oxygen species leading to an increased transcription of IL-8 by airway epithelial cells, further propagating the chemotactic neutrophil response (24). High concentrations of neutrophils have been found in the airways of severe asthmatic patients hospitalized for life-threatening attacks, suggesting a role for this cell type in the pathogenesis of acute allergic airway inflammation. Although steroids are known to increase neutrophil numbers by prolonging survival through decreased apoptosis (55), airway samples taken over time from this cohort showed an initial increase in neutrophil count that, interestingly, continued to increase throughout resolution of the exacerbation (56). This has led some to speculate that the neutrophil may not only be part of the problem, but may also help play a role in the recovery process.

Although not much is known regarding the functional relationship of immunoregulatory myeloid cells which are regulators of airway inflammation and steroid resistance in asthma, glucocorticoids induce the *in vitro* expansion of these cells (57). Eosinophils, mast cells, and airway epithelial cells have been shown to produce IL-25 in the lungs of asthmatics (58). IL-25, a member of the IL-17 family, regulates multiple aspects of mucosal immunity and has been shown to induce a population of pulmonary myeloid cells capable of producing Th2-associated cytokines IL-4 and IL-13. Recent evidence also indicates that blocking the glucocorticoid signal in a murine trauma model using the antagonist of the glucocorticoid receptor RU486 blocked the *in vivo* expansion of these cells (59). Further investigations are therefore necessary to provide mechanistic insight for the regulation of steroid resistance by these cellular phenotypes and identifying these regulatory cells as new therapeutic targets for asthma therapy.

In addition to driving Th2 responses in the airways, IL-25 has also been implicated in airway remodeling. Using a murine model, investigators blocked IL-25 in allergen-exposed mice and noted subsequent reduction in eosinophil count and Th2 cytokine levels. Reduction in airway smooth muscle hyperplasia, neovascularization, collagen deposition, and bronchial hyper-responsiveness were also noted in those animals undergoing IL-25 neutralization. Additionally, production of IL-33 and TSLP was also decreased when IL-25 was blocked (60).

### Glucocorticoid receptor inhibition by kinases

Glucocorticoids exert their effects by binding to GR in the cytoplasm which then translocates into the nucleus where it interacts with DNA to enhance or inhibit the transcription of genes affecting the inflammatory response. It has been demonstrated that GR function is reduced through phosphorylation by several kinases, most notably p38MAPK. Stress-induced phosphorylation of serine 134 on the GR in a p38MAPK-dependent manner (61) leads to steroid resistance by impeding nuclear translocation, protein stabilization, and binding to DNA, thus decreasing the transcription of anti-inflammatory genes (43, 62). p38MAPK activity is enhanced by IL-2, IL-4, and IL-13 and inhibited by p38MAPK inhibitors, where inhibition of its action correlates with decreased phosphorylation of the GR, resulting in increased nuclear translocation and ability to bind GRE (63, 64). Along with MAPKs, extracellular signal-regulated kinase (ERK) can phosphorylate the GC receptor when stimulated by superantigens, preventing nuclear translocation. Upon treatment with an ERK inhibitor, GC response is restored (65).

### Glucocorticoid receptor and transcription factors

Nuclear factor-kappa B is a transcription factor playing an important role in the pro-inflammatory response. It exists in the cytoplasm bound to an inhibitory protein, IκB. Upon binding of cell signaling molecules such as IL-1β, IL-2, GM-CSF, and TNF-α, a kinase cascade ensues leading to phosphorylation and ubiquitination of IκB, liberating NF-κB to translocate to the nucleus where it then transcribes pro-inflammatory cytokines (66). AP-1, a heterodimer of Fos and Jun, is a member of the leucine zipper transcription family which functions to enhance or inhibit transcription by dimerizing with other transcription factors. AP-1 has been demonstrated to physically interact with the GR to prevent binding to GREs (6, 67).

Once bound to glucocorticoid, GR translocates to the nucleus where it inhibits NF-κB and AP-1 pro-inflammatory cytokine transcription through the recruitment of histone deacetylase 2 (HDAC2) which reverses histone acetylation, thereby inhibiting gene transcription (68). However, in the presence of MAPK phosphorylation, GR is unable to translocate to the nucleus and has reduced ability to induce histone acetylation, precluding its ability to inhibit this NF-κB and AP-1-associated pro-inflammatory transcription process (6, 69). Additionally, HDAC2 activity is reduced by phosphoinositide 3-kinase (PI3K) δ phosphorylation (70). Oxidative stress increases the activity of PI3Kδ and thus is responsible for a pro-inflammatory state. Generation of oxidants occurs in the airway of asthmatic patients due to the presence of increased nitric oxide levels resulting in the formation of peroxynitrite, tyrosine nitration, and lipid peroxidation (71). Tyrosine nitration of HDAC2 is purported to inhibit its functional ability, and this appears to be an important mechanism of steroid resistance in asthmatic patients who smoke (72).

