

Immunomodulators in the treatment of asthma

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ABSTRACT

Asthma represents a syndrome of airway inflammatory diseases with a complex pathology. The immunologic pathogenesis is being increasingly revealed and provides opportunity for targeted biological intervention. This study was designed to describe current experience with immunomodulators as targeted therapy in asthma. A literature review was performed. Targeted therapies have included strategies to activate dendritic cells through the Toll-like receptor (TLR) 9 receptors, to interrupt the action of T_H2 cytokines with cytokine blockers and monoclonal antibodies, to promote development of T_H1 responses, to interrupt mast cell signaling, to block IgE mediated pathways, and to block TNF- α . Omalizumab is the only biological therapy that has an approved indication in asthma at this time. Improved understanding of the heterogeneity of asthma should allow for specific targeting of different disease phenotype-specific therapies including immunomodulators.

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Key words: Airway inflammation, asthma, cytokine blockers, dendritic cell, IgE, immunomodulators, monoclonal antibodies, omalizumab, T_H1 cells, T_H2 cells

The pathological changes in the asthmatic airway are multiple and complex. The airway lining epithelium exhibits sloughing/denudation, ciliary dysfunction, goblet cell hyperplasia, and mucous gland hypertrophy. The mucus produced by the asthmatic epithelium exhibits increased elasticity, increased viscosity, increased content of mucin, cellular debris, and surfactant dysfunction. There is subepithelial fibrosis. The submucosal tissues also show a significant inflammatory cellular infiltrate characterized by lymphocytes, eosinophils, neutrophils, increased numbers of mast cells, and new blood vessel formation (angiogenesis). The airway smooth muscle layer shows increased mass, consisting of both hypertrophy and hyperplasia, and isolated asthmatic airway smooth muscle cells have been shown *in vitro* to exhibit increased contractility. The smooth muscle layer is also characterized by mast cell infiltration and angiogenesis. In a majority of patients, these types of pathological changes are seen during active

disease but largely reverse during periods of disease control. In some patients failure to resolve or repair these changes is observed, with many of the aforementioned changes becoming fixed, in a process referred to as airway remodeling.^{1–3}

The immune responses underpinning the pathological changes seen in the asthmatic airway are also complex and involve both the innate and the adaptive immune systems. Among the processes involved are epithelial cell disruption and activation with cytokine production, dendritic cell activation and antigen presentation, activation and expansion of T_H2 cells with cytokine secretion, activation of resident inflammatory cells including mast cells, and recruitment from the bloodstream of other inflammatory cells including lymphocytes, eosinophils, and neutrophils, which also release mediators and cytokines.^{2,3}

The airway inflammatory response in asthma can be conceptualized as beginning in the airway lumen (Fig. 1). Here, allergens, viruses, or other pathogens interact with airway epithelial cells and dendritic cells. Some aeroallergens have enzymic properties capable of directly cleaving tight junctions between epithelial cells and/or activating the epithelial cells through protease-activated receptors. The activated epithelium and other tissue cells release factors including cytokines and chemokines that can prime or otherwise influence the response of the dendritic cell⁴ and cells of the innate (basophils, eosinophils, and mast cells) and of the adaptive immune systems (B and T lymphocytes).⁵ Both airway epithelial cells

