

Anti-interleukin Therapy in Asthma

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Asthma remains one of the most prevalent and costly diseases in the United States. Asthma accounts for a significant amount of direct medical expenditures and indirect cost from days lost at school and work. Modern understanding of its complex pathogenesis has allowed recognition of the heterogeneity of the disease across populations and the various inflammatory pathways that drive airway inflammation in asthma. Interleukins play important roles in both eosinophilic and noneosinophilic asthma, and anti-interleukin therapy will allow for a targeted, personalized approach to asthma management. With the success of anti-interleukin (IL) -4, IL-5, and IL-13 therapy in recent large trials among specific populations of asthmatics, it is likely that targeted anti-interleukin therapy will be approved for use in the near future. It will be important for clinicians and pharmacists to understand their risks, benefits, and proper indications.

OVERVIEW OF ASTHMA EPIDEMIOLOGY, CURRENT ASTHMA MANAGEMENT, AND UNMET THERAPEUTIC NEEDS

Asthma is a chronic inflammatory disorder of the airways marked by airflow limitation, bronchial hyperreactivity, and recurrent episodes of wheezing, dyspnea, and cough. The clinical spectrum of asthma ranges from infrequent, mild wheezing episodes, to severe daily symptoms and acute life-threatening episodes of respiratory failure. Asthma affects approximately 300 million people worldwide and asthma symptoms are reported in 11.6% of 6- to 7-year-old children worldwide. In the United States, almost 19 million adults and 7 million children are estimated to be diagnosed with asthma, accounting for over 14 million primary care visits and 2 million emergency room visits per year.^{1,2} The estimated cost per person exceeds \$3,300 per year and annual spending exceeds 50 billion in medical expense, lost work, and early death.² The prevalence of asthma has more than doubled over the past 3 decades in the United States.³ The prevalence rate is highest among Blacks and Puerto Ricans, who suffer a disproportionate burden of disease.⁴

Traditional management of asthma includes allergen avoidance, treatment of comorbidities, and pharmacologic therapy. Pharmacologic management is based on a stepwise approach after assessment of severity, symptom frequency and control. Formal guidelines have been published by the National Asthma Education and Prevention Program (NAEPP) as well as the Global Initiative for Asthma (GINA).^{5,6} Pharmacologic therapy includes short- and long-acting beta-agonists, inhaled and systemic corticosteroids, leukotriene modifying drugs, and anti-IgE therapy for

more severe disease. Although not formally approved by the FDA for an asthma indication, recent data have supported a beneficial role of long-acting anticholinergics in asthma.⁷ Lastly, bronchial thermoplasty has been approved by the FDA as a nonpharmacologic treatment for asthma that provides sustained reduction of asthma exacerbations through the bronchoscopic delivery of thermal energy to disrupt airway smooth muscle.⁸

Despite the widespread adoption of guideline-based approaches, approximately 10% of patients remain poorly controlled.⁹ These patients have significant asthma-related morbidity and account for a disproportionate amount of the financial burden of asthma. However, it is hoped that the recent recognition of various asthma phenotypes and endotypes, as well as our increasing understanding of asthma pathogenesis, will allow for a targeted, personalized approach to refractory asthma therapy (see **Figure 1**).

Biologic therapies provide a potential for this approach in asthma. Omalizumab is a monoclonal antibody that binds IgE and is, to date, the only biologic therapy approved for asthma. It has been approved for patients with difficult to control allergic asthma and has been shown in randomized controlled trials in children, adolescents, and adults to decrease exacerbation frequency, decrease the required dose of inhaled corticosteroid, and improve asthma-related quality of life measures.^{10,11} The cost of omalizumab, the fact that it requires frequent regular injections, and the dosing challenges for patients with high IgE levels or increased body weight have limited its use.

Nonetheless, the development of omalizumab highlights the potential to successfully target specific pathways in asthma. This is important because it is now increasingly recognized that

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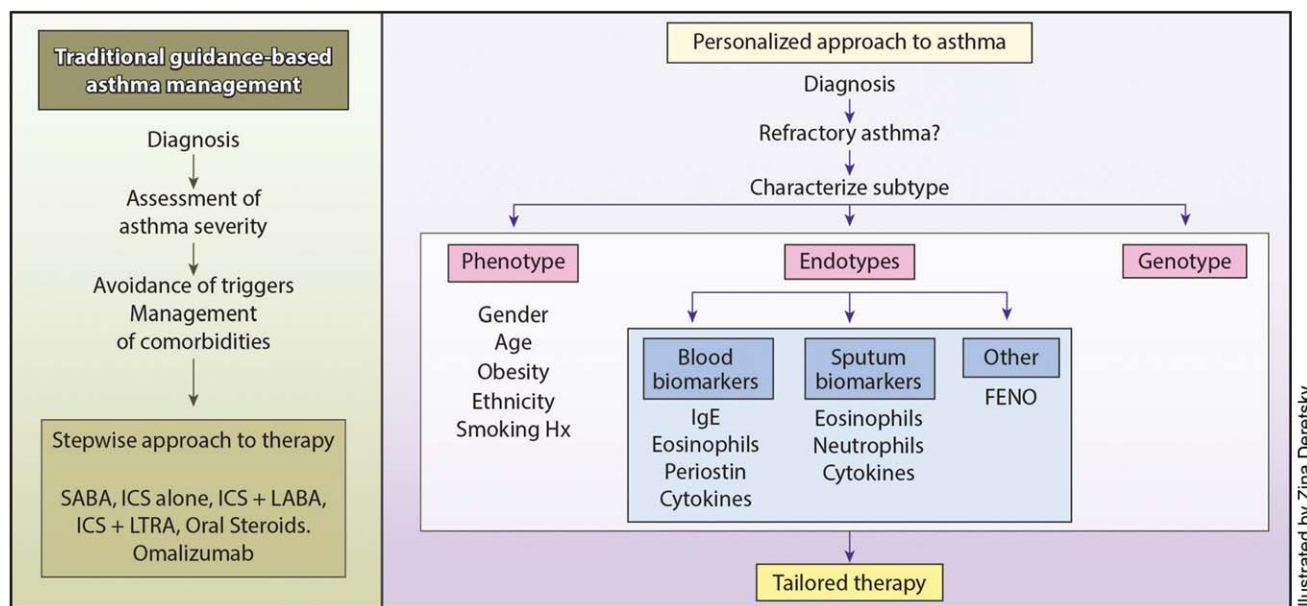