## Novel therapeutic options

Asthma is typically treated with ICS, along with the addition of long-acting β-agonists (LABA) and/or long-acting muscarinic agonist (LAMA) (73) if ICS alone do not control disease. For patients who remain poorly controlled on this regimen, escalating doses of ICS are provided, along with alternative therapies including the addition of anti-IgE therapy in qualifying patients (74). When all conventional asthma treatments fail, new targets for therapy offer hope. These new drugs target novel pathways in the asthmatic response to gain better control over the disease.

### Targeting cytokines

One potential option for treatment is the modulation of chemokines and cytokines to inhibit inflammatory cell migration into airways (Table 2). Inhibition of Th2-associated cytokines IL-4, IL-5, IL-9, and IL-13 offer promise as potential treatment targets.

**Table 2** Novel targets currently under investigation for the treatment of steroid-resistant asthma

| Target | Treatment | Stage of development for use in asthma (ref) |
|---|---|---|
| IL-4 | Pitrakinra/ dupilumab | Phase II clinical trials (17, 75, 76)/ phase II clinical trials (77) |
| IL-5 | Mepolizumab | Phase III clinical trials (16, 78, 79) |
| IL-9 | MEDI-528 | Phase II clinical trials (81, 82) |
| IL-13 | Lebrikizumab/ tralokinumab | Phase III clinical trials (82–85)/ phase II clinical trials (86) |
| IL-17 | IL-17 antibody | Animal model of allergic airway disease only (87–89) |
| IL-23 | IL-23 antibody | Animal model of allergic airway disease only (89) |
| IL-33 | IL-33 antibody | Animal model of allergic airway disease only (90) |
| CCR3 | CCR3 receptor antagonists | Phase II clinical trial (91, 92) |
| Kinase inhibitors | Imatinib | Phase II clinical trials (95) |
| Upregulation of HDAC2 | Theophylline | Currently available (96–98) |
| Airway smooth muscle | Bronchial thermoplasty | Currently available (99, 100) |

IL-4 monoclonal antibody appears effective for blocking the allergic airway response. The development of recombinant human IL-4, pitrakinra, was shown to block both IL-4 and IL-13 from binding to IL-4Rα/IL-13Rα1, resulting in improved FEV1 following allergen challenge and reduction in the need for rescue medications in humans subjects (17, 75, 76). Dupilumab, another IL-4Rα monoclonal antibody, was recently shown to reduce asthma exacerbations in subjects on high-dose ICS/LABA with sputum eosinophilia. Individuals were treated with either dupilumab or placebo for 4 weeks prior to withdrawal of LABAs, and then steroids were tapered over weeks 6–9. Those receiving drug experienced an exacerbation rate of 6% relative to those receiving placebo of 44% (77).

A human anti-IL-5 antibody, mepolizumab, was developed based on its ability to decrease eosinophil recruitment into the airways following allergen challenge in animals pretreated with antibody (78). In a recent meta-analysis, mepolizumab was shown to reduce eosinophil counts in the sputum and blood of subjects, but had no effect on other end points such as FEV1, peak flow, or histamine PC20 (79). However, the DREAM study (mepolizumab for severe eosinophilic asthma), a multicenter, randomized, placebo-controlled trial, did demonstrate a reduction in the rate of exacerbations in the treatment arm (16), suggesting that this may provide improvement in quality of life.

Humanized anti-IL-9 monoclonal antibody MEDI-528 was shown to have an acceptable safety profile in clinical trials with a trend toward less frequent exacerbations in the treatment group (80). While further study remains, MEDI-528 was able to reduce Th2-associated cytokines IL-4, IL-5, and IL-13 along with eosinophil and lymphocyte counts in BAL fluid from a mouse model. Additionally, MEDI-528 inhibited airway hyper-reactivity in response to a methacholine challenge in the same animal model of allergic airway disease (81).

An anti-IL-13 antibody, lebrikizumab, was developed with the ability to reduce Th2-associated cytokine and IgE production along with eosinophil recruitment. Trials in humans have demonstrated safety (82, 83) and have shown significant improvement in FEV1 in severe asthmatics compared to those treated with placebo. This effect was most pronounced in those with higher serum levels of periostin, a marker of high Th2 eosinophil-predominant disease, released from airway epithelial cells in response to increased circulating levels of IL-13 (84, 85). An alternative IL-13 monoclonal antibody, tralokinumab, was recently shown to produce a statistically significant improvement in FEV1 and decreased use of rescue medications in those on drug relative to placebo in atopic patients with moderate to severe asthma (86).

Th17-associated cytokine IL-17 is another target of interest. The use of an anti-IL-17 antibody was shown to block the effects of IL-17 following allergen challenge in a murine model where decreased levels of eosinophils, lymphocytes, and neutrophils were detected in BAL fluid from the treated animals (87). Treatment with anti-IL-17 antibody reduced levels of IL-4, IL-5, and IL-13 (88). Upstream blocking of IL-17 production by Th17 cells using an anti-IL-23 antibody was shown to be effective in reducing recruitment of neutrophils, eosinophils, and lymphocytes into the airways. Use of both anti-IL-17 and anti-IL-23 antibodies in humans is currently underway in clinical trials for other immune-mediated diseases such as Crohn's and rheumatoid arthritis and is likely to represent a future target in the treatment of asthma (89).