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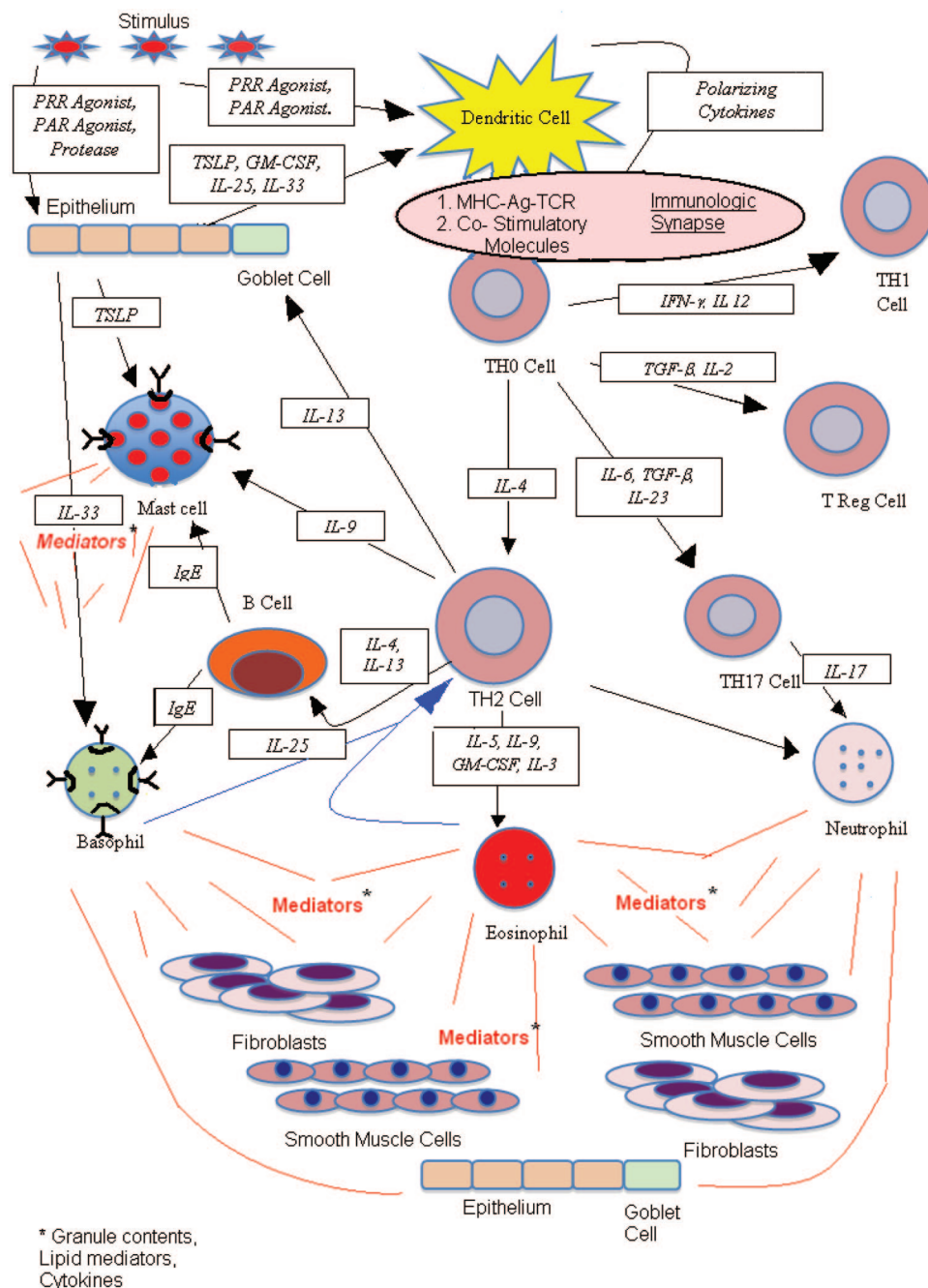


Figure 1. Schematic depiction of the components of the airway allergic inflammatory response. PRR, pattern recognition receptor; PAR, protease activated receptor; TSLP, thymic stromal lymphopoietin; GM-CSF, granulocyte-macrophage colony stimulating factor. Arrows indicate the direction in which the identified signal is acting. Red lines indicate the discharge of cellular mediators (including granular contents, lipid mediator, and cytokines).

and dendritic cells carry surface pattern recognition receptors for pathogens including Toll-like receptors (TLRs), nucleotide-binding oligomerization domain-like receptors, and retinoic inducible gene receptors, which recognize surface characteristics of pathogens and, on ligation, initiate responses of the innate immune system. In addition to pattern recognition receptors, antigen uptake by the dendritic cell may occur through either nonspecific mechanisms includ-

ing macropinocytosis or through a highly antigen-specific mechanism using immunoglobulin Fc receptors. For example, in sensitized individuals, expression of the high-affinity receptor for IgE (FcεR1) on airway dendritic cells probably strongly facilitates the processing of specific allergen, captured by bound IgE.⁶⁻⁸ The dendritic cell internalizes and processes antigen into fragments, which are then cognately presented in the context of MHC

molecules to naive (T_H0) CD4 lymphocytes. Factors associated with the antigen, the presence of TLR ligands, and factors from the activated epithelium can influence the dendritic cell activation status.⁵ The peptide presentation by the dendritic cell, in conjunction with patterns of costimulatory ligand-receptor interactions between the dendritic cell and the lymphocyte (the immunologic synapse) and cytokines released by the activated dendritic cell, together direct the subsequent differentiation of the antigen-specific T_H0 cell. The signals that the T_H0 cell receives lead it to differentiate along one of several possible lineages including T_H1 , T_H2 , T_H17 , and T-regulatory cells. The presence of IL-4 appears to favor differentiation of T_H0 cells into T_H2 -type cells and has an inhibitory effect on the development of a T_H1 response. IL-12 and IFN- γ have a negative developmental effect on T_H2 phenotype cells, while preferentially driving the differentiation of T_H0 cells toward a T_H1 phenotype. Factors favoring the development of T_H17 cells indicate a primary role for IL-6 with subsidiary roles for TGF- β and IL-23. Factors favoring the development of regulatory T cells continue to be elucidated.