Figure 1 Traditional and personalized approach to asthma therapy. SABA, short-acting beta-agonist; ICS, inhaled corticosteroid; LABA, long-acting beta-agonist; LTRA, leukotriene receptor antagonist; FENO, fractional exhaled nitric oxide.

asthma is a markedly heterogeneous disease. Among asthmatics, several factors, such as age of onset, obesity, ethnicity, and sex, are associated with differences in the pathogenesis, clinical phenotype, and response to traditional therapy. The classic view of asthma pathogenesis as a Th2 driven (“Th2 high”) disease marked by eosinophilia and IgE-mediated inflammation is inadequate to explain the full spectrum of asthma pathogenesis. Many patients have “Th2 low” disease and are more likely to have neutrophilic (rather than eosinophilic) inflammation, are less likely to respond to inhaled corticosteroids, and may have more severe disease.¹² These nuances are likely important when considering the most effective treatment approach for a given patient. In any given patient, even when Th2-mediated airway inflammation is the primary driver of the disease, there are likely subphenotypes that may respond differentially to specific therapies. Thus far, Th2 inflammation has been the primary target of biologic asthma therapy. The majority of the Th2-specific cytokines (interleukin-4, -5, -9, and -13) have been targeted, either directly or indirectly, by recently developed biologic therapies with varying degrees of success (see **Table 1**). In addition, inhibition of other interleukins, including IL-2, IL-8, IL-17, and IL-33 are either in development, or have been targeted in early phase trials for asthma. Herein, we review these novel therapies and their potential to play an important role in asthma.

OVERVIEW OF ASTHMA PATHOGENESIS

Asthma pathogenesis is marked by variable degrees of airway inflammation, bronchial hyperreactivity, mucus hypersecretion, and airway remodeling. These pathologic changes are mediated by airway epithelial cells and several important effector immune cells including dendritic cells, B- and T-lymphocytes, eosinophils, mast cells, and innate immune cells, all of which communicate

through important signaling molecules (cytokines) relevant to this review (see **Figure 1**).

Airway epithelial cells form the barrier against mechanical stress, oxidant stress, allergens, pollutants, infectious agents, leakage of endogenous solutes and have important roles in mucociliary clearance.¹³ Epithelial cell-derived cytokines and chemokines (including IL-25, IL-33, thymic stromal lymphopoietin [TSLP]), and granulocyte-macrophage colony-stimulating factor signal immune effector cells and initiate crosstalk with dendritic cells to shape characteristic asthmatic immune response patterns to allergic and infectious stimuli.¹³

Similar to airway epithelial cells, dendritic cells are directly exposed to the external environment. The pulmonary dendritic cell serves as an antigen-presenting cell and can be recruited to the airway in response to allergens and pathogens.¹⁴ They can be directly stimulated by surface binding of allergens or infectious agents or indirectly stimulated by airway epithelial cells (by mediators including IL-25, IL-33, TSLP, and granulocyte-macrophage colony-stimulating factor).¹⁵ Dendritic cells affect T-cell differentiation, and under the proper circumstances, generate a Th2 response commonly seen in atopic asthma.¹⁶

T cells subsets play important roles in the pathogenesis of asthma. Asthma has traditionally been considered a disease of Th2 predominance (“Th2 high”), characterized by expression of Th2-specific cytokines (IL-4, IL-5, IL-9, and IL-13).¹⁷ Some asthmatics demonstrate a Th1 inflammatory signature, which can develop under the influence of IL-18 and IFN- γ , and is characterized by further production of IFN- γ .¹⁸ More recently, Th17 cells, which are CD4⁺ T cells expressing IL-17, have been recognized to play a role in a subset of asthmatics with neutrophilic inflammation.¹⁹

Due to their production of IgE, B-cells play a critical role in asthma. IL-4, -5, and -13 all play specific roles in B cell survival,

Table 1 Important clinical trials with anti-interleukins and other targeted therapy in asthma

Cytokine	Medication	Mechanism	Dose and Interval	Key Findings	Biomarker/ Phenotype	Reference
IL-2	Daclizumab	– Monoclonal antibody to alpha subunit of IL-2	Intravenous, every 2 weeks after loading dose	Initial decrease improvement in FEV1, B-agonist use and asthma symptoms but was not sustained throughout study	None	Busse <i>et al.</i> , AJRCCM, 2013
IL-4/IL-13	Dupilumab	– Monoclonal antibody to alpha subunit of IL-4 receptor (IL-4 α)	Subcutaneous, weekly	Fewer asthma exacerbations among moderate-to-severe asthmatics with sputum eosinophilia when LABA and ICS withdrawn	Th2 phenotype	Wenzel <i>et al.</i> , NEJM, 2013
IL-5	Mepolizumab Reslizumab Benralizumab	- Monoclonal antibody to IL-5 - Monoclonal antibody to alpha-chain of IL-5 receptor	Subcutaneous, or IV (reslizumab) every 4 weeks Same	In two phase III trials, mepolizumab significantly reduced exacerbation frequency, improved measures of asthma control lung function among patients on high-dose ICS and allowed for a significant reduction in oral steroid dose in steroid dependent asthmatics. *Phase III trials of reslizumab resulted in significant reductions in asthma exacerbations and improvements in asthma symptoms and lung function	Th2 phenotype	Bel <i>et al.</i> , NEJM, 2014 (mepolizumab) Ortega <i>et al.</i> , NEJM, 2014 (mepolizumab) Castro <i>et al.</i> , AJRCCM, 2011 (reslizumab) *Teva Pharmaceuticals Industries LTD (unpublished)
IL-9	MEDI-528	– Monoclonal antibody to IL-9	Subcutaneously, every 2 weeks	No significant benefit in ACQ scores, exacerbation rates, or FEV1 in patients with moderate-to-severe asthma		Oh <i>et al.</i> , Respiratory Research, 2013
IL-13	Lebrikizumab	– Monoclonal antibody to IL-17	Subcutaneous, monthly	Significant improvement in FEV1 (5.5%) among patients with elevated serum periostin	Periostin	Corren <i>et al.</i> , NEJM, 2011
IL-17	Brodalumab Secukinumab	- Monoclonal antibody against IL-17 receptor A - Monoclonal antibody to IL-17	Subcutaneously, every 2 weeks Subcutaneously every week for 4 weeks then every 4 weeks	Overall no change in asthma control though there was a small improvement in patients with high reversibility subgroup	IL-17 levels in BAL fluid	Busse <i>et al.</i> , AJRCCM, 2013 (brodalumab) ClinicalTrials.gov (NCT01478360) (Secukinumab)
TSLP	AMG 157	– Monoclonal antibody to TSLP	Intravenously, every 4 weeks	Significantly reduced late asthmatic response, FENO levels and blood/sputum eosinophils	Allergic Asthma	Gavreau <i>et al.</i> , NEJM, 2014

