

Use of biologicals as immunotherapy in asthma and related diseases

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Recent advances in our understanding of the immune-mediated inflammatory pathways in asthma and other allergic diseases have resulted in the development of novel biological compounds for the treatment of these conditions. These compounds offer an advantage over glucocorticosteroid therapy by specifically targeting components of the immunologic cascade, thereby allowing patients to reduce or discontinue their glucocorticosteroid treatment. Another potential advantage of biological compounds is that they may provide additional anti-inflammatory benefits, over and above those provided by glucocorticosteroid therapy, for patients who continue to have evidence of refractory asthma. The anti-IgE monoclonal antibody omalizumab is already being used for the treatment of allergic asthma and a number of other biological therapies are currently in various stages of clinical development. The purpose of this review is to summarize the data from these studies and to provide a rationale for the use of these compounds in asthma and related allergic airway diseases.

KEYWORDS: anti-IgE • anti-IL-2 receptor • anti-IL-4 • anti-IL-5 • anti-IL-9 • anti-IL-13 • asthma • biologic therapy • omalizumab • TNF- α

Advances in our understanding of the immune-mediated inflammatory mechanisms involved in the pathogenesis of asthma and other related allergic diseases have resulted in the development of novel target-specific biological compounds for the treatment of these conditions. The only such agent currently approved for the treatment of allergic asthma is the anti-IgE monoclonal antibody (mAb) omalizumab, although its role in the treatment of other allergic airway diseases has not yet been clearly defined. However, many other biological compounds are currently under development for the treatment of asthma and related diseases, and several of these compounds are now in various stages of clinical development. The purpose of this review is to summarize the data from these studies and to provide a rationale for the use of these compounds in asthma and related allergic airway diseases. It should be emphasized that the review will focus on those biological compounds that have shown some evidence of therapeutic benefit in human studies, as several agents showing promise both *in vitro* and in experimental animal models have failed to confirm any clinical benefit in human clinical studies.

Anti-IgE mAb therapy

IgE is a central mediator in the pathogenesis of allergic asthma and related diseases, and is thus a logical target for asthma therapy [1]. In population-based studies, most asthmatic individuals have increased levels of IgE [2]. Mast cells and basophils have many high-affinity IgE receptors on their cell surface that bind circulating IgE [3], which results in further upregulation of these surface receptors [4]. Binding of IgE to receptors on basophils and mast cells results in subsequent crosslinking, which initiates an inflammatory cascade through the release of histamine, leukotrienes and platelet-activating factor [5].

Omalizumab is currently the only biologic therapy approved in Europe and North America for the treatment of moderate-to-severe persistent atopic asthma in adults and children 12 years and older whose symptoms were inadequately controlled with inhaled corticosteroids (ICS) [6]. Omalizumab is a recombinant DNA-derived humanized mAb to the Fc portion of the IgE antibody that prevents the binding of IgE to its high-affinity receptor (Fc ϵ RI) on mast cells and basophils [7]. Thus, the anti-IgE mAb does not bind IgE associated with the cell surface and,

theoretically at least, cannot therefore trigger receptor crosslinking, which might result in anaphylactic reactions. The decreased binding of IgE on the surface of mast cells leads to a decrease in the release of mediators in response to allergen exposure. In addition, omalizumab decreases FcεRI expression on basophils and airway submucosal cells [7].

The binding of the anti-IgE mAb to the circulating IgE leads to a measurable reduction in circulating IgE of 99% and to the downregulation of IgE receptors on mast cells and basophils [8,9]. After 3 months of treatment in patients with allergic rhinitis, histamine release from basophils was reduced by 90% and there was a reduction in the expression of FcεRI on basophils, monocytes and dendritic cells measured within 7 days of treatment [9,10]. Anti-IgE mAb treatment attenuates both the early- and late-phase allergen-induced asthmatic responses [11,12]. This may be due to the combined actions of reducing the circulating IgE levels and downregulating the cell-surface receptor expression. In asthmatic patients, there is a measurable decrease in the amount of serum IgE and the number of FcεRI-positive cells in the bronchial submucosa and epithelial tissue of patients following omalizumab treatment [13]. There are also marked decreases in the numbers of sputum and airway-tissue eosinophils in patients with allergic asthma who are treated with omalizumab [13]. This substantial reduction in airway eosinophilia is likely to be highly clinically relevant, given the relationship between eosinophilic airway inflammation and the future risk of asthma exacerbations and asthma severity [14,15]. FcεRI expression is known to be increased at baseline in allergic asthma patients and, thus, the ability of omalizumab to decrease the expression of FcεRI on antigen-presenting dendritic cells suggests an ability to alter allergen presentation and so decrease the allergic response [16].