IMMUNOMODULATION IN ALLERGIC ASTHMA

The antigen-presenting cell, or dendritic cell, plays a key role in initiating and propagating the allergic inflammation and much of the abnormal pathology can be linked to cytokine products of activated proliferating T_H2 cells, basophils, and mast cells. Knowledge of these processes has exposed many potential targets for immune modulation as described later. Strategies have included approaches aimed at skewing the dendritic cell toward promotion of T_H1 responses, anti- T_H2 or pro T_H1 cytokine-based biological therapies, or targeting of specific effector cells such as mast cells. There is increasing evidence for the role of cytokines derived from resident tissue cells in amplifying T_H2 -type allergic inflammation. Therapeutic agents aimed at interrupting such antigen-independent pathways will undoubtedly be soon explored. Anticytokine therapies might theoretically include (a) receptor-specific nonactivating/blocking antibodies, which prevent cytokine binding; (b) soluble receptors, which would competitively inhibit binding of cytokine to cell surface cytokine receptors; or (c) other compounds, such as mutant ligands, designed to block the cytokine receptor (Fig. 2).

DENDRITIC CELL-BASED THERAPIES

TLR-9 Agonists

The dendritic cell has been described as a sophisticated information transfer system linking the environ-

ment with the adaptive immune system.⁹ As described previously, the dendritic cell interacts with antigens through a number of important mechanisms among which are pattern recognition receptors including TLRs. A large number of animal studies have shown activation of TLR-9 through the use of CpG-rich unmethylated DNA segments commonly found in bacteria. These are also known as immunostimulatory DNA sequences and can result in skewing of immune responses toward T_H1 .¹⁰⁻¹³ Human studies have now been undertaken with TLR-9 agonist immunostimulatory DNA sequences covalently linked to allergen, delivered as a series of subcutaneous injections, with a goal of ameliorating allergic inflammatory disease. Creticos *et al.* reported a randomized, double-blind, placebo-controlled phase II trial of ragweed Amb-a 1, a major ragweed allergen, bound to a TLR-9 agonist (6 weekly injections versus placebo) in the treatment of ragweed-associated seasonal allergic rhinitis. They observed a diminution in peak seasonal symptoms of allergic rhinoconjunctivitis in the year in which treatment was administered and during the following year's ragweed season as well as reduction in the need for allergy medications.¹⁴ An accompanying report looking at nasal biopsy specimens of these patients showed increased amounts of IFN- γ , suggesting T_H1 -skewed immune responses, but there was no decrease in the amount of IL-4 or eosinophils in the nasal biopsy specimens.¹⁵ A separate study reported a diminished *ex vivo* ragweed-specific T_H2 response and a transient T_H1 biasing effect of this therapy in patients with ragweed allergic rhinitis.¹⁶ The initial proof of a concept clinical trial was followed by a larger multicenter randomized controlled clinical trial. Improvement was observed in the treatment groups, again over 2 years, but a higher treatment dose group apparently showed no incremental benefit over those receiving a lower dose equivalent to what had been studied in the first trial. Enthusiasm has waned somewhat for this single-allergen immunotherapy approach but it is believed that additional studies are being designed and other studies are ongoing to investigate therapy of allergic asthma by TLR-9 agonists alone or conjugated to allergen.¹³

The promotion of T_H2 -type allergic inflammation by cytokines released from resident tissue cells such as epithelial cells is an area of active investigation. Thymic stromal lymphopoietin, derived largely from activated epithelial cells, influences the expression of costimulatory molecules on dendritic cells, particularly OX-40 ligand. This represents a T_H2 polarizing signal that preferentially drives naive T_H0 CD4 T cells toward an allergic inflammatory T_H2 phenotype.^{17,18} Thymic stromal lymphopoietin can also cause immature cord blood-derived mast cells to secrete increased amounts of the T_H2 cytokines IL-5 and IL-13 and certain chemokines, as well as show an enhanced rate of mast cell