Table 1 Continued

Cytokine	Medication	Mechanism	Dose and Interval	Key Findings	Biomarker/ Phenotype	Reference
M1 Prime	Quilizumab	– Monoclonal antibody to M1 prime	Intravenously	Reduction in serum IgE, allergen specific IgE as well as a reduction in early and late asthmatic response	Allergic Asthma	Gavreau <i>et al.</i> Sci Trans Med, 2014

LABA, long-acting beta-agonist; ICS, inhaled corticosteroid; BAL, bronchoalveolar lavage; TSLP, thymic stromal lymphopoietin.

interaction with T cells and IgE secretion. Secreted IgE is primarily bound by means of the high-affinity Fc epsilon receptors on mast cells and basophils, and when cross-linked by aeroallergen, cause these cells to degranulate and release their inflammatory mediators.²⁰

Eosinophils are bone marrow derived granulocytes that have long been recognized as key effector cells in allergic asthma. Eosinophil differentiation in the marrow is mediated by IL-13, IL-5, and granulocyte-macrophage colony-stimulating factor,²¹ while eosinophil recruitment is mediated by IL-13, histamine, prostaglandin-2, and eotaxins (by means of the CCR3 receptor).²² In addition to releasing toxic granular proteins such as eosinophilic cationic protein and eosinophil derived neurotoxin, dozens of cytokines and chemokines are secreted by eosinophils, promoting both Th2 inflammation as well as airway epithelial damage.²³

Well recognized for their role in allergy and anaphylaxis, mast cells are another important immune cell in asthma. Maintained near mucosal surfaces by IL-9, these cells can be activated by stem cell factor binding to surface receptor c-kit, IgE crosslinking, or binding of tyrosine kinase.²⁴ Activated mast cells are an important source of histamine, cysteinyl leukotrienes, and prostaglandins (e.g., prostaglandin-2),²⁵ which are central mediators of bronchoconstriction, vasodilation, and the allergic inflammatory cascade.

More recently, a new type of effector leukocytes was described. Termed innate lymphoid cells, these cells are stimulated by IL-25 and IL-33 (seen in response to viral illness) and require the transcription factor ROR α for signaling. They have the potential to produce significant quantities of Th2 cytokines, stimulating eosinophils in the process.²⁶

It is clear that interleukins play a central role in the interaction with all of these specific cell types. They are either secreted by, or stimulate, important cells that trigger the inflammatory cascade seen in asthma. In the following sections, we review the current literature on the use of anti-interleukin therapy in asthma and discuss these promising and clinically important targets.

TARGETING CYTOKINES IN ASTHMA

Interleukin-5

Discovered in murine models in 1985,²⁷ IL-5 is hematopoietic cytokine secreted by Th2 cells and is a potent stimulator of eosinophils. It is one of the most well studied cytokines involved in asthma pathogenesis and has been an important therapeutic target in clinical asthma trials over the last 15 years. It has also been

associated with several other atopic and eosinophilic diseases.^{28–30} Unlike other Th2 cytokines (IL-4, IL-9, IL-13), IL-5 is extremely specific to eosinophils and is involved in eosinophil differentiation, maturation, migration, and prevention of apoptosis.³¹ Besides its link to eosinophils, there have been multiple observations that have suggested a role for IL-5 in asthma. IL-5 protein and mRNA have been observed to be elevated in the serum and bronchial tissue of asthmatics,³² and inhalation of IL-5 has been shown to provoke airway hyperresponsiveness and sputum eosinophilia in asthmatics.³³

With these observations in mind, several investigators have studied anti-IL-5 monoclonal antibodies as a therapy in asthma since the early 2000s. Leckie *et al.* conducted a randomized, placebo-controlled pilot trial of anti-IL-5 therapy in a group of 24 patients with mild asthma and showed that a humanized monoclonal antibody to IL-5 was safe, and resulted in a significant reduction in both circulating and sputum eosinophils but had no significant impact on airway hyperresponsiveness or on the late asthmatic response to allergen challenge.³⁴ In 2004, Kips *et al.* conducted another pilot study in a small group of 26 patients with severe persistent asthma who were randomized to treatment with anti-IL-5 therapy at escalating doses or placebo and again found a significant reduction in eosinophil count but no significant effect on clinical indices of asthma or lung function.³⁵ Given the small size of these initial trials, there was still optimism that IL-5 blockade may be efficacious if tested in a larger, higher powered trial. In 2007, a more definitive multicenter randomized controlled trial of 362 patients looked at the effect of three monthly serial infusions of mepolizumab at different doses in patients with moderate persistent asthma.³⁶ Again, as in the previous trials, mepolizumab was associated with a significant and sustained reduction in sputum and blood eosinophils with no statistical difference in clinical asthma indices or measures of lung function (peak expiratory flow or forced expiratory volume in 1 s).³⁶

The initial failure of anti-IL-5 therapy to improve clinically significant endpoints was disappointing and surprising and cast doubt upon the traditionally understood role of eosinophils in asthma. However, in 2009, two trials were published that reexamined anti-IL-5 therapy (mepolizumab) in a targeted subgroup of patients with severe asthma and persistent, eosinophilic airway inflammation.^{37,38}