There have been several randomized, placebo-controlled clinical trials examining the effectiveness of omalizumab in patients with allergic asthma (TABLE 1). The INNOVATE study consisted of a 28-week double-blind, placebo-controlled, multicenter, parallel group study. Patients were eligible for enrollment into the study if they had poorly controlled severe persistent asthma, despite being treated with Global Initiative for Asthma 2002 Step 4 therapy (short-acting β₂-agonist, medium- or high-dose ICS and long-acting β₂-agonist or leukotriene modifier) [17]. There were 209 patients in the treatment group and 210 in the placebo group. Results show a 26% reduction in the rate of clinically significant asthma exacerbations (with adjustment for baseline exacerbation history), as well as a decrease in severe exacerbations by 50% ($p = 0.002$) and a decrease in visits to emergency departments by 44% ($p = 0.38$) [18]. It should be noted, however, that the two study groups were not comparable in terms of previous exacerbations at the time of randomization and that the significant differences in favor of omalizumab reducing asthma exacerbations were only evident following a *post hoc* statistical adjustment of the baseline study subject characteristics. This statistical maneuver, while acknowledged as permissible, still detracts from the methodological rigor of the study and suggests some caution when interpreting the results. Ayers *et al.* published an open-label study in which 200 patients were treated for 52 weeks with omalizumab and

106 patients were treated with current best standard of care [19]. The results of this study indicate that patients receiving open-label omalizumab treatment (in addition to best standard of care) had approximately half the number of asthma-related exacerbations compared with those patients treated with best standard of care alone (4.92 vs 9.76 episodes per patient-year) [19]. In a subgroup analysis of this study, there was a 59% ($p = 0.001$) decrease in the annual rate of asthma exacerbations for those patients treated with omalizumab compared with those patients treated with best standard of care.

Three large randomized, placebo-controlled studies (enrolling 525, 546 and 246 patients, respectively) showed a significant decrease in the incidence of asthma exacerbations, as well as in hospitalizations and ICS usage, in those patients treated with omalizumab therapy [20–22]. In a subsequent meta-analysis of these three studies, the most striking clinical benefits related to omalizumab treatment were observed in those patients who were at highest risk of morbidity and mortality related to their asthma [23]. Two of the studies subsequently examined an additional time period beyond the initial 28–30-week outcome timepoints and found that there were sustained benefits observed even after a further 24 weeks had elapsed [24,25]. A pooled analysis of the study data by Bousquet *et al.* showed that omalizumab therapy significantly decreased the annual exacerbation rate of patients by 38% ($p < 0.0001$), as well as the emergency room visit rate by 47% ($p < 0.0001$) compared with placebo-treated control patients [26]. In terms of health-related quality-of-life (QoL) outcome measures, the INNOVATE trial showed statistically significant improvements in all four domains of the Asthma Quality of Life Questionnaire (asthma symptoms, environmental stimuli response, activity limitations and emotional dysfunction) [18]. This improvement in asthma QoL score following omalizumab therapy was also demonstrated in clinical studies by both Buhl *et al.* [27] and Finn *et al.* [28].

One of the significant limitations preventing the widespread uptake of omalizumab therapy into clinical practice is the high financial cost associated with the maintenance use of this medication. It can be challenging to evaluate the cost–effectiveness of biological treatments such as omalizumab, given that the initial drug costs are substantially higher than current standard-of-care treatments. Such a pharmacoeconomic evaluation requires careful comparison of the drug to opportunity costs of reduced emergency room visits and hospitalizations, days lost from work/school and the treatment of glucocorticosteroid-induced adverse effects. To that end, Bousquet *et al.* carried out a multivariate analysis of seven randomized controlled trials (RCTs) in 2007 to ascertain the baseline characteristics of those patients who would probably have the greatest predicted clinical response to omalizumab treatment [29]. Baseline serum IgE levels in this study showed poor correlation with disease severity and the study unfortunately did not identify any at baseline marker that could accurately predict which patients would probably have the greatest clinical response to omalizumab therapy. The study did, however, confirm that patients who had a positive response to treatment could be identified with a composite measure, based on the physician's overall

Table 1. Selected omalizumab studies in patients with asthma.