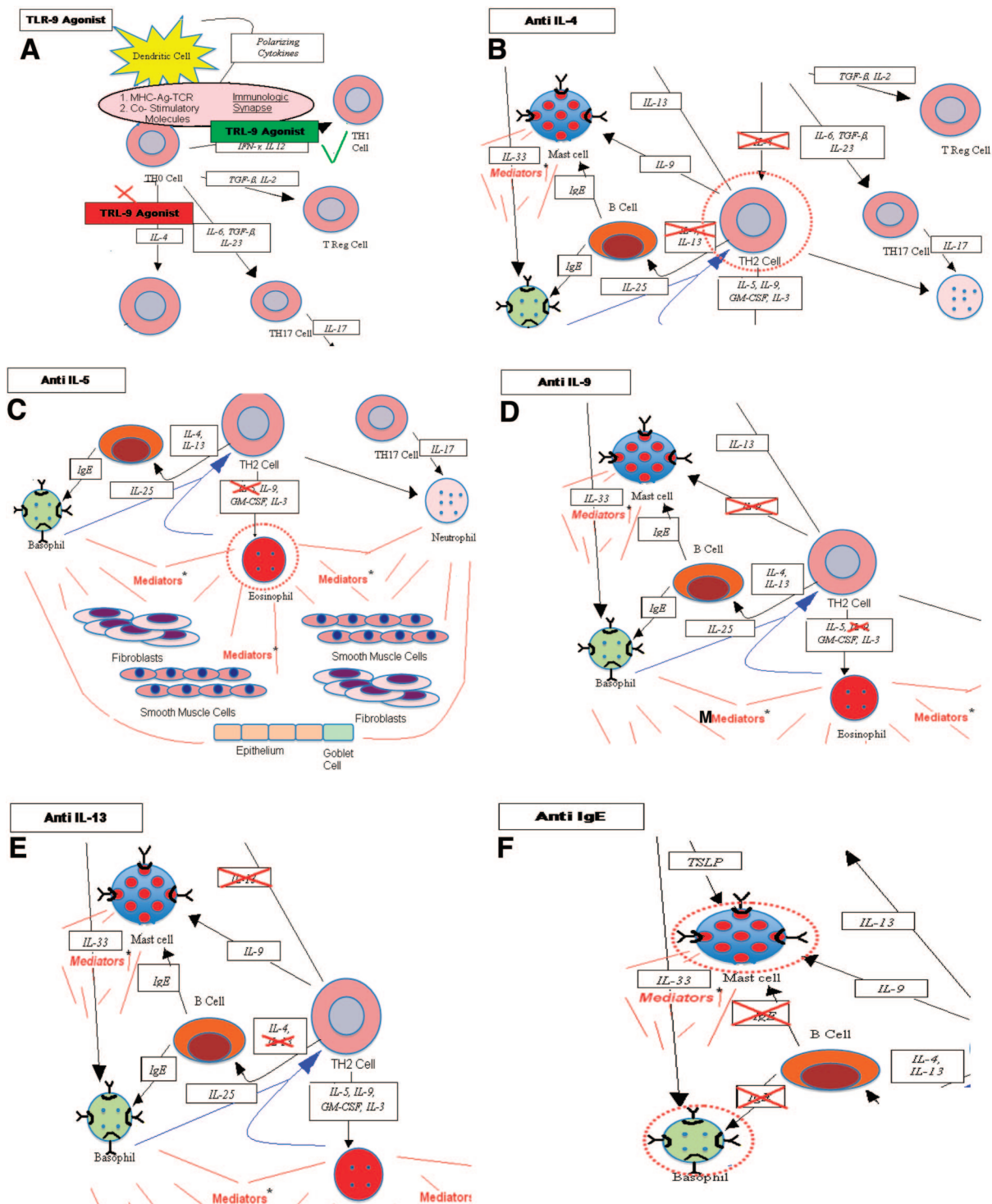


Figure 2. (A) Toll-like receptor (TLR) 9 agonists act on dendritic cells in conjunction with antigen and result in a preferential activation of antigen-specific T_H12 cells (green line) and inhibition of development of antigen-specific T_H2 cells (red X). (B) Anti-IL-4 agents block the development of T_H2 cells, whether the source of the IL-4 is from activated basophils or eosinophils or from the stimulated T_H0 cells. This, in turn, has the potential to diminish production of all T_H2 products—indicated by a broken red circle surrounding the T_H2 cell. (C) Anti-IL-5 agents block the development and release from the bone marrow of eosinophils. This has the potential to result in less tissue eosinophilia in allergic inflammation and less exposure to mediators from activated eosinophils—indicated by the broken red circle surrounding the eosinophil. (D) IL-9 is a product of activated T_H2 cells, which can directly activate mast cells. Anti-IL-9 could inhibit this. (E) IL-13 is a product of activated T_H2 cells with activities in determining isotype switch to IgE of allergen-specific B cells, direct activation mast cells, and effects on airway goblet cell activity and mucus production. Anti-IL-13 has the potential to inhibit these various activities. (F) Anti-IgE results in loss of high-affinity IgE receptors of mast cell and basophils with diminished capacity for activation after allergen exposure. Anti-IgE reduces high-affinity IgE receptors on dendritic cells, which may in turn decrease allergen processing and presentation.

maturation.¹⁹ More recently, a novel IL-1 family member cytokine, IL-33, has also been shown to increase T_H2 allergic inflammation. IL-33 has a very high level of expression in high endothelial venules of lymphoid tissue is felt to play a role in the chemotaxis of T_H2 cells and was shown to be capable of increasing cytokine production from T_H2 polarized lymphocytes.²⁰ More recently, IL-33 has been shown to cause a direct activation of basophils, resulting in increased secretion of IL-4, IL-13, and IL-8 as well as enhanced FcεR1-dependent mediator release and cytokine production. It is also capable of activating as human eosinophils.²¹ Targeting these allergen-independent cytokine pathways that promote T_H2-type allergic inflammatory disease might offer an important potential to interrupt chronic allergic inflammation.

T_H2 CYTOKINE BLOCKING THERAPIES

The cytokines produced by T_H2-type lymphocytes, basophils, and mast cells (IL-4, IL-5, IL-9, and IL-13) have been linked to many of the pathological findings in asthma detailed previously. Such knowledge has led to the development of several anticytokine agents with potential utility in interrupting the T_H2-dependent allergic inflammation characteristic of asthma. Human studies so far have included attempts to target most of the major T_H2 cytokines including IL-4, IL-5, IL-9, and IL-13 and joint targeting of IL-4 and IL-13.