Persistent eosinophilic inflammation was defined as sputum eosinophil count greater than 3% despite oral or high-dose inhaled corticosteroids. Nair and colleagues showed that in a

small group of patients ($n = 19$) who had persistent sputum eosinophilia on prednisone, 5 months of treatment with mepolizumab during a protocolized reduction in prednisone dose resulted in significantly fewer asthma exacerbations (12 vs. 1; $P = 0.002$) while allowing for significantly greater reduction in prednisone dose (47% vs. 83%; $P = 0.04$).³⁷ A randomized, double-blinded, placebo-controlled trial by Halder *et al.* compared 12 months of mepolizumab vs. placebo in 61 patients on high-dose inhaled steroids and persistent symptomatology and sputum eosinophilia. In this trial, the investigators found a significant reduction in the number of severe exacerbations (3.4 vs. 2.0; $P = 0.049$), an increase in Asthma Quality of Life Questionnaire score (0.55 vs. 0.19; $P = 0.02$) and, predictably, a reduction in blood and sputum eosinophils but no change in other symptoms, forced expiratory volume in 1 s (FEV1) or airway hyperresponsiveness.³⁸ A recent follow-up of this patient cohort was published and suggested that after cessation of mepolizumab the exacerbation rate slowly returned to that of the placebo group by 12 months with blood eosinophil counts equalizing by 6 months.³⁹ Both of these trials provided evidence that in a select group of asthma patients with eosinophilic inflammation, inhibition of IL-5 could result in clinically important benefits.

The success of anti-IL-5 therapy in both of these small studies of patients with eosinophilic airway inflammation renewed optimism in anti-IL-5 therapy and set the stage for a large, multicenter randomized controlled trial. Although only a phase II study, the Dose Ranging Efficacy And safety with Mepolizumab in severe asthma (DREAM) trial involved 81 centers in 13 different countries and evaluated the efficacy of three different doses of mepolizumab (75 mg, 250 mg, 750 mg) given at 4-week intervals for a year. Importantly, only patients with evidence of eosinophilic airway inflammation and a history of recurrent, severe asthma exacerbations in the year leading up to enrollment were included in the study. The primary outcome, rate of clinically significant asthma exacerbations, was reduced in all three groups who received mepolizumab. However, other measures of asthma control—scores on the asthma control questionnaire and the Asthma Quality of Life Questionnaire, and FEV1—were minimally affected. The overall reduction in exacerbation rate was 48%, 39%, and 52% in the 75 mg, 250 mg, and 750 mg groups, respectively, suggesting a limited benefit to higher doses. The treatment was tolerated well and there were no significant differences in side effects when compared with placebo.⁴⁰

More recently, two important clinical trials with mepolizumab were published in the *New England Journal of Medicine* and further support its efficacy in the treatment of eosinophilic asthma. In the first study, by Bel *et al.*, among patients requiring oral glucocorticoids for asthma control, treatment with subcutaneous mepolizumab at 4 week intervals over 20 weeks allowed for significant reduction in steroid dose and a reduced exacerbation rate.⁴¹ In the same issue of the Journal, Ortega *et al.* reported the results of double-blinded randomized placebo-controlled trial of mepolizumab (both IV and subcutaneous) among patients with eosinophilia requiring high-dose inhaled corticosteroid and demonstrated a significant reduction in asthma exacerbations and improvements in lung function and measures of asthma control

over 32 weeks.⁴² These new studies offer further hope that anti IL-5 therapy may soon be available for eosinophilic asthma patients

Besides mepolizumab, two other anti-IL-5 therapies have been developed, and are also being studied, reslizumab and benralizumab. In 2011, Castro and colleagues published a phase II trial that evaluated the efficacy of the novel anti-IL-5 agent, reslizumab.⁴³ In contrast to previous studies with anti-IL-5 therapy, treatment with reslizumab resulted in small but significant improvements in both asthma control scores and lung function in patients with persistent eosinophilic asthma. Notably, patients with higher baseline Asthma Control Questionnaire scores and nasal polyps seemed to benefit most from reslizumab.⁴³ Recently, the results from two positive phase III trials with reslizumab were presented at the European Respiratory Society meeting. In these similarly designed, randomized, double-blind, placebo-controlled trials, intravenous reslizumab given every 4 weeks resulted in significant reductions in asthma exacerbations (50% and 60%, respectively; $P < 0.0001$ for both) as well as improved lung function and asthma control symptoms.^{44,45} These trials, when published, will mark the first phase III publications with reslizumab demonstrating clinically significant benefits in asthma with anti-interleukin therapy.

Benralizumab is a humanized monoclonal antibody to the alpha subunit of the IL-5 receptor and has the theoretical benefit of inducing antibody-dependent cell-mediated cytotoxicity of target cells (eosinophils).⁴⁶ Benralizumab has been evaluated in two small phase I trials and has been shown to be safe and to result in significant reductions in airway, bone marrow, and peripheral blood eosinophilia.^{47,48} Larger future trials will determine if benralizumab has the same clinical benefits as mepolizumab and reslizumab.

While anti-IL-5 therapy is not yet approved for treatment of asthma, the current body of evidence suggests that it is safe and efficacious in a select group of asthma patients. In its current formulations, it must be given intravenously (reslizumab, mepolizumab) or subcutaneously (mepolizumab, benralizumab) over 4-week intervals. As the cost of this therapy will be significant, the appropriate selection of patients to ensure maximal benefit will be vital. In addition to the recently completed reslizumab and mepolizumab trials, there are multiple phase III trials ongoing that should help further define a role for this promising therapy in asthma.^{49,50}

