

EXPERT OPINION

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Th2 cytokine antagonists: potential treatments for severe asthma

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Introduction: Asthma is a major disease burden worldwide. Treatment with steroids and long acting β -agonists effectively manage symptoms in many patients but do not treat the underlying cause of disease and have serious side effects when used long term and in children. Therapies targeting the underlying causes of asthma are urgently needed. T helper type 2 (Th2) cells and the cytokines they release are clinically linked to the presentation of all forms of asthma. They are the primary drivers of mild to moderate and allergic asthma. They also play a pathogenetic role in exacerbations and more severe asthma though other factors are also involved. Much effort using animal models and human studies has been dedicated to the identification of the pathogenetic roles of these cells and cytokines and whether inhibition of their activity has therapeutic benefit in asthma.

Areas covered: We discuss the current status of Th2 cytokine antagonists for the treatment of asthma. We also discuss the potential for targeting Th2-inducing cytokines, Th2 cell receptors and signaling as well as the use of Th2 cell antagonists, small interfering oligonucleotides, microRNAs, and combination therapies.

Expert opinion: Th2 antagonists may be most effective in particular asthma subtypes/endotypes where specific cytokines are known to be active through the analysis of biomarkers. Targeting common receptors and pathways used by these cytokines may have additional benefit. Animal models have been valuable in identifying therapeutic targets in asthma, however the results from such studies need to be carefully interpreted and applied to appropriately stratified patient cohorts in well-designed clinical studies and trials.

Keywords: antagonists, asthma, CRTH2, ERK, GM-CSF, IL-13, IL-25, IL-33, IL-4, IL-4R α /IL-13R α 1, IL-5, IL-9, JAK, microRNA, mouse models, patient stratification, small interfering oligonucleotides, ST2, STAT, Th2 cytokine, therapy, TSLP

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1. Introduction

Asthma is an allergic disease of the airways with 300 million sufferers worldwide, according to WHO estimates, with a concomitantly high healthcare cost. The disease is prevalent in all age groups, however, the incidence is highest in children and in the developed world. As countries develop (e.g., India and China) the disease burden increases with rising socioeconomic status.

1.1 Features and pathogenesis of asthma

Although asthma is now recognized as a heterogeneous syndrome, involving different subtypes/endotypes (discussed below), it has common clinical features of

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Article highlights.

- Anti-Th2 cytokine therapies, for example, anti-IL-5, anti-IL-13, and IL-4R α /IL-13R α 1 antagonists are showing promise in the treatment of asthma.
- Because of the complexity of the disease and redundancy in the function of cytokines, combination therapies may be more effective than single factor approaches.
- Mouse models of disease are valuable tools for understanding pathogenesis, the roles of specific factors in disease, and in target discovery.
- Targeting of factors discovered using mouse models and human studies needs to be carefully performed in well-defined patient cohorts that have disease where these factor(s) have demonstrable activity by biomarker analysis.
- New approaches involving the targeting of infections or microRNAs also hold promise for the future.

This box summarizes key points contained in the article.

wheezing, breathlessness, dyspnea, and cough [1-4]. These symptoms result from the effects of airway inflammation underpinned by the influx of T-lymphocytes, neutrophils, macrophages, eosinophils, and mast cells, in combination with mucus hypersecretion, and hyperresponsiveness of the airways to non-specific stimuli that cause airway obstruction (Figure 1). CD4⁺ T helper type 2 lymphocytes (Th2 cells), eosinophils, and mast cells are associated with allergic mild to moderate asthma and when activated release a range of pro-inflammatory and pro-fibrotic mediators that promote airway remodeling, mucus hypersecretion, bronchoconstriction, and airway hyperresponsiveness (AHR) (Figure 2) [4,5]. Th2 responses are also enhanced in exacerbations and more severe asthma, however other responses (i.e., Th1), neutrophils, and macrophages pre-dominate in airway infiltrates in these patients, who are often resistant to steroid therapy [6-10].

1.2 Current asthma treatments

The mainstay of current therapies for asthma is combination treatments with inhaled corticosteroids and broncho-relaxing long-acting β -agonists. Corticosteroids are anti-inflammatory and suppress the activity of numerous inflammatory and structural cell types including eosinophils, macrophages, mast cells (DCs), dendritic cells, and lymphocytes (including Th2 cells), as well as epithelial and smooth muscle cells, as we have recently reviewed [9]. They function by crossing cell membranes and binding to glucocorticoid receptors in the cytosol and these complexes then travel to the nucleus. There they inhibit the expression of pro-inflammatory cytokine genes (including Th2 cytokines) and induce the gene expression of anti-inflammatory factors. Corticosteroids are effective in managing the symptoms in most patients with mild to moderate asthma, however, 10 – 20% of patients have severe asthma, many of who are refractory to these therapies. Indeed, steroid resistant patients make up only a small

proportion (5 – 10%) of asthmatics but account for more than 50% of asthma-related healthcare costs because they are difficult to treat and often require admission to outpatient facilities [9]. Furthermore, steroid therapy has limitations; it treats the symptoms not the causes of disease, is linked to non-compliance, has waning efficacy, and its use for treatment may have serious systemic side effects. Thus, there is an urgent need to develop new therapies that target the underlying causes of asthma and more severe forms of the disease.

1.3 Need for new therapies

In order to develop new treatments it is fundamentally important to understand the mechanisms of asthma pathogenesis. This can be achieved through a combination of the use of animal models, *in vitro* studies with human cells, and clinical studies. Nevertheless, it is becoming obvious that the results of such studies must be carefully interpreted and translated so that the most appropriate clinical trials are performed in selected cohorts of patients. Different treatments may be effective in suppressing the induction of asthma and in reversing established disease and may be specific to different asthma endotypes. Thus, understanding the critical factors that drive disease in each patient, or patient group, will result in tailored medication for maximum benefit.

Asthma results from a complex network of inflammatory pathways and there are many immunological and cellular contributors, which vary in the different endotypes [4]. Nevertheless, it is known that Th2 cells and the cytokines that these and other inflammatory cells release are critically important in the pathogenesis of mild to moderate forms of the disease, and are elevated in more severe forms. It has also been recently recognized that innate immune and myeloid cells can also release Th2 cytokines, and may be critical players in the inception and exacerbation of asthma [11,12]. The major Th2 cytokines that are thought to contribute to asthma pathogenesis are interleukin (IL)-4, IL-5, IL-9, IL-13, and granulocyte macrophage-colony stimulating factor (GM-CSF) [4]. Recently, IL-25, IL-33, and thymic stromal lymphopoietin (TSLP) have been linked to the development of Th2 responses and the inception of asthma. Furthermore, Th2 receptors and signaling pathways have a significant role in the development and production of Th2 responses. It is important to consider that individual features of disease may be regulated independently of each other (e.g., inflammation and AHR [13-16]) and may need to be targeted separately. Also, the inflammatory response in asthma may have redundant components where multiple effector molecules or cells can drive disease (e.g., eosinophil recruitment into tissues) and thus combination therapy rather than targeting a single mediator may be required for maximal therapeutic benefit [4].