Targeting IL-4

IL-4 has been shown to have an important role in the development of allergic inflammation at several levels and is an attractive target for therapy. In the presence dendritic cell-presented allergen, IL-4 (possibly basophil derived) strongly influences the differentiation of T_H0 cells into T_H2 cells. Furthermore, along with IL-13, IL-4 is a primary signal influencing the B lymphocyte to undergo isotype switch from IgM to IgE. IL-4 has also been shown to up-regulate the high-affinity and low-affinity IgE receptor expression on mast cells and basophils. Finally, IL-4 can also contribute to inflammatory cell recruitment *via* vascular cell adhesion molecule 1/very late antigen 4-dependent mechanisms and can also directly contribute to goblet cell hyperplasia. In mouse models of asthma, many studies have shown that IL-4 removal or blockade profoundly abrogates the allergic inflammatory response and other features of asthma.²²

A solubilized IL-4 receptor fragment (altrakinept), consisting of the extracellular portion of human IL-4R- α chain, which competitively inhibits the binding of IL-4 to its receptor, appeared promising in early human studies of mild to moderate asthma. Delivered by nebulizer, it appeared safe and well tolerated and had a serum half-life of ~5 days. After a single dose it

resulted in a reduction in exhaled nitric oxide and stabilization of asthma symptoms, despite inhaled corticosteroids withdrawal.²³ In a subsequent 12-week proof of concept study including 62 atopic patients with mild-to-moderate persistent asthma, weekly administration by nebulizer safely permitted withdrawal of inhaled steroid while maintaining asthma control. Thus, in contrast to the control group, patients treated with soluble IL-4 receptor showed no decline in forced expiratory volume in 1 second (FEV₁) or increase in symptoms after inhaled corticosteroids therapy withdrawal. However, the study discontinuation rate due to asthma exacerbations was similar between the soluble IL-4 receptor and placebo groups.²⁴ Although no further reports of clinical studies have been published, it is understood that a larger, phase III trial failed to confirm this benefit or efficacy suggested by the earlier, smaller trials (see Ref. 25).

IL-4 and IL-13 overlap in several functions and share a common α -chain in their receptors. Recent research has suggested that the soluble IL-4R under certain conditions may even enhance IL-13 responses. This observation that may be relevant to the soluble IL-4 receptor's ultimate lack of efficacy in clinical trials.²⁶

An alternative approach to targeting IL-4 has been the use of an anti-IL-4 humanized monoclonal antibody (pascolizumab). This antibody effectively blocked IL-4 responses *in vitro* and showed a favorable *in vivo* pharmacokinetic profile in cynomolgus monkeys. A phase II clinical trial in humans has been reported with unimpressive results for asthma, and further development was discontinued.²⁷

Targeting IL-5

IL-5 plays an important role in the differentiation of CD34-positive hematopoietic precursor cells into eosinophils, their subsequent maturation, and passage out of the bone marrow into peripheral blood. IL-5 also induces activation of mature eosinophils and promotes their survival.²⁸ Because eosinophils express the profibrotic growth factor TGF- β , IL-5 has also been linked to airway remodeling.²⁹ A series of animal studies highlighted the important role of IL-5 in asthma^{30,31} and showed that removal of IL-5 resulted in improved parameters of asthmatic disease activity.^{32,33}

In humans, a 12-week double-blind, placebo-controlled, randomized controlled trial of an anti-IL-5 monoclonal antibody (mepolizumab) in patients with mild to moderate asthma was reported. Although the study showed a rapid, dose-dependent and sustained reduction in eosinophil counts in blood and sputum, no differences in bronchial hyperresponsiveness, late-phase allergic responses, or other asthma outcomes were identifiable between the two treatment groups.³⁴

A separate study in patients with severe persistent asthma of another anti-IL-5 monoclonal antibody (re-

lizumab) yielded similar findings; *i.e.*, eosinophil numbers were decreased in peripheral blood but changes in asthma outcomes were not observed over 10 weeks of treatment.³⁵ A subsequent study using mepolizumab confirmed the suppression of eosinophilia in blood, in bone marrow, and in airway lavage fluid with a limited reduction in the number of the eosinophils seen in airway biopsies.³⁶ The reduction in airway eosinophil levels was associated with decreased expression of eosinophil TGF- β mRNA, decreased levels of TGF- β in bronchoalveolar lavage fluid (BAL) as well as decreased levels of tenascin, lumican, and procollagen 3 in the bronchial subepithelial basement membrane.³⁷ These findings suggest that anti-IL-5 therapies might have potential in regulating tissue remodeling in asthma.