Interleukin-13 and Interleukin-4

Interleukin-13 (IL-13) is another key cytokine involved in Th2-mediated airway inflammation and is an important therapeutic target in clinical asthma trials. IL-13 is a nonglycosylated protein that shares 30% homology with IL-4, another important mediator of allergic asthma.⁵¹ IL-13 is secreted by mast cells, basophils, eosinophils, and, most importantly, Th2 cells. It exerts its effects on many different cells including B cells, T cells, mast cells, macrophages, and epithelial cells. It has been found to be elevated in patients with both atopic and nonatopic asthma⁵² with higher levels being seen in patients with more severe asthma and those who are resistant to glucocorticoids.⁵³ In murine models of asthma, transgenic overexpression of IL-13 has been shown to

cause airway inflammation, increased mucous secretion, subepithelial fibrosis, and eotaxin production.⁵⁴ Furthermore, IL-13 has also been shown to increase airway hyperresponsiveness through augmentation of airway smooth muscle cells' response to calcium.⁵⁵

Many of IL-13's effects are mediated through binding to an IL-13 receptor- α that then forms a heterodimer with IL-4 receptor- α . This membrane bound complex has been termed the Type 2 receptor and may be formed with IL-4 or IL-13 as the signaling cytokine. The type 2 receptor has an intracellular domain that interacts with janus kinase resulting in phosphorylation of the intracellular domain which allows docking of an important transcription factor, STAT6.⁵⁶ STAT6 is subsequently phosphorylated and forms homodimers. Dimerization and phosphorylation of STAT6 allows translocation to the nucleus and results in IL-13 dependent gene expression and effects. One of the genes induced by this mechanism is the POSTN gene, which encodes periostin. Periostin is a recognized marker of IL-13 activity and a component of the subepithelial fibrosis seen in asthma.⁵⁷ Unlike IL-13, periostin may be easily measured in serum and is a potentially important clinical biomarker of this phenotype of asthma.

Lebrikizumab and tralokinumab have, to date, been the two most studied anti-IL-13 antibodies in clinical trials. They are both humanized monoclonal IgG4 antibodies to IL-13 and potent inhibitors of its function.

The largest trial of anti-IL-13 therapy was published by Corren *et al.* in 2011 and evaluated the efficacy of lebrikizumab among 219 patients with moderate to severe asthma not controlled on inhaled glucocorticoids. The primary and secondary endpoints for this study were FEV1 improvement at week 12 and exacerbation rates through week 24, respectively.⁵⁸ The investigators prespecified subgroups based on Th2 status and periostin levels. In this study, the treatment arm had a significant improvement in lung function with an increase in FEV1 by 5.5% ($P = 0.02$). The majority of the improvement, however, was seen in the subgroup with elevated periostin levels at baseline (increase in FEV1 by 8.2%; $P = 0.03$). While there was a trend toward a reduction in exacerbation over the treatment period (61% in high periostin group), it did not reach statistical significance ($P = 0.05$). In contrast to other studies of biologic therapy in asthma, this study demonstrated that in an easily identifiable subgroup (high periostin) of asthma patients, lung function could be improved. Unfortunately, two more recent trials in mild asthmatics did not yield such promising results. In a phase II trial by Noonan *et al.*, 212 patients with mild asthma were randomized to placebo or three different doses of lebrikizumab. There was no improvement in the primary endpoint, prebronchodilatory FEV1, but there was reduced risk of treatment failures among all patients irrespective of periostin levels.⁵⁹ In a smaller study by Scheerens *et al.*, the investigators evaluated the effects of 12 weeks of lebrikizumab compared with placebo in a small group ($n = 29$) of mild asthmatics. There was no statistically significant difference in their primary endpoint, late asthmatic response to allergen in this patient population.⁶⁰ The failure of these last two trials suggests that mild asthmatics or those who are controlled on inhaled corticosteroids are less likely to derive significant benefit from lebrikizumab.

In 2013, Piper *et al.* published results of a phase II trial evaluating the efficacy of tralokinumab in 194 patients with moderate to severe asthma randomized to either one of three treatment arms with escalating doses of medication (150 mg, 300 mg, 600 mg) at 2-week intervals or placebo. Unfortunately, there was no difference in their primary endpoint, change in Asthma Control Questionnaire score; however, there was a statistically significant reduction in β_2 -agonist use and a statistically significant increase in FEV1 in the patients who received the highest dose of tralokinumab.⁶¹ There was an apparent benefit in the subgroup with elevated IL-13 levels in their sputum. This trial suggested that tralokinumab, like lebrikizumab, may have beneficial effects on lung function in select patients at proper dosing.

Similar to IL-13, IL-4 has been shown to play an important role in Th2-mediated asthma through related molecular mechanisms. As with IL-13 and IL-5, IL-4 has been shown to be elevated in the serum and bronchoalveolar lavage fluid in patients with asthma and, in nebulized form, has been shown to induce airway hyperresponsiveness in asthmatics. Polymorphisms in the IL-4 gene and IL-4 receptor have also been associated with atopic asthma and elevated IgE levels.⁶² In murine models, IL-4 deficient mice have been shown as far back as the early 1990s to have a reduction in airway inflammation in response to allergens.⁶³ In addition to the already discussed overlapping effects of IL-13, other key processes IL-4 is believed to be responsible for include up regulation of low and high affinity IgE receptors, induction of vascular cell adhesion molecule-1 (VCAM-1) which directs eosinophils to sites of inflammation,⁶⁴ and stimulation of undifferentiated T cells toward a Th2 response.

As IL-4 binds IL-4R α /IL-13R α (Type 2 receptor) and signals through a Janus Kinase/Stat6 pathway, it is not surprising that many of the effects of IL-4 are similar to IL-13 (airway inflammation, mucous production, IgE secretion). However, IL-4 may also bind two homodimers of IL-4R α and form what is termed the Type 1 receptor. IL-13 is unable to do this. This type of IL-4 signaling through type 1 receptors result in activation of GATA3, an important transcription factor that leads to Th2 differentiation and proliferation and Th1 inhibition.⁶⁵ There are almost certainly other IL-4 specific effects as a result of type 1 receptor formation important in asthma that are not yet fully elucidated.

Inhibition of IL-4 activity was an initial attractive target for drug trials. There have been multiple different formulations directed at specifically inhibiting IL-4 including soluble IL-4 receptor to bind free IL-4, anti-sense RNA to decrease IL-4 expression, and monoclonal antibodies to IL-4 (e.g., pascolizumab).