2. Th2 cytokine antagonists for asthma

Th2 cells, and other inflammatory cells, release a range of cytokines that have common and/or unique effects [4]. Many

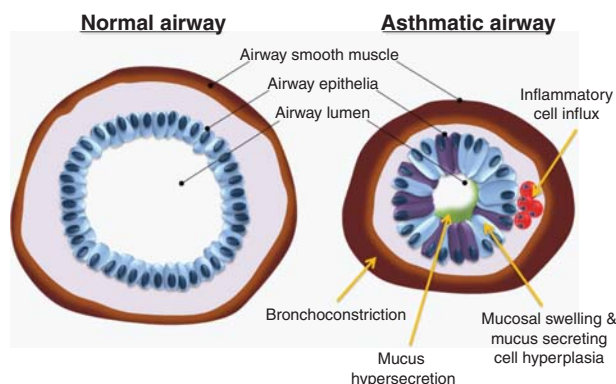


Figure 1. Pathological features of the asthmatic airway. In the asthmatic airway (right), the infiltration and activation of inflammatory cells (red cells), subsequent mediator release results in mucosal swelling (blue cells), mucus secreting cell hyperplasia (purple cells), mucus hypersecretion (green), and bronchoconstriction. These immunopathological features result in airway narrowing and widespread but variable airflow obstruction that is characteristic of asthma.

of these cytokines and their effects were first discovered using animal models, particularly mice. Acute models of allergic airway disease (AAD) in mice can be used to assess the roles of cytokines and effects of targeting their expression on airway inflammation, cytokine release, immunoglobulin (Ig)E levels, mucus hypersecretion, AHR, and early features of tissue remodeling (Figure 2) [6-8,10,17-32]. They have many [6,7,10,17-32] or individual factors [8] of asthma, and can be used to assess: The role of immune factors [6,17-23]; the contribution of immune cells and factors in adoptive transfer studies [20,23,24] and in factor deficient [19,22,24] or transgenic animals [14,20]; the impact of deleterious (e.g., *Chlamydia* [17,18,20,23,24], *Haemophilus* [7,10]) or protective [2,25-27] infection and Treg-inducers [2,25-28]; effects before, during, or after sensitization [7,10,25] or in established disease [28]; the effects of different stages of life [6,17,18,23]; and even nutritional interventions [29,30]. Chronic mouse models can be used to assess longer-term remodeling events [31-33]. Commonly used mouse models involve systemic sensitization followed by airway challenge with ovalbumin. This enables the assessment of systemic and local pulmonary immune responses, as well as antigen-specific T cell responses. There are also powerful molecular tools that can be combined with ovalbumin-induced models of AAD such as ovalbumin T cell receptor transgenic mice [18,20]. House dust mite is another commonly used antigen to induce Th2 responses after direct sensitization through the airway mucosa rather than systemically with an adjuvant [27,34,35]. Rat models of AAD have also been used to investigate airway epithelial specific responses [36,37].

In vitro studies using human immortalized cell lines or primary cells from the circulation (peripheral blood mononuclear cells) or the respiratory tract (broncho-alveolar

lavage or sputum) have provided valuable insights into the contributions of Th2 cytokines to asthma pathogenesis [38-42]. Many *in vivo* association studies in both children and adult patients [43,44] have also linked Th2 cytokines with the perception and progression of asthma. Furthermore it is possible to perform antigen or infectious challenge studies of asthmatics to further investigate the roles of particular factors in the acute phases of disease [45]. Results from experimental and clinical studies guide clinical trials as an ultimate test of new therapeutics for asthma, however, they must be well designed to test therapeutics in the most appropriate sub-group(s) of asthmatics (e.g., [45-49]).

There is the potential to target each of the Th2 cytokines (Table 1), or their receptors and signaling pathways for therapeutic benefit, particularly in different endotypes where specific cytokines may have a major influence on the expression or progression of disease. Since there may be redundancy in activity, and pathologic features may be independent of each other, combination therapies that target multiple cytokines or common receptors that are utilized by multiple cytokines may be more effective than single target strategies.

2.1 Th2 cytokine antagonists

The classic Th2 cytokines IL-4, IL-5, IL-9, and IL-13, as well as GM-CSF, which is released by Th1 and Th2 cells, all have specific effects in asthma [4]. Targeting these cytokines in patient groups with evidence of increased function of these cytokines, or the pathophysiological outcomes of their effects, may be effective in targeting the mechanisms that lead to disease and thus suppress asthma (Table 1).

2.1.1 IL-4

IL-4 has many pro-asthmatic effects and can induce: Th2 cell differentiation (and suppression of Th1 responses); B cell isotype switching and IgE production; mast cell proliferation; eosinophilic influx; maintenance of antigen-induced inflammation; eotaxin production; mucus hypersecretion; and AHR, and may also contribute to airway remodeling (Figure 2) [4,50-55]. IL-4 signals through the IL-4R α /IL-13R α 1 to induce its effects [56].

Increased IL-4 expression or administration of recombinant IL-4 to the airways of mice does not induce inflammation or AHR, but induces IgE release and mucus hypersecretion [53,57-59]. The use of IL-4-deficient ($^{-/-}$) mice has also implicated IL-4 in promoting some features of asthma. When acute AAD was induced in IL-4 $^{-/-}$ mice they had attenuated eosinophilic inflammation, mucus hypersecretion, IgE production, and in some cases also had reduced AHR [54,60,61]. In chronic models IL-4 $^{-/-}$ mice had attenuated inflammation but enhanced remodeling and AHR [33]. Thus, IL-4 is implicated in some features of asthma pathogenesis including eosinophilic inflammation, IgE production, and mucus hypersecretion and possibly AHR but not necessarily airway remodeling.

Targeting IL-4 using anti-IL-4 monoclonal antibodies (mAb) in mouse models has been shown to attenuate some