A possible explanation for the observations of less impressive reductions of eosinophils in the airways than in the blood or BAL might be that airway eosinophils do not express the IL-5 receptor making, them less amenable to modulation by this therapy than circulating eosinophils.^{38,39} Specific strategies to reduce tissue eosinophils in the airways might prove more beneficial in asthma treatment and these might include targeting either the shared β -chain between IL-3 and IL-5 or eotaxin. Anti-IL-5 monoclonal antibodies may be useful in other diseases characterized by high levels of eosinophils in blood and tissues including the hypereosinophilic syndrome, eosinophilic esophagitis, and other eosinophilic enteritides, chronic rhinosinusitis with nasal polyps. Preliminary data in patients with hypereosinophilic syndrome (HES) and eosinophilic esophagitis (EE) treated with anti-IL-5 have revealed promising results including improvement in clinical parameters and levels of tissue in eosinophilia.^{40–43} A recent randomized, double-blind, placebo-controlled trial in patients with HES showed anti-IL-5 therapy was able to result in clinical stabilization, decreased peripheral blood eosinophil levels, and decreased oral steroid requirements.⁴⁴

Targeting IL-9

IL-9 has been shown to have a direct effect on the development and activation of human mast cells and to contribute to goblet cell hyperplasia in the airways and to other aspects of airway remodeling.²⁹ In mouse models of asthma, blocking IL-9 reduces allergen-induced airway inflammation and airway hyperresponsiveness.

Early human studies of an anti-IL-9 monoclonal antibody (Medi-528) have been conducted. These include two phase 1 dosing studies, which have been completed in healthy subjects without major adverse events. Phase II studies are currently in progress in patients with symptomatic moderate to severe asthma. Trial results still are not available.⁴

Targeting IL-13

IL-13, in addition to its role in promoting IgE isotype switching by B cells, has important effects on epithelial cell maturation, goblet cell hyperplasia, mucous production, generation of extracellular matrix proteins, and enhanced contractility of airway smooth muscle cells. IL-13 is also involved in the recruitment of eosinophils, monocytes, macrophages, and T cells.^{45,46} These properties make it a key potential target in the treatment of allergic inflammatory diseases including asthma.⁴⁷ In animal models including mice and primates, reduction of lung inflammation, decreased airway responsiveness, and diminished mucus production have been associated with anti-IL-13 monoclonal antibodies or by using a soluble form of IL-13 receptor.^{46,48,49}

Human anti-IL-13-specific monoclonal antibodies are under development and some are in phase I and II trials. A phase I clinical trial with CAT 354 showed that increasing single doses of i.v.-administered anti-IL-13 monoclonal antibody in 34 patients with mild asthma were well tolerated at all doses with no identified safety concerns.⁴ No outcome data related to asthma improvement are available.

Joint Targeting of IL-4 and IL-13

IL-4 and IL-13 have a common α -chain in their respective receptors. Blocking this shared component of the receptor has the potential to inhibit signaling by both IL-4 and IL-13, two potent T_H2 cytokines with several overlapping properties.

An IL-4 mutant protein, pitakinra, that inhibits the effects of both IL-4 and IL-13 through its ability to bind to the IL-4R α -chain, has been developed both as a subcutaneous injection and as an inhaled therapy. A phase III study of the inhaled formulation in allergen-induced asthma in humans, involving twice-daily inhalation for 27 days shows decreases in the late-phase allergic responses, decreases in exhaled nitric oxide, and improved pulmonary function in asthmatic patients.⁵⁰ Additional studies are under way.

Promoting T_H1 Cytokines

As mentioned previously, IL-12 and IFN- γ have a negative developmental effect on T_H2 phenotype cells, while preferentially driving the differentiation of T_H0 cells toward a T_H1 phenotype. An alternative biological approach to the treatment of allergic asthma therefore might include use of recombinant T_H1 cytokines, such as IL-12, with a goal of down-regulating T_H2-type responses.

In animal models of allergic asthma, administration of IL-12 during sensitization suppresses allergen-induced T_H2 cellular responses in favor of T_H1 responses, inhibits airway hyperresponsiveness, and re-

sults in diminished airway eosinophilia after allergen challenge.

In humans, a clinical trial involving injection of recombinant human IL-12 in patients with mild asthma resulted in a decrease in the number of circulating blood eosinophils after allergen challenge, but not in sputum eosinophilia, the late-phase asthmatic responses, or in airway hyperresponsiveness. This therapy was accompanied by flu-like symptoms, abnormal liver function tests, and cardiac arrhythmia.⁵¹

T_H17 Cells as a Target

The T_H17 cells have been identified as a distinct T-helper cell lineage that follows a distinct differentiation pattern requiring IL-6 and TGF- β .⁵² This novel T-helper subset is characterized by the production of IL-17 cytokines, which produce tissue inflammation by inducing the release of proinflammatory and neutrophil immobilizing cytokines. The T_H17 cells have been shown experimentally to have an important role in the development of autoimmune diseases but may also contribute to the pathogenesis of classically recognized T_H2-mediated allergic diseases.⁵³ Experimental evidence suggests that IL-17 decreases tissue eosinophil recruitment and bronchial hyperreactivity but may, on the other hand, increase neutrophil infiltration and increase mucus proteins.⁵⁴ Additionally, more recent evidence suggests that IL-17 is associated with a steroid-resistant phenotype in asthma.⁵⁵ These observations may eventually lead to targeting this pathway in the treatment of severe neutrophilic asthma.