Soluble IL-4 receptors (sIL-4r) was initially promising in murine models and a phase I human study in 25 patients with moderate asthma showed that IL-4R group had a reduction in beta-2 agonist use with a preservation of FEV1, forced expiratory flow and asthma symptom scores when compared with placebo.⁶⁶ This led to a larger, phase II trial published in 2001 in which patients with moderate asthma underwent discontinuation of an inhaled corticosteroid and were followed over a 12-week period. The treatment arms received once weekly nebulizations of soluble IL-4 receptor at various doses. Among patients who received the high dose they had a significantly reduced decline in their FEV1

when compared with the placebo. There was no statistically significant change in asthma symptom score though there was a trend that favored the treatment arm.⁶⁷

Pascolizumab is a humanized monoclonal antibody to IL-4 and has been evaluated in murine models and phase I and II trials. A preclinical study by Hart *et al.* demonstrated that pascolizumab, when exposed to human cell lines *in vitro*, was able to decrease IgE production, IL-5 synthesis and proliferation of T and B cells.⁶⁸ A subsequent phase II trial was terminated early for lack of efficacy.⁶⁹

While both IL-4 and IL-13 clearly play an important role in allergic asthma, some of the lack of efficacy in therapies targeted at either one of these interleukins individually may be explained by the duplicative roles they share. With this in mind, therapies that inhibit both pathways have been developed. Agents with this ability include a recombinant form of IL-4 (pitakinra, Aero-vance) and a monoclonal antibody to IL-4 receptor- α (AMG 317 and dupilumab). Both of these mechanistic approaches have been efficacious in specific subsets of asthma patients.

Pitakinra is a recombinant mutated form of human IL-4 that has high affinity for IL-4 receptor- α and thus acts as a competitive antagonist inhibiting the downstream effects of both IL-13 and IL-4. Initial trials in animal models showed that it was capable of inhibiting Th2 cytokine production, eosinophilic inflammation, goblet cell hyperplasia, and airway hyperresponsiveness.⁷⁰ In phase II trials, pitakinra has been shown to attenuate the decline in FEV1 in response to allergen challenge among mild atopic asthmatics with the inhaled formulation seemingly superior to the subcutaneous route.⁷¹ A phase IIb study of moderate to severe asthmatics demonstrated a reduction of exacerbation frequency among subgroups of patients with eosinophilic asthma, elevated exhaled nitric oxide, and a specific IL-4 receptor polymorphisms during withdrawal of inhaled corticosteroids.⁷²

AMG 317 and dupilumab are humanized monoclonal antibodies to IL-4 receptor alpha and thus inhibit both IL-4 and IL-13 signaling. In a phase I trial, AMG 317 was shown to be safe and reduce total IgE levels. A phase II trial failed to demonstrate overall clinical efficacy but did result in a significant improvement in Asthma Control Questionnaire symptom scores among patients with moderate to severe asthma.⁷³

The most promising results of dual inhibition of IL-4 and IL-13 came in a study done by Wenzel *et al.* using dupilumab in 104 patients with moderate to severe eosinophilic asthma who were uncontrolled on inhaled corticosteroids and long-acting beta-agonist. In this multicenter, randomized placebo-controlled phase II trial, four weekly injections of the study drug during discontinuation of long-acting beta-agonist followed by a scheduled inhaled glucocorticoid taper resulted in a significant reduction in asthma exacerbations (6% vs. 44%; $P \leq 0.001$), improvements in lung function, and measures of asthma control. In this 12-week study, dupilumab also resulted in a significant reduction in markers of Th2 inflammation (exhaled nitric oxide, IgE, eotaxin-3, thymus and activation regulated chemokine [TARC]).⁷⁴ While practicing clinicians are unlikely to discontinue long-acting beta-agonist and inhaled glucocorticoids to induce exacerbations as was done in this study, this proof of concept study suggested a

potential role for dupilumab in a specific subset of patients with poorly controlled eosinophilic asthma. Ongoing and future trials of dupilumab will likely better define its role in asthma therapy.⁷⁵

In summary, it is clear that IL-4 and IL-13 play key roles in the pathogenesis of asthma in a significant portion of the patients. Key phase II trials with lebrikizumab and dupilumab have yielded promising results with significant benefits to carefully selected patients. However, at this time it is not yet clear how these results will impact real-world clinical medicine.

Interleukin-17

In contrast to the traditional Th1/Th2 dichotomized theory of asthma, Th17 cells have, more recently, been recognized to play a role in eosinophilic and neutrophilic airway inflammation in asthmatics. Interleukin-17 is secreted by Th17 cells and consists of a family of several closely related cytokines including IL-17A-F. IL-17E is also known as IL-25. IL-17A and IL-17F are proinflammatory cytokines that are elevated in the airways of asthmatics and correlate with airway hyperresponsiveness to methacholine.⁷⁶ *In vitro*, IL-17 acts upon bronchial epithelial cells and causes increased release of CXCL8, a potent neutrophil chemoattractant.⁷⁷ IL-17 levels in the airways of asthmatics have been shown to correlate with sputum neutrophil counts and asthma severity.⁷⁸ In mice, IL-17 has also been shown to mediate neutrophilic airway inflammation resistant to corticosteroids.⁷⁹

The largest study of anti-IL-17 therapy in human subjects was completed using brodalumab. Brodalumab is a human anti-IL-17RA monoclonal antibody that blocks the biological activity of IL-17, IL-17E, and IL-17F and was initially designed and studied in a variety of autoimmune disease including psoriasis.⁸⁰ In 2013, Busse *et al.* published the results of a randomized, double-blinded, placebo-controlled trial of brodalumab at three different doses in 302 subjects with moderate-to-severe asthma inadequately controlled on inhaled corticosteroids. In this trial, there was no significant difference in the primary endpoint, change in Asthma Control Questionnaire scores, or any of the secondary endpoints. There was small improvement in Asthma Control Questionnaire scores noted in a prespecified subgroup of patients with a postbronchodilator FEV1 improvement of greater than 20% but the significance of this was unclear.⁸¹

Secukinumab is a monoclonal antibody to IL-17A with proven efficacy in psoriasis is also being tested in an ongoing phase II trial among poorly controlled asthmatics.⁸²