Author	Study type	Patient population	Duration of study	Patients (n)	Major study results	Ref.
Humbert <i>et al.</i> (INNOVATE study)	Double-blind, parallel group, multicenter	Severe asthma ($\geq$ two exacerbations per year) Age 12–75 years Positive skin-prick test	28 weeks	209 in treatment group 210 in placebo group	26% decrease in clinically significant exacerbations 50% decline in severe exacerbations 44% decrease in ER visits	[18]
Ayres <i>et al.</i>	Open-label, parallel group, 49 centers	Moderate-to-severe asthma for over 2 years ($\geq$ one ER visit and $\geq$ one oral corticosteroid per year) Positive skin prick to two clinically relevant allergens	52 weeks	206 in treatment group (omalizumab plus best standard of care) 106 in best standard of care group	Measured in asthma-related deterioration-related incidents (course of steroid, missed school work and unscheduled physician visit) 50% decrease in treatment group 60% decrease in exacerbations	[19]
Busse <i>et al.</i>	Phase III double-blind, placebo-controlled multicenter	Severe allergic asthma of at least 1 year duration (with daily ICS use) Age 12–75 years Positive skin prick to one allergen	28 weeks: week 1–16 steroid reduction phase; week 16–28 stable steroid phase	268 in omalizumab group 252 in placebo group	59% decrease in annual rate of asthma exacerbation Decrease in ICS dose in treatment group Improved daily asthma scores in treatment group	[20]
Holgate <i>et al.</i>	Randomized, placebo-controlled, multicenter	Severe allergic asthma (needing ICS) Age 12–75 years Serum IgE 30–700 IU/ml Positive skin-prick test	6–10 week run-in period to switch to fluticasone 16 week stable fluticasone 16 week to decrease fluticasone	126 in omalizumab group 109 in placebo group	Greater decrease of fluticasone use with omalizumab	[21]
Soler <i>et al.</i>	Double-blind, Randomized placebo-controlled, parallel group, multicenter	Moderate-to-severe asthma for at least 1 year (stable) Age 12–75 years Positive skin-prick test Serum IgE 30–700 IU/ml	28 weeks: week 0–15 steroid stable phase; week 16–28 steroid reduction phase	274 in omalizumab group 272 in placebo group	58% fewer exacerbations per patient in steroid stable phase 50% fewer exacerbations per patient in steroid reduction phase	[22]
Holgate <i>et al.</i>	Meta-analysis of three studies	Moderate-to-severe allergic asthma with daily ICS use	16-week stable steroid phase and 12–16 week steroid-reduction phase. Studies 1 and 2 had additional 24-week extension phase	1412 total patients enrolled in all groups of the 3 studies. Pooled subgroup analysis of only high-risk asthma patients included 135 in omalizumab and 119 in placebo group	Annualized rate of significant asthma exacerbation episodes was decreased in high-risk group on omalizumab	[23]
Buhl <i>et al.</i>	Double-blind extension to Soler <i>et al.</i>	Moderate-to-severe asthma for at least 1 year (stable) Age 12–75 years Positive skin-prick test Serum IgE 30–700 IU/ml	24-week extension to 28-week trial	254 in omalizumab group 229 in placebo group	Lower ongoing beclomethasone use in omalizumab group Disease control sustained in more patients in treatment group	[24]
Lanier <i>et al.</i>	Double-blind extension to Busse <i>et al.</i>	Severe allergic asthma of at least 1 year duration (with daily ICS use) Age 12–75 years Positive skin prick to one allergen	24-week extension to 28-week study	245 in omalizumab group 215 in placebo group	Continued significant decrease in exacerbation rate and sustained decrease in ICS use in treatment group	[25]

ER: Emergency room; ICS: Inhaled corticosteroid.

assessment, which included patient interview, review of medical notes, symptom diary, rescue medication use and spirometry and peak expiratory flow measurements. Patients who scored 'marked improvement' or 'complete control' on the physician's overall assessment were termed responders and analysis of clinical outcomes in this subgroup was better than that of the overall patient population. Based on these data, Bousquet and his colleagues concluded that it was difficult to predict *a priori* which patients were likely to respond well to omalizumab therapy and their recommendation, based on the study, is that an assessment should be made after 16 weeks of treatment to decide if the patient is a 'responder' who should be continued on treatment.