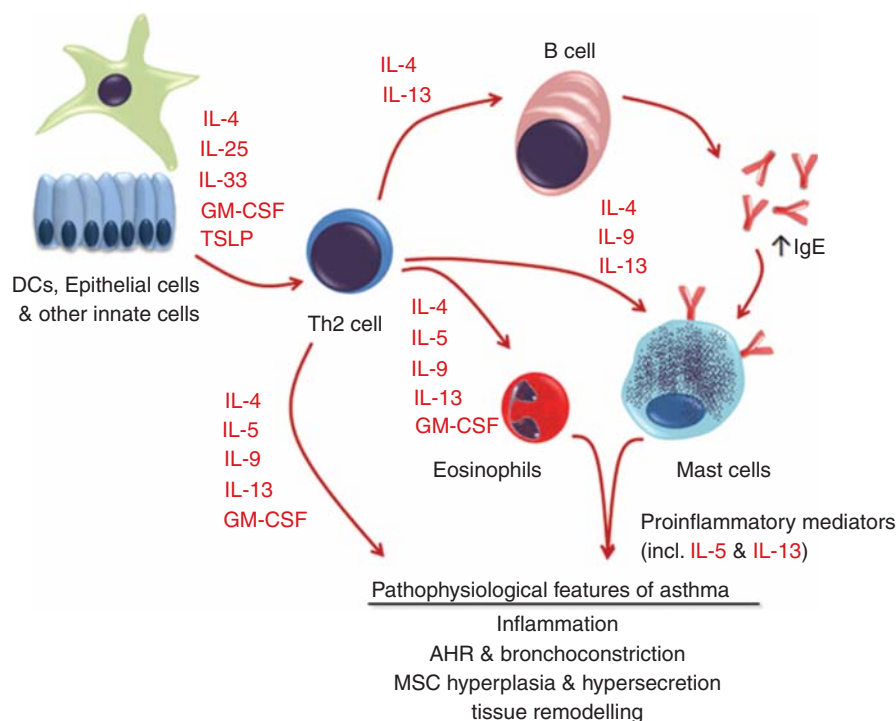


Figure 2. Role of Th2 cells and cytokines in the pathogenesis of asthma. Th2 cell differentiation, recruitment, and activation is induced by Th2-inducing cytokines such as IL-4, IL-25, IL-33, GM-CSF, and TSLP that are produced by dendritic cells (DC), epithelial cells, and other innate immune cells and are present in the extracellular environment. Activated Th2 cells secrete the Th2 cytokines, IL-4, IL-5, IL-9, IL-13, and GM-CSF which promote IgE production and eosinophil and mast cell recruitment and activation in the lung. Th2 cytokines produced by Th2 cells and the release of pro-inflammatory mediators by activated eosinophils and mast cells in the asthmatic airway drive the pathophysiological features of asthma.

features of AAD (e.g., IgE production, AHR) when administered during sensitization but not with treatment before or after allergen challenge [57,62]. Other studies showed that IL-4 mAb treatment also reduced IL-13 levels but not to sufficiently low levels to inhibit the development of AHR [63]. Thus, mouse models have shown that IL-4 contributes to the development of AAD but is less important in disease progression. Furthermore, anti-IL-4 treatment may have beneficial effects in suppressing the development of asthma.

A humanized anti-IL-4 neutralizing mAb has been generated that inhibits IL-4 bioavailability *in vitro* [64], but a Phase II trial of anti-IL-4 (pascolizumab) in established asthma was discontinued because of a lack of clinical effects [64]. Nevertheless, this antibody has not yet been used in clinical trials to suppress the development of asthma during early life when allergen sensitization takes place. Anti-IL-4 treatment during this period may suppress the establishment of Th2 responses (IL-5, IL-13, GM-CSF) and their pro-asthmatic sequelae. An IL-4 antagonist, soluble IL-4R α , has been assessed as a treatment in established moderate persistent asthma and has been shown to improve forced expiratory volume in one second (FEV₁) and reduce asthma symptom scores [65]. However, inhibition of IgE function, which is a major product of IL-4 action, with anti-IgE mAb (omalizumab) has limited efficacy

but does reduce clinical symptoms and exacerbations in moderate to severe allergic asthma in children, adolescents, and adults [66,67]. Its efficacy in non-allergic asthma remains unknown. Once again targeting specific populations may be required to be able to demonstrate the greatest therapeutic effect (e.g., anti-IgE therapy for poorly controlled asthma or viral-induced asthma where IgE receptor-bearing cells are thought to contribute).

In summary, targeting IL-4 has some beneficial effects but efficacy may be greatest for disease prevention.

2.1.2 IL-5

IL-5 plays a pivotal role in the development, maturation, and mobilization of eosinophils from the bone marrow [4,68]. It is also implicated in the development of AHR (Figure 2) [69]. This cytokine signals through the IL-5R α in conjunction with the beta common (β c) chain and induces the enhanced expression of its alpha-receptor [68].

Increased IL-5 expression or administration of IL-5 to the airways of mice induces eosinophilic inflammation and structural changes [70-72], which were further increased when AAD was induced [73]. The use of IL-5^{-/-} mice showed that the absence of IL-5 suppresses eosinophilic inflammation, mucus hypersecretion, early signs of airway remodeling, and AHR in

Table 1. Th2 cytokines that contribute to the pathogenesis of asthma, how their roles have been determined using mouse models (Upper Table), the inhibitors that have been developed and their effects in AAD in mice (Upper Table) and in clinical trials in humans (Lower Table).

Cytokine	Role	Deficient Mice	Over-expressing mice/ recombinant treatment	Neutralizing antibody/ inhibitor in AAD	Summary of importance of cytokines found in mouse models
IL-4	Th2 cell differentiation B cell isotype switching and IgE production; Mast cell proliferation; Eosinophilic efflux; Maintenance of antigen-induced inflammation; Eotaxin production; Mucus hypersecretion; AHR Airway remodeling	Implicated IL-4 in promoting some features of asthma after acute AAD, mice had attenuated eosinophilic inflammation, IgE production, and mucus hypersecretion	Does not induce inflammation or AHR Induces IgE release and mucus hypersecretion	Attenuated IgE, AHR when administered during sensitization (but not with treatment); Decreased IL-13 but did not inhibit AHR	Contributes to the development of AAD but not progression; Anti-IL-4 treatment may suppress development of AAD
IL-5	Development, maturation, mobilization, and chemotaxis of eosinophils; Implicated in development of AHR	Suppressed eosinophilic inflammation, mucus hypersecretion, airway remodeling, and AHR	Induced eosinophilic influx and structural changes, increased further after AAD induction	Suppressed eosinophils (effective up to 12 weeks before AAD); Administration close to induction of AAD suppressed mucus hypersecretion, AHR, and remodeling	Important in eosinophilic inflammation and AHR; Anti-IL-5 treatment may suppress airway inflammation, remodeling, and acute AHR in asthma
IL-9	Promotes T-cell growth and mastocytosis; May control phenotype of mast cells and other cell types	Suppressed eosinophilic airway inflammation and AHR	Lung-specific overexpression increased airway inflammation, MSC hyperplasia, and AHR		
IL-13	Induces all hallmarks of asthma (IgE production, eosinophilic airway inflammation, mucus hypersecretion, airway remodeling, and AHR)	Suppressed MSC numbers, may or may not have AHD in acute AAD Pulmonary eosinophilic inflammation	Increased baseline mucus secretion, airway remodeling, and AHR (all further enhanced after allergen challenge)	Suppressed airway inflammation, mucus production, and AHR	Antagonizing IL-13 may have potential as a therapy for chronic asthma but additional suppression of eosinophilic inflammation would be required
IL-4Rα/IL-13Rα1	Common receptor for both IL-4 and IL-13 signaling	Reduced eosinophilic inflammation, mucus hypersecretion, IgE production, and AHR	Increased serum IgE and mucus production	Targeting IL-4Rα/ IL-13Rα1 is beneficial in asthma; Results more effective than antagonizing IL-4 alone	

Table 1. Th2 cytokines that contribute to the pathogenesis of asthma, how their roles have been determined using mouse models (Upper Table), the inhibitors that have been developed and their effects in AAD in mice (Upper Table) and in clinical trials in humans (Lower Table) (continued).