Targeting IL-25

IL-25 (IL-17E) is also a member of the IL-17 family, but is distinct from all the other IL-17 family members in that it has been shown to provoke T_H2 cell-mediated inflammatory responses in animal studies. Animal studies have also shown that a neutralizing antibody against IL-25 significantly abrogates airway hyperresponsiveness, reduces IL-5 and IL-13 production, and reduces tissue eosinophil infiltration, goblet cell hyperplasia, and a serum IgE.⁵⁶ In humans it has been shown that eosinophils and basophils likely represent the primary source of IL-25 and that its major affect may be on T_H2 memory cells.⁵⁷ Although human studies still have not been reported, this might become an important potential therapeutic target.

INHIBITION OF MAST CELL ACTIVATION

Cross-linking of IgE-Fc ϵ R1 complexes on the mast cell surface by allergen leads, within minutes, to the so-called "early phase" of the allergic reaction, which involves mast cell degranulation and the synthesis of lipid mediators. Cytokines and chemokines liberated after mast cell activation initiate the "late phase,"

which peaks some hours later and involves a recruitment and activation of inflammatory cells.

Syk Tyrosine Kinase Inhibitors

The high-affinity receptor for IgE, Fc ϵ R1, is expressed as a $\alpha\beta\gamma 2$ -tetramer on mast cells and basophils and as a $\alpha\gamma 2$ -trimer on human antigen-presenting cells, monocytes, eosinophils, platelets, and smooth muscle cells. The extracellular domain of the α -chain contains the IgE binding site, whereas the signaling motives (immunoreceptor tyrosine-based activation motives [ITAMs]) are located in the intracellular sequences of the β - and γ -chains. After cross-linking of IgE-Fc ϵ R1 complexes, phosphorylation of the ITAMs by the sarcoma tyrosine kinases, Lyn and Fyn are key events that initiate receptor signaling. Syk tyrosine kinase next binds to the phosphorylated ITAMs of the Fc ϵ R1 γ -chain and, in turn, phosphorylates several other substrates playing an important role in propagating and amplifying the mast cell activation signal.⁵⁸

Syk tyrosine kinase has become a therapeutic target for inhibition of mast cell activation triggered by IgE cross-linking. An inhibitor of this tyrosine kinase, R112, has been studied in human clinical trials. In allergic rhinitis, intranasal administration of R112 followed by allergen challenge inhibited nasal obstruction and rhinorrhea and inhibited prostaglandin D₂ production.⁵⁹ In a 2-day, outdoor park study, during which patients with allergic rhinitis were exposed to high pollen counts, treatment with this agent effectively decreased global allergy symptoms.⁶⁰ Improvement was rapid in onset and had ~4 hours duration of action. A Syk tyrosine kinase inhibitor R343 is also under development as a potential dry-powder inhaled therapy for asthma. Although Syk activity is not restricted to mast cells and basophils, it is critical and nonredundant in initiating IgE-mediated activation of these cell types, making it an attractive therapeutic target in allergic diseases.⁶¹

ANTI-IgE

Omalizumab, an IgE-specific humanized IgG₁ monoclonal antibody, binds to the Fc-region of the IgE molecule, prevents IgE from interacting with high- or low-affinity IgE receptors (Fc ϵ R1 and Fc ϵ R11), and results in rapid decreasing levels of circulating free IgE. Omalizumab does not directly bind to receptor-bound IgE but down-regulates Fc ϵ R1 expression on circulating basophils, on skin mast cells, and on circulating dendritic cells. It has the potential to inhibit allergen-induced mast cell activation regardless of the allergen specificity of the IgE molecules. The mechanisms of action of omalizumab might also include reduced presentation of allergen by dendritic cells to T cells. Early studies in asthma showed that this antibody inhibited

both early asthmatic responses and late asthmatic responses to inhaled allergen in patients with asthma.⁶²

Demonstration of anti-inflammatory properties of omalizumab was provided in a 16-week placebo-controlled bronchial biopsy and sputum study of 45 patients with corticosteroid naive mild persistent asthma. Omalizumab-treated subjects showed a reduction in sputum and tissue eosinophils, a reduction in the number of IgE⁺ and FcεR1⁺ cells in the submucosa, a decrease in the number of cells staining positive for IL-4, and a decreased overall number of CD4 and CD8 lymphocytes. However, perhaps surprisingly, bronchial hyperresponsiveness to methacholine was not significantly changed.⁶³

Omalizumab is administered by subcutaneous injection. Although the level of circulating free IgE decreases rapidly after the first dose of omalizumab, up to 16 weeks of treatment might be required before optimal clinical effects are seen. Large placebo-controlled clinical trials in adults, adolescents, and children with poorly controlled IgE-mediated asthma have shown that omalizumab improves symptom control, decreases the frequency of asthma exacerbations, and allows patients to be managed with lower doses of inhaled corticosteroids.^{64,65} Recent studies have indicated efficacy of omalizumab in patients with more severe asthma and in pooled analyses of clinical trials, omalizumab significantly reduced asthma exacerbations by 38%, emergency department visits by 61%, hospital admissions by 52%, and unscheduled doctor visits by 47% when compared with control subjects.^{66,67}

Omalizumab has also been studied in other allergic diseases including the treatment of allergic rhinoconjunctivitis, as an adjunctive therapy with allergen immunotherapy, in chronic urticaria and angioedema, atopic dermatitis, allergic bronchopulmonary aspergillosis, latex allergy, and others. Additional study is ongoing and none of these conditions are approved indications for use of this drug.