IL-33 can enhance Th2 responses through an increased expression of IL-5 and IL-13, leading to eosinophil influx and IgE production. Treatment with an IL-33 monoclonal antibody in a murine model of allergic airway disease attenuates these features; however, its role in human allergic airway disease remains to be determined (90).

### Targeting chemokine receptors

Targeting the receptors responsible for mediating the traffic of inflammatory cells is an alternate pathway under investigation for the treatment of asthma. Chemokine receptors CCR1, CCR2, CCR3, CCR4, CCR5, and CCR8 have been implicated in allergic airway disease through their interactions with chemokine ligands expressed by mast cells, eosinophils, and T-helper lymphocytes among other cells involved in the inflammatory response (91). The most promising of these, CCR3, expressed largely on eosinophils, is the only chemokine receptor for which an antagonist has been developed and tested in clinical trials. Unfortunately, despite demonstrating safety, it did not show efficacy in a phase III clinical trial for the treatment of allergic rhinitis (92). Development of these small molecules to target chemokine receptors for the treatment of asthma may prove to be problematic due to their functional redundancy and binding specificity (93).

### Additional targets for treatment

As discussed, certain kinases can phosphorylate glucocorticoid receptors to diminish their glucocorticoid response. p38MAPK inhibitors currently under development can interfere with the phosphorylation of GR to decrease the downstream release of inflammatory mediators by increasing GR nuclear translocation and have been shown to be safe in animal models (94). *In vitro* studies using peripheral blood mononuclear cells (PBMCs) have examined the effects of p38 MAPK inhibition on steroid responsiveness. Peripheral blood mononuclear cells from severe asthmatics exposed to lipopolysaccharide in addition to a p38 MAPK inhibitor expressed less IL-8 after the addition of dexamethasone than cells not pretreated with p38 MAPK inhibitor. This finding suggests that use of these inhibitors may attenuate inflammatory responses in the presence of steroid (64). The use of tyrosine kinase inhibitor imatinib mesylate in severe asthma represents a promising new therapy (95). Administration of imatinib was shown to attenuate airway hyper-reactivity, eosinophil accumulation, and Th2-associated cytokines IL-4 and IL-13 in a murine model of allergic airway disease. Its proposed mechanism of action is through the inhibition of tyrosine kinase C-kit activity, resulting in decreased production of

stem cell factor by airway epithelial cells leading to reduced mast cell activity in the airways. This drug is currently in phase II clinical trials for use in asthma.

The use of drugs to potentiate the production of HDAC2 when levels are decreased as a consequence of oxidative stress is another option for treating refractory asthma. One such drug, theophylline, is speculated to restore steroid responsiveness in previously resistant individuals. The mechanism through which this is thought to occur is increased activity of HDAC2 secondary to NF-κB suppression due to direct inhibition of oxidant activated PI3Kδ by theophylline (96, 97). In a study conducted in severe asthmatics controlled on high-dose ICS and other therapies including theophylline, withdrawal of theophylline was shown to worsen control of disease, while re-treatment restored asthma control, suggesting utility in using this drug to augment treatment (98). Another target in the treatment of severe asthma is the airway itself. To reduce bronchoconstriction, a new procedure termed bronchial thermoplasty has been developed to reduce airway smooth muscle mass. For this procedure, a catheter is used to deliver 65°C of thermal energy to the airway in order to reduce smooth muscle mass through uncoupling of contractile tissue. This procedure typically takes place in three sessions scheduled approximately 3 weeks apart. The Asthma Intervention Research 2 Trial for bronchial thermoplasty (AIR2 trial) is a double-blind, randomized, sham-controlled clinical trial examining the efficacy and safety of bronchial thermoplasty in severe asthmatics. Initial data showed that treated patients had a statistically significant improvement in their Asthma Quality of Life Questionnaire (AQLQ) and less missed days of work/school from exacerbations. However, this study also notes that the sham treatment conferred benefit as well, and those receiving the intervention had an increased rate of hospitalization following treatment (99). Recently published data on the AIR2 trial at 5 years of follow-up show that treated patients continued to experience a reduction in their rate of exacerbations between years 2–5, despite remaining on a lower average dose of steroids relative to the 12 months prior to treatment. Additionally, no structural airway abnormalities were observed on CT scan (100).

## Conclusions

Asthma is a complex disease affecting millions of individuals worldwide with 10 percent of asthmatics being refractory to conventional treatment. Novel therapies are currently being explored to circumvent steroid refractory disease. The European Network for Understanding Mechanisms of Severe Asthma (ENFUMOSA), Severe Asthma Research Project (SARP), and Unbiased BIOmarkers in the PREDiction of respiratory disease outcomes (U-BIOPRED) are currently working to phenotype and subphenotype asthmatic patients. Microarray studies comparing PBMCs from corticosteroid-sensitive and insensitive asthmatic patients have identified 11 discrepant genes which could be used to profile individuals at risk for steroid-resistant asthma (101). Future trends may include phenotyping and genotyping individuals to determine the best course of treatment for each patient.

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## Conflicts of interest

The authors have no conflicts of interest to disclose.

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