IL-2

IL-2 has been shown to play an important role in regulatory T-cell growth, survival, and function as well as being an eosinophil chemoattractant.^{83,84} It has diverse immunomodulatory effects and has been studied as a therapeutic in the treatment of melanoma and renal cell carcinoma. Its role in the pathogenesis of asthma is suggested by a study by Park *et al.* in 1994 that showed that IL-2 and soluble IL-2 receptor were significantly elevated in the bronchoalveolar lavage (BAL) fluid from 25 patients with symptomatic asthma. The concentrations of IL-2 in BAL fluid

correlated directly with BAL eosinophil percentage and had an inverse correlation with FEV1.⁸⁵

Daclizumab is a humanized monoclonal antibody directed toward the alphaCD25 subunit of the high affinity IL-2 receptor and inhibits the biologic function to IL-2. Daclizumab has been approved for the prevention of organ rejection among transplant recipients. Given its relative safety in renal transplant recipients and the likely importance of IL-2 in the pathogenesis of asthma, daclizumab was evaluated in a randomized, double-blinded, placebo-controlled study in 115 patients with moderate-to-severe asthma.⁸⁶ After the initial phase of the study, the daclizumab group demonstrated a modest but significant improvement in FEV1 (4.4 vs. 1.5; $P = 0.05$), daytime asthma symptom score reduction and a decrease in beta-2 agonist use. However, during the second phase of this trial, when inhaled corticosteroids were tapered, the beneficial effects of daclizumab disappeared with no significant difference in exacerbations, FEV1 or other measures of asthma control evident. No further trials have been performed with daclizumab in asthma.

IL-9

Initially discovered in the 1980s, interleukin-9 is a pleiotropic cytokine secreted by T lymphocytes with a variety of effects on inflammation and immunity. It has been most commonly associated with a Th2 phenotype of allergic inflammation. Overexpression of IL-9 in mice lung is associated with the development of a typical asthma phenotype marked by eosinophilic infiltration, mast cell hyperplasia, epithelial cell hypertrophy, and mucous production.⁸⁷ Similarly, IL-9, IL-9 mRNA, and IL-9R have been found to be elevated in the lungs of asthmatics when compared with nonasthmatic controls.⁸⁸

MEDI-528 (Developed by MedImmune under license from Generea) is an antibody to IL-9, which has been studied in early phase trials. Unfortunately, the largest trial to date, a multicenter, 24 week, large phase IIb study in 329 subjects with moderate-to-severe persistent asthma failed to demonstrate a improvement in the primary endpoint, Asthma Control Questionnaire-6 score, or any other relevant asthma outcomes.⁸⁹

IL-8, IL-25, IL-33, and Other Important Targets in Asthma

IL-8 is an important cytokine in neutrophilic airway inflammation. It acts as a chemoattractant for neutrophils and there is some evidence that it may trigger calcium release, contraction and migration of airway smooth muscle cells.⁹⁰ IL-8 has been found to be elevated in the BAL fluid in asthmatic patients and correlates with myeloperoxidase activity (an enzyme expressed in neutrophils) but not sputum eosinophilia.⁹¹ There are significant concerns with IL-8 modulation and a potential decrease in host immune response to infection; however, early phase trials have not shown an increased risk of pneumonia.^{92,93} In 2012, Nair *et al.* published the results of a small, double-blinded, placebo-controlled trial of 34 patients with severe neutrophilic asthma which showed a significant reduction in mild exacerbations and a 14% reduction in sputum neutrophils but no other differences in relevant clinical endpoints.⁹³

IL-25 is also known as IL-17E and is likely an important mediator of allergic airway inflammation. IL-25 induces gene

expression of IL-4 and IL-13. Similar to other relevant cytokines, its role in asthma has been most suggested by evidence in murine models where blocking its activity leads to a decrease in airway eosinophilia, goblet cell hyperplasia, serum IgE secretion, and airway hyperresponsiveness.⁹⁴ To date, no specific inhibitors of IL-25 have been studied in humans.

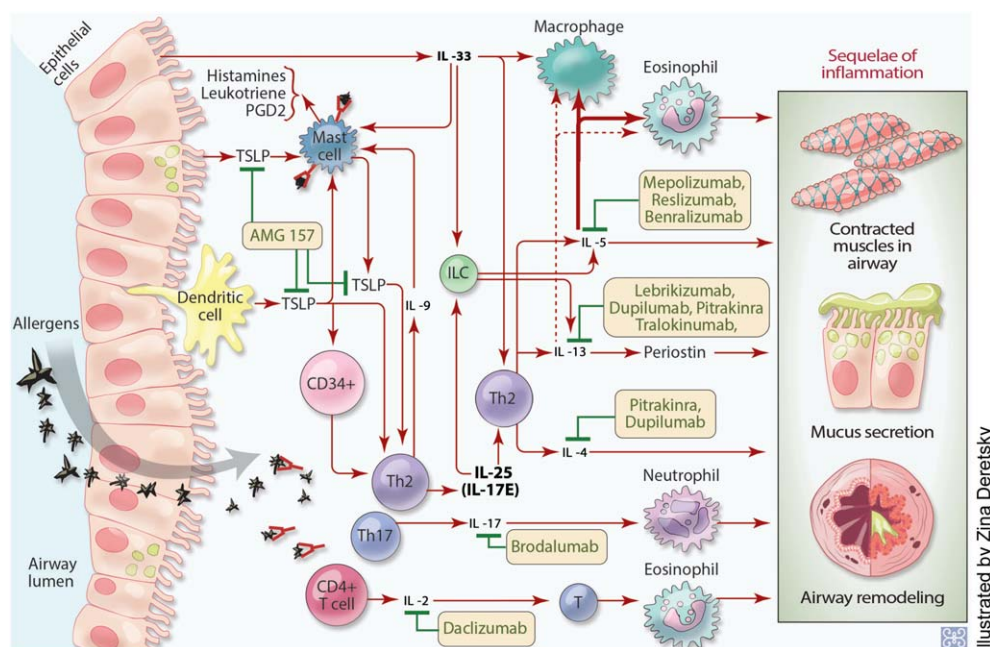
A member of the IL-1 family of cytokines, IL-33 is proinflammatory and induces T cells, basophils, eosinophils, and mast cells to secrete Th2 cytokines. IL-33 acts through the ST2 receptor that leads to NF- κ B and MAP kinase pathway activation. In murine models, antibodies to ST2 leads to reduced mucous production, Th2 cytokine production, and airway inflammation.⁹⁵ Increased expression of IL-33 has been found in airway epithelial cells and in BAL fluid samples from asthmatic patients.⁹⁶ While inhibition of IL-33, either directly or by means of blocking of the ST2 receptor, remains an attractive option for asthma, no human studies have been performed. Anti-IL-33 therapy is currently under development by the pharmaceutical company AnaptysBio.