Cytokine	Role	Deficient Mice	Over-expressing mice/ recombinant treatment	Neutralizing antibody/ inhibitor in AAD	Summary of importance of cytokines found in mouse models
GM-CSF	Controls induction of numerous inflammatory cell types including eosinophils; Enhances eosinophil chemotaxis, degranulation, and cytokine production Critical for initiation of natural killer cell and Th2 inflammatory responses and associated airway lesions (IgE production, airway eosinophil influx, mucus production, epithelial cell hyperplasia)		Enhanced allergic sensitization, airway inflammation, and fibrosis	Anti-GM-CSF antibodies administered inhibited airway inflammation, mucus production, and suppressed AHR Monoclonal antibody neutralization reverses Th2-associated pathology, when administered during challenge suppressed AHR	
IL-25	Promotes chemotaxis of Th2 cells; May contribute to DC-induced development of Th2 cells; Activates NK and NKT cells to secrete IFN- γ May function early in innate immune activation to promote Th2 immune responses; Induces DC-induced Th2 cell differentiation and Th2 cell chemotaxis; Promote release of cytokines (IL-4, IL-5, IL-13)	Induces Th2-like inflammatory response, as well as increased tissue eosinophil influx, IgE, MSC, and lung hyperplasia			
IL-33				Anti-IL-33 antibody inhibits airway inflammation; Monoclonal antibody blockade of IL-33R attenuates hallmark features of AAD	
TSLP			TSLP receptor ^{-/-} mice are less susceptible to the induction of acute AAD	Antibody mediated blockade of TSLP receptor suppresses AAD by inhibiting DC migration to the airways and CD4 ⁺ T cell priming	

Target cytokine	Compound	Mechanism of action	Phase of development	Prediclinical/clinical efficacy
IL-4	Pascolizumab (SB 240683)	Humanized cytokine antagonist	Not yet studied in human clinical trials	High binding affinity, long half-life, and low immunogenicity
IL-5	Mild asthma: mepolizumab Severe asthma: reslizumab (SCH55700)	Humanized cytokine antagonist, blocks binding of IL-5 to the IL-5R α chain	Small initial safety and efficacy clinical trials underway	Well tolerated, reduced eosinophil numbers in blood and sputum. No significant improvement in late-phase reaction, AHR, or lung function.
IL-9	MEDI-529	Humanized cytokine antagonist	Phase IIa Studies	Acceptable safety profile, no serious adverse events, linear PK profile
IL-13	Lebrikizumab	IgG4 humanized monoclonal cytokine antagonist	Small clinical trials have occurred	Improved prebronchodilator FEV1, did not reduce asthma symptoms
IL-4R α /IL-13R α 1	AMG 317	IgG2 humanized monoclonal receptor antagonist to IL-4R α	Phase II clinical trials	Safe, well tolerated, fewer asthma exacerbations were seen in the higher AMG 317 treatment groups

acute AAD, which could be reversed by re-constitution of IL-5 [72,74]. Similar effects were observed in IL-5R α ^{-/-} mice [73]. The induction of epithelial hypertrophy and sub-epithelial fibrosis but not chronic inflammation and AHR in chronic AAD in IL-5^{-/-} mice demonstrates that this cytokine regulates inflammation and AHR, but not airway remodeling in the long term [75].

Anti-IL-5 neutralizing antibodies have been developed and have strong and sustained suppressive effects on eosinophils, being effective for up to 12 weeks in mice [57,76]. Administration of anti-IL-5 just prior to the induction of acute AAD results in suppression of mucus secretion, AHR, and remodeling, but not antibody or T cell cytokine production [68,73,77,78]. In chronic models of AAD, treatment with anti-IL-5 after disease had developed was effective in suppressing airway inflammation and remodeling but not AHR, suggesting that these features are independently regulated and additional strategies for suppressing chronic AHR are required. Thus, mouse models have shown that IL-5 is important in regulating eosinophilia and acute AHR and that anti-IL-5 treatment may have beneficial effects in suppressing airway inflammation, remodeling, and acute AHR in asthma.

The extensive studies in mouse, as well as other animal models of asthma (including non-human primates) have led to the development of humanized anti-IL-5 mAbs (e.g., mepolizumab), which block the binding of IL-5 to the IL-5R α chain. Small initial safety and efficacy clinical trials have demonstrated similar effects as observed in chronic mouse models. Treatment of mild (mepolizumab) and severe (SCH55700/reslizumab) asthmatics with a single infusion of these anti-IL-5 mAbs [69,79,80] was well tolerated and provided proof-of-principle that targeting IL-5 reduced eosinophil numbers in the blood and airways (sputum) of patients. However, no significant clinical improvement was observed in the late-phase reaction, AHR, or lung function [69,79-82]. The lack of demonstrable clinical efficacy may have been due to small sample size, trial design [83], or failure to decrease tissue eosinophil numbers to therapeutically beneficial levels [84]. Indeed, in a subsequent clinical trial mepolizumab was shown to only partially decrease eosinophil numbers in bronchial mucosa [82]. Building on these studies, atopic asthmatic patients were given three infusions of mepolizumab, which reduced eosinophil levels in the bronchial mucosa by approximately 50% and correlated with decreased expression of eosinophil TGF- β 1 and extracellular matrix proteins (tenascin, lumican, and procollagen III) strongly suggesting a role for this granulocyte in remodeling of the airway wall [81]. Further studies indicated that mepolizumab acts in part by attenuating eosinophil maturation and potential local tissue eosinophilopoiesis [85]. More recently, the anti-eosinophil effects of mepolizumab and reslizumab were investigated in asthmatics selected for elevated levels of eosinophils in their sputum ($\geq 3\%$) [46-48]. In these studies the numbers of eosinophils in sputum were decreased to almost zero. Collectively, these studies showed that this approach to stratification of patients

based on sputum cell counts for anti-eosinophil therapy resulted in decreased frequency of exacerbations, a steroid-sparing effect, improved FEV₁, and long-term improvement of asthma control (reslizumab) [46-48].

Thus, anti-IL-5 has substantial promise for the treatment of poorly controlled eosinophilic asthma [19,86]. In the future, therapeutic approaches that reduce eosinophil numbers to very low levels in lung tissue of patients where these granulocytes are present and activated should result in enhanced therapeutic outcomes. Approaches that target eosinophils directly, such as the cytotoxic anti-IL-5R α mAb benralizumab, may further enhance therapeutic outcomes [87-89]. Such anti-eosinophil approaches should significantly benefit the approximately 30% of asthma patients presenting at outpatient clinics that have diagnosed eosinophilic bronchitis [90]. Similar therapeutic strategies are urgently required for neutrophilic and other forms of asthma where our knowledge of pathogenic pathways is more limited.

2.1.3 IL-9

IL-9 is produced by Th2 cells and the recently described Th9 cells and drives T cell proliferation and mast cell-driven inflammation, mucus production, AHR, and airway fibrosis (Figure 2) [4,91-95].

Adoptive transfer studies show that Th9 cells and overexpression of IL-9 in the lung may promote mast cell-driven eosinophilic inflammation, mucus hypersecretion, and AHR in mice, which is reversible by the neutralization of IL-9 [93,96,97]. Thus, anti-IL-9 may have beneficial effects in suppressing these features of asthma, particularly in patients with a mast cell component to their disease.

A human anti-IL-9 mAb (MEDI-528) has been developed [98] and is in trials for long-term treatment of moderate to severe asthma [99,100]. Initial results have demonstrated only small improvements in lung function and the trials are ongoing.

2.1.4 IL-13

IL-13 can induce all of the hallmark features of asthma: IgE production; eosinophilic airway inflammation; mucus hypersecretion; airway remodeling, and AHR (Figure 2) [4,18,100,101]. IL-13 primarily signals through the IL-4R α /IL-13R α 1 receptor complex, but in some circumstances signals independently of this complex [102].