Pragmatic clinical studies are now emerging that address the use of omalizumab therapy in 'real-life practice'. One such study looked at 154 patients in France and found that patients who started treatment with omalizumab had 62% fewer exacerbations requiring systemic corticosteroid treatment, 65% fewer emergency department visits and 29% fewer hospitalizations compared with the previous year [30]. As indicated earlier, the theoretical risk of omalizumab-related adverse effects are negligible, in large part due to the fact that the mAb binds only to the free IgE in circulation and cannot crosslink IgE molecules already bound to the cell surface. Nonetheless, the safety of omalizumab therapy has now been evaluated extensively, with more than 5000 patients previously enrolled in Phase IIb/III RCTs. In these studies, the prevalence of adverse events was infrequent, with the most common events reported being local injection-site pain and bruising, headache, sinusitis, nasopharyngitis and upper-respiratory tract infections. It is important to note that, in these studies, the reported adverse events between the omalizumab-treated group and the control groups (whether placebo or standard therapy) were similar in frequency and nature [31]. More recently, a Joint Task Force Report from the American Academy of Allergy, Asthma and Immunology and the American College of Allergy, Asthma and Immunology had the opportunity to review the safety data for omalizumab from the various clinical trials conducted to date, and from postmarketing surveillance data provided by the manufacturer [32]. The task force concluded that approximately 40,000 patients received omalizumab during the period from June 1, 2003 to December 31, 2005 and that 35 patients had 41 episodes of anaphylaxis associated with omalizumab administration during this time. This corresponds to an anaphylaxis-reporting rate of 0.09% from treated patients. There were no fatalities in any of the reported cases.

There are a number of unanswered questions regarding omalizumab therapy in the treatment of asthma and related allergic diseases. Although omalizumab is already approved for the treatment of moderate-to-severe persistent atopic asthma in adults and children 12 years and older, it is not clear what role the compound has in patients with less-severe asthma. It is also unclear whether ongoing treatment with omalizumab might attenuate or reverse aspects of airway remodeling or result in significant improvements (more than two doubling-dose concentrations) in airway hyper-responsiveness (AHR). Similarly, while studies have suggested that omalizumab may have substantial clinical benefits in the

treatment of some related diseases, such as allergic rhinosinusitis, acute bronchopulmonary aspergillosis, atopic dermatitis and food allergies, its potential role in the treatment of other allergic diseases has yet to be clearly defined.

Anti-TNF- α monoclonal antibody therapy

TNF- α is a 26-kDa membrane-anchored precursor protein that is cleaved into a biologically active homotrimer form. The homotrimer binds to the TNF- α receptors 1 and 2, and this interaction results in a broad cascade of intracellular signaling, including the upregulation of several proinflammatory proteins, such as IL-8, IL-6 and TNF- α [33–35]. TNF- α is considered to be critical in the innate immune response, which is the initial form of host defense to a variety of insults, prior to the activation of the T-cell-mediated, immunogen-specific adaptive immune system [36]. TNF- α is produced mainly by macrophages but also by other proinflammatory cells, such as monocytes, B cells, neutrophils, dendritic cells, eosinophils and mast cells [37]. A number of TNF- α mAbs have been developed and are currently approved for the treatment of several rheumatological diseases. TNF- α levels are markedly increased in the synovial fluid and serum of patients with rheumatoid arthritis [38] and treatment with TNF- α mAbs has resulted in significant improvement in disease activity scores [39].

A possible link between increased TNF- α levels and the pathogenesis of asthma was established a number of years ago. Increased levels of TNF- α mRNA [40] and TNF- α protein [41] have been shown to be present in the airways of asthmatic individuals and experimental inhalation of TNF- α by nonasthmatic normal individuals resulted in the development of AHR and airway neutrophilia [42]. The mechanism by which TNF- α acts and results in AHR is not yet fully understood, but it is possible that TNF- α exerts a direct bronchoconstrictor effect on airway smooth muscle [43] or that TNF- α facilitates the release of cysteinyl-leukotrienes [44] and histamine from mast cells [45]. Additional known effects of TNF- α include increasing the cytotoxic action of eosinophils on endothelial cells [46], activating and promoting cytokine release by T cells [47] and acting as a chemoattractant to neutrophils and eosinophils [48]. It is also possible that in refractory asthma, other as-yet undefined mechanisms might be working. These include TNF- α -induced neutrophil chemotaxis to the airways [42] and upregulation of TGF- β , which leads to the activation of airway fibroblasts and their differentiation into myofibroblasts, which, in turn, are involved in the pathogenesis of airway remodeling [49].