More recently, two important trials have been published on antibodies to the novel targets TSLP and M1 prime in mild allergic asthma. TSLP is an epithelial-cell-derived cytokine that acts upon several innate immune cells including dendritic cells, mast cells, and CD34+ progenitor cells.⁹⁷ TSLP overexpression in murine lung epithelium has been associated with eosinophilic infiltration, airway inflammation, increased levels of IgE, and hyperresponsiveness to methacholine,⁹⁸ suggesting that inhibition of TSLP may attenuate the asthmatic response. AMG 157 is a humanized monoclonal antibody that binds directly to TSLP. Gauvreau *et al.* showed that in 31 patients with mild atopic asthma (confirmed by a positive skin-prick test), three monthly infusions of AMG 157 resulted in a significant decrease in the late allergic response to allergen and a reduction in fractional exhaled nitric oxide levels and blood and sputum eosinophils when compared with placebo. FEV1 was not affected though there was a significant increase in the methacholine PC20 among the group given AMG 157.⁹⁷

Based upon the recognized role of IgE in asthma and the success of anti-IgE antibody therapy, quilizumab, a humanized monoclonal antibody to the M1 prime segment of membrane bound IgE was developed to decrease IgE production by B cells.⁹⁹ Gauvreau *et al.* published the results of a phase II trial that evaluated the efficacy and safety of quilizumab in patients with mild allergic asthma. They found that quilizumab reduced serum total and allergen-specific IgE levels, decreased early and late asthmatic responses by 26% and 36%, and decreased allergen induced sputum eosinophilia. The reduction in serum IgE levels was sustained at 6 months posttreatment.⁹⁹

In contrast to these early phase positive trials targeting M1 protein and TSLP in allergic asthma, a recently developed toll-like receptor-9 (TLR-9) agonist, Qb10, was recently found to be unhelpful in allergic asthma after initially promising results.¹⁰⁰

In conclusion, as more is discovered about the pathogenesis and heterogeneity of asthma, anti-interleukin therapy will likely play an important role in the future of modern asthma management. It is clear that in specific subsets of asthma patients, anti-interleukin therapy is effective and improves both clinical indices



Illustrated by Zina Deretsky

Figure 2 Inflammatory pathways in asthma. Inhaled allergens bind initially to IgE which bind to Fc receptors on mast cells causing the release of mediators of bronchoconstriction (histamine, cysteinyl leukotrienes, and prostaglandin D2 [PGD]). Epithelial cell derived cytokines include IL-33 and thymic stromal lymphopoietin (TSLP) which act upon mast cells dendritic cells and undifferentiated Cd34+ progenitor cells to promote a Th2 response. In allergic asthma, Th2 cells secrete cytokines including IL-4, IL-5, IL-9, and IL-13 play important roles in allergic asthma including: eosinophil recruitment, macrophage activation, IgE production, mucous hypersecretion, and airway hyperresponsiveness. IL-25 and IL-33 stimulate the recently discovered innate lymphoid cells (ILC) causing further secretion of IL-5 and IL-13 contributing to Th2-mediated inflammation. Th17 cells secrete IL-17, an important mediator of airway inflammation that has been associated with sputum neutrophilia and steroid resistant asthma. Interleukin-2 plays an important role in regulatory T cell function and also serves as eosinophil chemoattractant.

of asthma control and lung function. The logistics of selecting this subset of asthmatics may prove challenging at the moment. In aggregate, the trials of anti-interleukin therapies have suggested that these therapies are unlikely to be beneficial unless prescribed in a thoughtful and tailored way. The traditional categorizations of intermittent, mild, moderate, and severe, while important, are, by themselves inadequate to identify those who will benefit. A successful targeted approach will require further characterization of a subphenotype or endotype based on clinical characteristics, inflammatory cell type, serum biomarkers, and potentially pharmacogenomics. Unfortunately, biomarkers such as periostin, exhaled nitric oxide, sputum eosinophils, and sputum IL-13, while useful in clinical trials are not yet universally available to practicing clinicians or routinely used. Furthermore, if more than one anti-interleukin therapy gets approved for asthma, the clinician will have to determine which is best for their specific patient without the benefit of head to head trials. Given their likely significant cost, these therapies will likely, at least initially, be reserved for the most severe asthmatics that remain uncontrolled on traditional therapy. As a disproportionate amount of poorly controlled asthmatics are either uninsured or underinsured, the widespread use of these medications could lead to a significant financial burden on either the healthcare system or individual payers. To reduce costs and maximize likelihood of a benefit, it will be important to identify improved selection methods to find those patients whom would derive the most benefit.

In summary, the pathogenesis of asthma is heterogeneous and may be marked by eosinophilic, neutrophilic, or mixed airway

inflammation. Specific interleukins play a significant role in all types of asthma including both the “Th2 high” and “Th2 low” phenotypes. Anti-interleukin therapy, including anti-IL-5, -IL-4, and IL-13 therapy, have shown the most promise in randomized, placebo-controlled trials with improvements in exacerbation frequency, lung function, and other measures of asthma control. It is likely that anti-interleukin and other targeted therapy will be approved for specific types of asthma in the future and it will be important for clinicians and pharmacists to understand their indications, benefits, and risks.

CONFLICT OF INTEREST

R. M. D. has no conflicts of interest. M. E. W. has received consulting honoraria from Sanofi, Regeneron, Novartis, Boehringer-Ingelheim, Medimmune, Cytos, Teva Pharmaceutical Industries, and GlaxoSmithKline.

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