Overexpression of IL-13 in mice increases baseline mucus secretion and induces airway remodeling and AHR, and this effect is further increased after allergen challenge [103]. Induction of acute AAD in IL-13^{-/-} mice results in suppressed mucus secretion and in some studies AHR [63,104]. The fact that AHR still develops in some studies indicates that redundancy exists in the IL-13-related mechanisms that regulate altered smooth muscle reactivity, and other compensatory factors are able to induce alterations in airways reactivity in AAD. Furthermore, eosinophilic inflammation is still a predominant feature of the inflammatory response in the absence of IL-13 suggesting

that targeting IL-13 alone may not give maximal therapeutic benefit. The potential for combination therapies has been investigated by depleting IL-4 or IL-5 in IL-13^{-/-} mice. IL-4 depletion suppressed airway eosinophilia and AHR but not the levels of peripheral eosinophils, whereas as IL-5 neutralization inhibited eosinophilia in both compartments and AHR [63,75]. In models of chronic AAD, IL-13^{-/-} mice have reduced airway inflammation, mucus hypersecretion, and airway remodeling but low levels of AHR persist [33]. Recently, we have shown using a range of methods that IL-13 promotes susceptibility to *Chlamydia* respiratory infection, which is linked to the induction and exacerbation of asthma [105]. Thus, targeting IL-13 may not only suppress features of asthma but also infection-associated induction and exacerbation of asthma [3,6,17,18].

Anti-IL-13 neutralizing antibodies have been developed and a naturally occurring soluble IL-13R α 2-Fc fusion protein, which is a competitive inhibitor of IL-13, is also available [61,106]. Treatment with either of these agents during the allergen challenge phase in models of acute AAD suppressed eosinophilic airway inflammation, mucus hypersecretion, and AHR [9,61,107]. In established chronic AAD anti-IL-13 suppressed cytokine and IgE production, airway inflammation, mucus hypersecretion, and remodeling but had only small effects on AHR [32,108-110].

Thus, mouse models have shown that IL-13 promotes eosinophilic inflammation and remodeling and contributes to AHR, and anti-IL-13 treatment may have beneficial effects in suppressing these features of asthma. However, add-on therapy may be required to further suppress eosinophilic inflammation.

Anti-IL-13 neutralizing antibodies have been successfully trialed in steroid-resistant asthmatics with increased circulating periostin levels, which is a surrogate measure of IL-13 activity [49]. Treatment for 12 weeks resulted in a significant (5.5%) improvement in lung function (FEV₁) with the greatest effects in those with higher levels of circulating periostin. Further studies are required to determine the optimal therapeutic strategies and patient groups. Two humanized anti-IL-13 antibodies that target different epitopes of IL-13 were also trialed in mild atopic asthmatics [45]. Subjects were administered the antibodies then challenged with allergen. Treatment suppressed early- and late-phase asthmatic responses (FEV₁ and area under the curve), although there were no changes in AHR or sputum eosinophils. Importantly, however, these subjects were not selected on the basis of increased IL-13 or periostin levels.

Thus, anti-IL-13 treatment may be effective in asthmatics with increased levels of this cytokine and where there is demonstrable biological activity.

2.1.5 GM-CSF

GM-CSF induces multiple signaling pathways that control the proliferation and activation of numerous inflammatory cell types that are important in asthma (e.g., eosinophils, DCs, macrophages, and neutrophils) (Figure 2) [4,111]. It contributes

to the early development of eosinophils and is critical for their survival and contribution to immune responses as well as in the activation of antigen presenting cells. GM-CSF signals through the activation of GM-CSFR α in conjunction with IL-3/IL-5/GM-CSF β c receptors [19,112,113].

The absence of the β c chain results in significant attenuation of Th2 development and all of the hallmark features of asthma in models of acute AAD [19]. Overexpression of GM-CSF in the airways of mice induces pulmonary sensitization to allergen, and increases airway inflammation and remodeling alone or upon allergen challenge [114,115]. Anti-GM-CSF treatment during challenge in models of acute AAD suppresses airway inflammation, mucus hypersecretion, and AHR [116].

Thus, mouse models show that GM-CSF promotes airway inflammation and remodeling, and AHR and anti-GM-CSF treatment may have beneficial effects in asthma.

A human anti-GM-CSF neutralizing antibody has been developed but results from trials have not yet been reported.

2.2 Th2-inducing cytokine antagonists

Th2 cytokines auto-induce the cell that releases them. However, recently, innate lymphoid cells (e.g., nuocytes) have been identified (through the use of gene reporter mice) as early sources of Th2 cytokines (e.g., IL-5 and IL-13) after allergen exposure and these cells may be important for the induction of subsequent adaptive Th2 responses [4,12,117]. Also recently, other cytokines have been identified, IL-25, IL-33, and TSLP, that have important roles in the induction of Th2 responses by regulating innate lymphoid development (Figure 2). Since IL-25, IL-33, TSLP and innate lymphoid cells are important in the early onset of Th2 responses these cytokines and cells may also be targets to prevent the induction and progression of asthma (Table 1). Treatments that target these cytokines in humans have not yet been trialed.

2.2.1 IL-25

IL-25 activates natural killer T cells, which are known to have a role in the induction of AHR in models of AAD [27], and asthma, and this cytokine contributes to the differentiation of Th2 cells, allergic inflammation, and angiogenesis (Figure 2) [4,118-123].

Administration of IL-25 to mice induces IL-4, IL-5 and IL-13 production, IgE synthesis, eosinophilic airway inflammation, mucus hypersecretion, and some features of airway remodeling [124]. IL-25 also induces the development of Th2 cells in models of acute AAD in mice [21,118,119]. An important recent study showed that IL-25 also induces the production of Th2 cytokines from a novel myeloid cell population (T2M cells) and this process is resistant to steroid treatment [12]. Furthermore, by using novel IL-13 reporter mice it has been demonstrated that IL-25, along with IL-33, induces the development of nuocytes, which are an early source of IL-13 [11].

Anti-IL-25 neutralizing antibodies have been developed that suppress Th2 responses and associated inflammation

and AHR in models of acute and chronic AAD in mice but they do not affect airway remodeling [21,31,124,125]. Furthermore, blocking IL-25 during the allergen challenge phase also suppressed AHR, indicating that anti-IL-25 has the potential to be used as a therapy in established disease [126].

2.2.2 IL-33

IL-33 is released early following epithelial cell damage [127]. It induces eosinophil differentiation, the release of IL-5 and IL-13, and the influx of Th2 cells into the airways resulting in airway inflammation, as well as potentially, dendritic cell (DC)-induced Th2 cell development and the pro-inflammatory activity of mast cells (Figure 2) [4,128-133]. This may occur through the ability of IL-33 to bridge innate and adaptive immunity [134]. IL-33 signals through T1/ST2 receptors on Th2 cells.

Administration of IL-33 to the lung induces Th2 responses, eosinophilic inflammation, IgE, mucus hypersecretion, and signs of airway remodeling [135].