There are currently three commercially available TNF- α mAb therapies available for the treatment of rheumatoid arthritis; infliximab (a chimeric mouse/human mAb), etanercept (a soluble fusion protein with 2 p75 TNF receptors and the Fc fragment of human IgG1) and adalimumab (a human mAb). While there have recently been several studies examining the therapeutic benefit of these agents in treating asthma, none of them are currently approved for the treatment of asthma or any related allergic diseases (TABLE 2). Howarth and his colleagues conducted an initial small, open-label study using etanercept to treat patients

Table 2. Selected antihuman TNF- α studies in patients with asthma.

Author	Study type	Patient population	Duration of study (weeks)	Patients (n)	Major study results	Ref.
Howarth <i>et al.</i>	Open-label, uncontrolled, proof-of-concept of etanercept	Severe asthma Age 30–67 years	12 of etanercept	17 with severe asthma	Improved asthma symptoms, lung function and bronchial hyperresponsiveness	[50]
Berry <i>et al.</i>	Double-blind, placebo-controlled, crossover pilot study	Treatment-refractory asthma (symptomatic with daily ICS)	10	Ten	Improved airway hyperresponsiveness, asthma-related QoL scores and FEV ₁	[51]
Erin <i>et al.</i>	Double-blind, placebo-controlled, parallel group	Moderate-to-severe asthma (treated with ICS) Asthmatics for over 1 year	12	38 (20 placebo; 18 infliximab)	No change in primary end point (lung function) Mild decrease in rate of exacerbations and decreased level of TNF- α in sputum of treatment group	[52]
Morjaria <i>et al.</i>	Double-blind, randomized, placebo-controlled parallel group	Severe asthma refractory to ICS	12	39 (20 placebo; 19 etanercept)	Very small improvement in asthma control questionnaire score in treatment group No change in asthma QoL score, lung function or exacerbation rates	[54]

FEV₁: Forced expiratory volume in 1 s; ICS: Inhaled corticosteroid; QoL: Quality of life.

with refractory asthma who were still symptomatic despite being treated with oral corticosteroids and regular inhaled bronchodilators [50]. The results of this study indicated that there were significant improvements in AHR, forced expiratory volume in 1 s (FEV₁) and in asthma QoL scores in those patients treated with etanercept. Subsequently, a randomized placebo-controlled study assessing the therapeutic benefits of etanercept in ten patients with refractory asthma was published by Berry and colleagues in 2006. The results of this trial were consistent with the earlier open-label study, confirming that etanercept treatment resulted in improvements in AHR, FEV₁ and asthma-related QoL scores [51]. However, a randomized placebo-controlled study of infliximab treatment in 38 patients with moderate-to-severe asthma did not show any significant improvements in lung function, although there was a reduction in the number of mild asthma exacerbations in the infliximab-treated group [52]. One possible explanation to account for the differences in the results of the infliximab study, compared with the two etanercept studies, is that the study population in the infliximab study was made up of patients with moderate asthma and that the greatest treatment benefit of anti-TNF- α mAb therapies is possibly to be derived in the severe-asthmatic group, as seen in anti-IgE treatment [53]. Similarly, it is unclear whether anti-TNF- α mAb treatment may be more effective in those patients with neutrophilic asthma, given the biological role for TNF- α in neutrophil chemotaxis. Earlier this year, Morjaria *et al.* published a study using etanercept in 39 patients with severe asthma and confirmed that 12 weeks of etanercept treatment resulted in small but significant improvements in patient asthma control questionnaire responses, but did not result in any differences in asthma-related QoL scores, lung function, peak expiratory flow rates or AHR [54].

There have been serious questions raised regarding the safety of anti-TNF- α mAb therapies, particularly in more generalized patient population settings. The side effects that are most often linked with TNF- α blockers include serious infections (including reactivation of TB), malignancy, congestive heart failure, demyelinating disease, drug-induced lupus-like syndromes and autoimmune diseases [55]. It is recommended that patients be screened for TB exposure prior to initiating anti-TNF- α mAb treatment, as there is a small but real risk of reactivating tuberculous disease. Clearly, this would make any recommendation regarding anti-TNF- α mAb therapies for the treatment of asthma less generalizable to patients in the Third world. A recent meta-analysis examined the literature regarding TNF- α blockers and infection risk, and found that there is not a significantly increased risk of serious infection with rituximab, but that high doses of anakinra are linked to increased risk of infection in patients with comorbidities [56]. In a study of patients with chronic obstructive pulmonary disease, there was a slight increase in the malignancy rate in the treatment group, as well as an increase in the number of cases of pneumonia, although these differences were not statistically significant [57]. There have also been rare reports of aplastic anemia, pancytopenia, vasculitis and demyelinating events with etanercept [58]. Thus, based on the results of several RCTs to date, as well as on the safety concerns, it is fair to state that the precise role of anti-TNF- α mAb therapy in the treatment of asthma has not yet been defined. Further RCTs in well-characterized subgroups of patients with severe refractory asthma are required before any conclusions regarding the potential therapeutic benefit of anti-TNF- α mAb treatments can be made.