TNF- α -BASED THERAPY

Recent studies have suggested that TNF- α may serve as a marker of severity in asthma and possibly as a target for biological therapy of asthma.⁶⁹

TNF- α is important in the innate immune response. It is a cytokine principally produced by macrophages in response to activation of membrane-bound pattern recognition molecules, such as Toll-like receptors, but is also produced by several other proinflammatory cells including monocytes, dendritic cells, B lymphocytes, T lymphocytes, neutrophils, mast cells, and eosinophils as well as structural cells including fibroblasts, epithelial cells, and smooth muscle cells. TNF- α has been linked to several chronic inflammatory dis-

eases including rheumatoid arthritis and Crohn's disease in which anti-TNF- α therapy has proved useful in treatment.

The possibility that TNF- α contributes to the inflammatory response seen in the asthmatic airway is supported by several observations. A biopsy study showed that TNF- α mRNA is 30-fold higher in the airways of severe asthmatic patients in contrast to well-controlled asthmatic patients. This finding was paralleled by increased levels of TNF- α protein in BAL and mucosal biopsies.⁷⁰ Administration of recombinant TNF- α to normal subjects led to development of airway hyperresponsiveness and airway neutrophilia.⁷¹ Silvestri *et al.* examined the level of four cytokines TNF- α , IL-8, IL-6, and IL-13 in the circulation of severe and mild/moderate asthmatic patients and normal controls. They report that TNF- α and IL-8 levels are higher in those with severe asthma and are inversely correlated with baseline forced expiratory volume. They also report correlations between serum TNF- α levels, exhaled nitric oxide, and circulating neutrophil counts. They suggest that circulating levels of TNF- α and IL-8 may serve as biomarkers to identify those severe asthmatic patients in whom anti-TNF- α therapy may be efficacious.⁷²

Based on these observations and a clear unmet clinical need in corticosteroid refractory asthma, a small number of recent clinical trials have evaluated the efficacy of anti-TNF- α therapy in asthma. Biological agents targeting the TNF- α axis include infliximab (a chimeric mouse/humanized monoclonal antibody), etanercept (a soluble fusion protein combining TNF- α receptors with an Fc fragment of human IgG1), and adalimumab (a fully human monoclonal antibody).

In a 12-week open-label uncontrolled study of etanercept in patients with severe asthma, Howarth *et al.* reported a significant improvement in methacholine airway hyperresponsiveness, an improvement in FEV₁ and in improvement in quality of life.⁷⁰ This was followed by a randomized controlled crossover trial of etanercept in 10 patients with severe asthma in which it was confirmed that treatment resulted in an improvement in airway hyperresponsiveness, FEV₁, and asthma-related quality of life. There was no effect of etanercept therapy on the number of sputum eosinophils or neutrophils. It was, however, shown that the membrane-associated TNF on circulating mononuclear cells appeared to be a predictor of the therapeutic response to etanercept.⁷³ A subsequent 12-week long randomized, placebo-controlled, paralleled group study of etanercept in a patients with severe asthma showed no beneficial effect.⁷⁴ In a randomized placebo-controlled trial, Erin *et al.* reported the efficacy of infliximab in patient's with moderate asthma. No improvement in morning peak flow occurred but there were decreases in diurnal variation of peak expiratory

flow rate and a 50% reduction in the number of mild asthma exacerbations. There were no significant improvements in lung function.⁷⁵ It has been stated that unpublished longer-term randomized, placebo-controlled, paralleled group studies of patients with moderate severe asthma have questioned the efficacy of anti-TNF- α therapy in asthma, discussed by Brightling *et al.*⁶⁹

CONCLUSION

Recent increases in our understanding of the pathobiology of asthma and of the innate and adaptive immune responses underlying those changes, including the role of various cellular elements and cytokines, has resulted in the identification of a large number of potential therapeutic targets for biological agents in asthma. Many such therapies have been developed and subjected to clinical study but, to date, vast majority of these have not received Food and Drug Administration approval for treatment of asthma or other allergic diseases. Omalizumab is a notable exception. Asthma is clearly a complex disease or syndrome of diseases and the possibility that separate populations of asthmatic patients are responsive to different biological therapies emphasizes the likely importance of tailoring novel therapies to individual patients. It is anticipated that as the heterogeneity of asthma becomes more fully understood that new targeted biological therapies will realize their potential as therapeutic agents. This is a very exciting time in the treatment of allergic diseases and the future seems full of promise.

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