Anti-IL-33 antibody inhibits airway inflammation in models of acute AAD in mice [136]. Neutralizing antibodies have been developed against the T1/ST2 receptor, which prevent Th2 stimulation and could potentially be used to block IL-33 activity [137].

2.2.3 TSLP

TSLP is an innate cytokine that promotes DC-induced development of Th2 responses, Th2 cell chemotaxis, and the release of cytokines by these cells (Figure 2) [4,138-142]. TSLP may also increase airway contractility, mast cell function, and suppress the generation of antigen-specific regulatory T cells (Tregs) [143,144]. Overexpression of TSLP in the lungs of mice results in severe Th2-mediated inflammation, and TSLP receptor^{-/-} mice have reduced acute AAD [145,146].

Neutralizing antibodies have been developed against the TSLP receptor, which suppress AAD by inhibiting DC trafficking and priming of T cells [147]. However, TSLP is not increased in all endotypes of asthma, but may be particularly important in severe asthma [148,149].

2.3 Th2 cell receptor antagonists

An alternative to targeting Th2 cytokines is to block the receptors that they bind to and signal through (e.g., IL-4R α /IL-13R α 1 and T1/ST2) or through receptors that mediate the mobilization of Th2 cells (CRTH2).

2.3.1 IL-4R α /IL-13R α 1

Directly targeting IL-4 may be too selective to account for redundancy and pathways that operate in parallel. However, suppressing the activity of the common IL-4 and IL-13 receptor complex, IL-4R α /IL-13R α 1, has the potential to inhibit the responses induced by both of these cytokines [4,150].

IL-4R^{-/-} mice have reduced eosinophilic inflammation, mucus hypersecretion, IgE production, and AHR in models of acute AAD [54,60,61]. The effects were greater than in

IL-4^{-/-} mice presumably since both the effects of IL-4 and IL-13 were reduced. In chronic models, IL-4R^{-/-} mice had reduced mucus hypersecretion, but there were no differences in airway fibrosis or AHR [33].

Anti-IL-4R α mAb has been tested for its ability to prevent AAD in mice. Treatment before allergen challenge suppressed eosinophilic airway inflammation, IgE levels, mucus hypersecretion, and AHR [58,151,152]. However, cytokine responses (IL-5, interferon (IFN)- γ) were not affected and IL-4 levels were actually increased. An IL-4 molecule (recombinant IL-4 variant, pitrakinra) with attenuated function, which blocks IL-4R α /IL-13R α 1 signaling, has also been developed and tested in mouse models [110,153]. Treatment during sensitization reduced IgE levels, airway eosinophilia, IL-4 and IL-5 release, mucus hypersecretion, and AHR. However, as with anti-IL-4 treatment the inhibitor had no effect when delivered after sensitization.

Thus, mouse models show that targeting the common IL-4R α /IL-13R α 1 may suppress eosinophilic influx, mucus hypersecretion, IgE release, and AHR but not T cell activity or airway remodeling, with the greatest effects when delivered prophylactically.

A human anti-IL-4R α mAb has been developed, which blocks IL-4 and IL-13 signaling, and has been tested as a treatment in established stable asthma [154]. Treatment suppressed asthma exacerbations, particularly in subjects with more severe asthma but did not alter stable asthma control, β -agonist use, or lung function. It is possible that increasing the length of treatment or the doses used or by the selection of specific asthma phenotypes may improve efficacy. A recombinant IL-4 variant that potently inhibits both IL-4 and IL-13 activity has been trialed in humans with atopic asthma [155]. Treatment was effective in preventing allergen challenge-induced reductions in FEV₁, and reduced spontaneous exacerbations. Most recently this IL-4R α antagonist has been shown to be particularly effective in asthmatics with specific alleles and SNPs of this receptor, providing further evidence for patient-specific therapies [155].

Thus, targeting the IL-4R α /IL-13R α 1 is beneficial in asthma, and may be more effective than antagonizing IL-4 alone, and remains a potential therapeutic approach.

2.3.2 ST2

T1/ST2, or ST2, is an IL-1 family receptor that can be membrane bound, soluble, or a variant [156]. It is selectively and specifically expressed on Th2 cells and is the IL-33 receptor. Blocking ST2 with antibodies or soluble ST2 in mouse models suppresses Th2 responses [157-159]. There have been no studies that have targeted this receptor in humans.

2.3.3 CRTH2

The mobilization Th2 cells is mediated by another receptor on these cells termed the chemoattractant receptor-homologous molecule expressed on Th2 cells receptor (CRTH2), which may also be a therapeutic target. Prostaglandin D₂ (PGD₂) is

produced in response to allergens, oxidative stress and cytokines by mast cells, macrophages, eosinophils, dendritic cells, Th2 cells, and endothelial cells [160,161]. PGD₂ signals through the D-type prostanoid receptor (DP₁), the thromboxane receptor (TX), or CRTH2 [162]. CRTH2 is expressed on eosinophils, basophils, macrophages and preferentially on Th2 lymphocytes, and mediates the migration of these cells toward PGD₂ [162]. Importantly, its activation induces the production of pro-inflammatory cytokines in Th2 cells, delays Th2 cell apoptosis, and induces the production of PGD₂ from Th2 cells [161,163,164]. Thus, CRTH2 antagonists may have potential as asthma treatments. There are three classes of CRTH2 antagonists, tetrahydroquinoline, phenylacetic acids and indole-acetic acids, and ramatroban and its analogues [165]. The latter are the most potent and work by either decreasing ligand-binding capacity or decreasing the binding affinity of PGD₂ to CRTH2 [166,167].

CRTH2^{-/-} mice have reduced IL-4 and IL-5 production, AHR, and IgE secretion in AAD [168]. In WT mice with allergic dermatitis ramatroban analogs reduce mucus cell hyperplasia and airway eosinophilia [169].

Although several Phase II clinical trials have been performed, the available data is limited. Nevertheless, oral treatment of steroid-naïve moderate asthmatics, for 28 days with a CRTH2 antagonist (OC000459) had favorable results for peak flow, circulating IgE, FEV₁, and quality of life [170].

2.4 Th2 cell signaling antagonists

Antagonizing common receptors (IL-4R α and IL-3/IL-5/GM-CSF β c receptors) may reduce the effects of IL-4, IL-13, IL-3, IL-5, and GM-CSF. However, common and pivotal downstream intracellular signaling molecules that are induced by these receptors could also be targeted and have broad effects in suppressing Th2 responses.

The induction of the common IL-4R α /IL-13R α 1 induces intracellular signaling through signal transducer and activator of transcription (STAT)6. This is a critical mediator of allergic airway responses, although STAT6-independent pathways also play important roles [13,22,171]. Specific STAT6 inhibitors (AS1517499 or inhibitory peptides) have shown promising efficacy by suppressing IL-13 responses, eosinophil influx into the airways, lung inflammation, mucus hypersecretion, and airway and bronchial smooth muscle hyper-responsiveness in mouse models of acute asthma [172,173]. As STAT6 is less commonly utilized than other pathways in the immune system, including those of the β c pathway, its inhibition is less likely to have significant side effects.

IL-3, IL-5, and GM-CSF all activate Janus Kinase (JAK) 1 and JAK2 following ligand-receptor binding leading to tyrosine phosphorylation of the β c on six critical residues. This induces the recruitment, phosphorylation and activation of signal transducer and activator of transcription STAT1 and 5 [174-176]. The STATs then form homo- or hetero-dimers, translocate to the nucleus and bind to the promoters of specific gene networks. The MAPK pathway extracellular signal regulated kinase (ERK) is also critical for the proliferation

and anti-apoptotic effects of IL-3, IL-5, and GM-CSF. Cytokine stimulation of ERK is initiated by JAK2-induced phosphorylation of a single residue on the β c chain, which eventually leads to the activation of c-Fos, c-Jun, bcl-2, and bcl-x [174-176]. Small molecule inhibitors of JAK-mediated tyrosine phosphorylation of the residues on the β c chain may hold the potential to suppress the allergic pathways driven by these three cytokines. Natural endogenous inhibitors within these pathways already exist and terminate signal transduction. These negative feedback signals include those that control the level of cytosolic phosphorylated substrates like the tyrosine phosphatase SHP1 [177], or the inhibitor of JAK2, cytokine signaling (SOCS)1 [178].

Broadly targeting either STATs, JAKs, or ERK by using synthetic inhibitors may lead to unwanted side effects as these signaling molecules play a multitude of roles in various immune responses. Nevertheless therapeutic strategies could be designed to bring aberrant responses back to normal levels rather than completely abrogating them.

2.5 Th2 cell antagonists

The development, function of Th2 cells can be regulated by Th1 cells and Tregs and the cytokines they release (IFNs and IL-12, and IL-10 and transforming growth factor- β , respectively) [2]. Thus, these antagonists of Th2 cell function may be potential asthma therapies.

2.5.1 Th1 cytokines

Th1 cells and cytokines may be utilized to reduce Th2 cell activity [2]. Treatment with IFNs (IFN- α , IFN- β , and IFN- γ) and IL-12 to suppress Th2 responses, AAD and asthma have been attempted in both mice and/or humans but have failed both due to a lack of efficacy and adverse effects [4]. Long-term IFN- α treatment of severe asthmatics did suppress Th2 responses, improved lung function, and reduced medication, symptoms, and exacerbations [179,180]. Th1 responses and IFN- γ suppress mucus production and eosinophilia in models of AAD [181] but this cytokine is also implicated in the pathogenesis of severe asthma, exacerbations, and in steroid resistance [4,8]. IL-12 treatment of mild asthmatics suppressed eosinophilic inflammation but did not suppress AHR or asthmatic responses [182]. Thus, along with their lack of efficacy, Th1 cytokines may induce inflammation and steroid resistance [4], and are not considered viable therapeutic approaches to the treatment of asthma.

2.5.2 Treg-inducers

Treatment of asthma may be better achieved by enhancing the numbers or effects of Tregs that suppress the activity of both Th1 and Th2 cells [2]. In healthy individuals, Tregs play a critical homeostatic role in modulating excessive inflammation and subsequent tissue damage. Allergic and/or asthmatic subjects have reduced numbers of Tregs and/or their Tregs have reduced suppressive capacity [183,184]. Numerous bacteria and their components, as well as TLR agonists, have been

shown in many studies to induce Tregs and this approach may be able to attenuate the features of asthma [25-28,185-188], as we have recently reviewed [2]. However, this is an emerging concept, which is not as advanced as the anti-cytokine field and developments in this treatment approach are eagerly awaited.

2.6 Small interfering oligonucleotides

Recent studies have investigated the therapeutic potential of post-transcriptional gene silencing (PTGS) to modulate the expression of specific pathogenic RNA species and interfere with disease pathogenesis. This can be achieved using synthetic single stranded anti-sense oligonucleotides (ASOs) or small interfering RNAs (siRNAs) that are complementary to their target and may be modified to prevent degradation [189-191]. The inhibition of gene transcription is initiated by the enzymatic cleavage of long, non-coding dsRNA species into siRNA that separate into single-stranded passenger and guide RNA strands. The guide strand typically binds to a region of a target messenger RNA (mRNA) with perfect complementarity and the bound product interacts with the catalytic Argonaute subunit of the RNA-induced silencing complex (RISC) leading to either enzymatic cleavage of the bound mRNA or translational inhibition.

ASOs are being developed into therapeutic strategies to modulate the effects of T cells in the control of allergic inflammation. Studies in mice have investigated the use of AIR645, which targets the IL-4R α , and showed that it suppresses allergen-induced AHR and airway inflammation in AAD [192].

Several ASOs are now in various stages of clinical development [193]. AIR645 did not have sufficient therapeutic benefit to justify further development [194]. As is the case with anti-cytokine therapy, combinatorial silencing of multiple pathogenic signaling molecules and/or pathways is likely to deliver the maximum clinical benefit. In this regard, TPI ASM8 is a combinatorial ASO that targets CCR3 and the β c-chain of IL-3/IL-5/GM-CSF receptors. In clinical studies, ASM8 reduced sputum eosinophil and total cell influx following allergen challenge, early asthmatic responses and late phase bronchoconstriction [195,196].

2.7 miRNAs

MicroRNAs (miRNAs) are short non-coding RNAs that regulate gene expression post-transcriptionally by directly blocking translation of their target mRNAs and/or by repressing protein production *via* mRNA destabilization [197,198]. The understanding of the role of miRNAs in the regulation of the immune system and inflammatory processes is only just emerging. Importantly, miRNAs have the potential to control differentiation and cell function by targeting one or more pivotal mRNA transcripts that encode critical transcriptional factors. These properties of miRNAs suggest that selective modulation of their activity may hold great promise for the treatment of human disease [199]. Recent reports

document significant therapeutic benefit of targeting miRNA in non-human primates and rodents [200-202]. Therapeutic silencing of miR-122 in primates with chronic hepatitis C virus infection induced prolonged suppression of viremia and normalization of the interferon pathway, which correlated with improved liver histology [203]. Recently we demonstrated a critical role for miRNAs in the regulation of inflammation in mouse asthma models of Th2 driven asthma [34,204,205]. To do this we employed antagomirs (chemically modified single stranded RNA analogues, complementary to the target miRNA) to induce long-lasting silencing of specific miRNA and the *in vivo* inhibition of miRNA function in the lung [34,204]. This approach demonstrated the critical importance of specific miRNAs in regulating gene expression programs that control inflammation and AHR in models of house dust mite-induced allergic eosinophilic asthma. Blocking the function of miR-126 suppressed the development of allergic inflammation by inhibiting the downstream expression of the Th2 specific transcriptional factor GATA-3. Furthermore, targeting miR-145 function was as effective as treatment with the anti-inflammatory glucocorticoid dexamethasone. These studies highlight, for the first time, the potential of miRNAs as targets for new non-steroidal anti-inflammatory treatments of asthma and other respiratory diseases.

2.8 Combination therapies

In asthma there are different endotypes, and complex and redundant regulatory mechanisms, which operate parallel pathways that underpin similar disease processes. Thus, developing new treatments for asthma is difficult. It is unlikely that presentation of any endotype will be underpinned predominantly by a single cytokine/inflammatory mediator or pathway, and thus will not be amenable to single target therapy. For example, collectively studies using models of AAD suggest that it may be necessary to block IL-4, IL-5, and IL-13 to inhibit many aspects of the disease concurrently, which could be achieved through targeting the IL-4R α /IL-13R α 1 and at the same time neutralizing IL-5 or the IL-5/IL-13/GM-CSF β c-receptor. Thus, the use of anti-IL-4R α mAb or IL-4R α antagonist in combination with anti-IL-5 mAbs and/or anti-GM-CSF would block IL-4, IL-13, IL-5, and GM-CSF responses. This could be further refined with the future development of a β c-receptor antagonist which in combination with an IL-4R α antagonist. This would mean that a combination of two agents might suppress IL-4, IL-13, IL-5, and GM-CSF responses.

IL-25, IL-33, and TSLP are often induced simultaneously and IL-33 can act synergistically with TSLP to enhance the activity of mast cells, and with IL-3 promotes Th2 cytokine release from basophils and eosinophils [206]. Therefore, the future development of agents that target these cytokines in combination, particularly during the early phases of disease, perhaps in high-risk individuals, may be an alternative approach to targeting end-effector cytokines such as IL-5 and IL-13. Thus,

other combinations of therapies that not only target Th2 cytokines but also Th2-inducing cytokines may also be effective.

Therapies that combine Th2 antagonists with those that induce Th2 suppressive responses, such as Tregs, or with anti-inflammatories such as steroids may also be trialed in the future. However, even blocking of multiple Th2 cytokines may only be appropriate for some endotypes. Severe asthma may be underpinned by infection and other cytokines released by cells such as Th1 and Th17 cells [3-10,17-20,23,105,207-211]. Therefore, again, the use of different combinations of agents may be required for different endotypes with IL-4, IL-5, and IL-13 being targets in mild to moderate asthma whereas suppressing IL-5, IL-17, and IFN- γ may be more beneficial in severe asthma [32].

Thus, it is likely that combination therapy that targets the critical mediators will be required for maximal therapeutic benefit. Nevertheless, targeting of single mediators or cells is showing efficacy in individuals where the target has demonstrable activity at the time of treatment. In addition, it must be remembered that complex therapies have their own pharmacokinetic and regulatory problems, and are unlikely to be approved in the near future due to the regulatory and safety concerns. Depleting a range of cytokines may have off-target effects and predispose to infection, which may even increase asthma severity and exacerbations, or malignancy. These possibilities could be minimized by short-term treatment that aims to normalize aberrant responses rather than completely inhibit them. Existing treatments that target the minimum number of pivotal components (e.g., IL-4R α /IL-13R α 1) may be most acceptable in the near term.

3. Patient stratification for optimal treatment

In general, most asthmatics present with similar physiological symptoms, although these vary in severity and responsiveness to steroids [9]. It has been long recognized that asthma is a heterogeneous disease with multiple phenotypes [1,4]. Recent clinical trials in moderate-to-severe asthma using various inhibitors of Th2-related factors have often been disappointing, despite showing efficacy in animal models. With multiple Th2-related targets now in clinical trials, the identification of molecular biomarkers for disease stratification is likely to be most important for optimal treatment. Identification of patient cohorts, using molecular biomarkers, who are most likely to respond to targeted therapy will be important in future design of clinical trials. The classification of patients into either Th2^{high} or Th2^{low} phenotypes based on the expression of biomarkers may be useful in predicting those most likely to respond to targeted therapy. Indeed, increased expression of the molecular biomarkers periostin, CLCA1, and SERPINB2 are robust predictors of a Th2^{high} phenotype [212]. They are likely to be useful in identifying those most likely to respond to therapies targeting Th2-related factors, such as in the trial with anti-IL-13 by Corren *et al.* [49]. More effort is required to classify asthmatics into distinct molecular phenotypes, such as Th2^{high} or Th2^{low}

and validation of new therapeutics should be performed in the relevant asthma subtypes. Such studies should then lead to the development of optimized “personalized medicine” for asthmatic patients.

4. Conclusion

In order to develop the most effective asthma therapies we must utilize the full spectrum of tools available to us. The contribution of Th2 cytokines to asthma and their targeting has been identified and elucidated in mouse models and validated in *in vitro* and *in vivo* human studies. This has led to the development of antagonists for use in human trials. Th2 antagonists show substantial promise in the treatment of asthma. Antagonists against IL-4 have limited beneficial effects and these are predominantly linked to factors associated with the inception of disease. Anti-IL-5 has been demonstrated to have substantial beneficial effects in poorly controlled and/or eosinophilic asthma and targeting IL-13 is effective in asthmatics that show this cytokine is biologically active. Nevertheless, these treatments remain to be fully explored and it has become clear that it is critical to perform trials in well-characterized patients that have demonstrable activity of the target of the therapeutic agent in the diseased state at the time of treatment. Further studies using such highly characterized patient cohorts at a cellular and molecular level are eagerly anticipated. A unified approach to inhibiting Th2 cytokine function may be achieved by targeting receptors that are utilized in common by these cytokines such as the IL-4R α /IL-13R α 1 and IL-3/IL-5/GM-CSF β c receptors. Targeting Th2 initiating factors may be beneficial in suppressing the inception of disease, and enhancing endogenous antagonists of Th2 cells, such as Tregs, may also be effective in this regard. Animal models and human studies have revealed the complexity of asthma pathogenesis and the presence of many compensatory mechanisms that regulate pathogenic pathways. Thus, combination therapies may prove to be the most beneficial approach to treatment in the future.

5. Expert opinion

Current treatments for asthma are corticosteroids and long acting β -agonists. They suppress the symptoms of disease but do not treat the causes of asthma, efficacy wanes over time, many patients are not compliant and 5 – 10% of asthmatics are refractory to this treatment approach. Th2 cytokine

antagonists show promise for treating the causes of asthma and they may eventually become the standard approach to therapy in specific sub-groups of patients. In isolation, IL-5 and IL-13 antagonists have been successfully used in specific endotypes of asthma. Their efficacy and broader applicability may be further enhanced by targeting other pivotal factors such as commonly used cytokine receptors, which control multiple cytokine signals. Indeed, targeting the IL-4R α /IL-13R α 1 that suppresses IL-4 and IL-13 signaling has been shown to be beneficial in asthma. In the future therapies must be tailored to treat specific cohorts characterized by common factors that underpin active disease at the time of treatment. Mild to moderate asthma is likely to benefit from combination therapies targeting IL-5 and IL-13 or their receptors, whereas severe asthmatics may benefit from anti-IL-5 and anti-IFN- γ therapies. The ultimate goal is to provide tailored targeted intervention for specific individuals in well-defined patient cohorts.

There is much debate over the use of mouse models to identify therapeutic targets. The data presented demonstrate that if interpreted carefully and applied to appropriate patient cohorts in well-designed clinical trials, mouse models are valuable preclinical tools for understanding disease pathogenesis and target identification.

In the future, entirely new approaches may be taken in asthma treatment that will target the fundamental causes and exacerbators of asthma. These include not only the Th2-inducing cytokines but also respiratory infections by viruses and bacteria for the induction and exacerbation of asthma [3,6,7,17,18], as we have recently reviewed [3,207,208]. For example, treatments such as macrolides that target infections but are also immune-modulatory may prove beneficial [213]. Targeting miRNAs that are shown to be important in asthma pathogenesis may also be novel approaches to prevention and treatment.

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