IL-5 antagonist therapy

Various *in vitro* and experimental animal models have confirmed the important role of IL-5 in the pathogenesis of airway inflammation in asthma [59–61]. IL-5 induces CD34⁺ progenitor cells to differentiate into eosinophils [62], which, in turn, release a wide range of mediators, cytokines and growth factors strongly implicated in the airway inflammatory changes present in asthma. A characteristic feature of the allergen-induced late-phase asthma response is airway eosinophilia, and the presence of both eosinophils and their inflammatory products correlate closely with asthma severity as well as with the degree of AHR [63,64]. Inhalation of IL-5 in individuals with asthma results in sputum eosinophilia and AHR [65], while asthmatic patients have increased levels of IL-5 protein in their serum and increased IL-5 mRNA-positive cells in their bronchial biopsy samples [66,67]. Clinical indices of disease severity correlate closely with expression of IL-5-mRNA or IL-5-receptor expression [68]. As such, eosinophils and the immune-mediated pathways that lead to eosinophil maturation and recruitment to the airways are important potential targets for therapies for asthma and other related diseases [69].

Animal studies confirm that anti-IL-5 mAb therapy result in decreases in eosinophil infiltration in both the airways and lung tissues compared with placebo [70–72], and have suggested that these changes are associated with functionally relevant improvements in the immune-mediated airway dysfunction that is present in these models. To date, two anti-IL-5 mAb therapies have been evaluated in human clinical trials. SCH55700 (reslizumab) is a humanized antihuman IL-5 mAb that is based on a rat IgG2a antibody against human IL-5. Reslizumab incorporates antigen-recognition sites for human IL-5 in consensus human IgG4(κ) constant regions using complementarity-determining region-grafting technology [73]. Reslizumab has been studied to evaluate the safety, biologic activity and pharmacokinetics of the compound (but not its efficacy) in patients with severe-to-persistent asthma [74]. Four concentrations of the mAb were compared with placebo and a dose-dependent decrease in circulating eosinophils was observed following treatment. At the two higher

concentrations studied, there was an improvement in baseline FEV₁ ($p = 0.019$) but there was no significant change in any other measures of disease activity. There is currently an RCT underway examining the safety, efficacy and clinical effects of reslizumab in patients with poorly controlled asthma [201].

A second humanized mAb to IL-5 (SB-240563 [mepolizumab]) was studied by Leckie and colleagues in an RCT of 24 patients with mild allergic asthma (TABLE 3) [75]. Two concentrations of mepolizumab (2.5 or 10 mg/kg, given as a single intravenous infusion) were compared with placebo. The effects of treatment were examined on blood and sputum eosinophils and on airway responses to inhaled allergen challenge administered at 1 and 4 weeks after treatment. The results of the study indicated that a single dose of mepolizumab significantly reduced blood and sputum eosinophils for at least 4 weeks, but did not have any significant effect on allergen-induced late responses or AHR. These observations led the authors to question the role of eosinophils in mediating AHR and to suggest that the lack of clinical efficacy for mepolizumab might be due to redundancy of immunologic pathways involved in the pathogenesis of AHR. However, there were significant concerns regarding the study methodology, specifically related to the small sample size and to the lack of an allergen-induced increase in AHR following placebo treatment [76]. These limitations indicate that caution needs to be taken when interpreting the results of this study and suggest that there may yet be a role for mepolizumab in the treatment of asthma and related diseases.

In a subsequent bronchial biopsy study published in 2003, Flood-Page and colleagues assessed 24 patients with mild asthma who were receiving either mepolizumab therapy or placebo [77]. While they again confirmed that there was a dramatic and absolute (100%) reduction in blood eosinophils following mepolizumab therapy, and that there was no clinically significant effect on AHR or lung function, they did make the important observation that there was only a modest reduction in tissue eosinophilia (55% decrease in airway eosinophils), suggesting that higher doses or a longer duration of mepolizumab treatment may be

Table 3. Selected mepolizumab studies on human patients with asthma.

Author	Study type	Patient population	Duration of study	Patients (n)	Major study results	Ref.
Leckie <i>et al.</i>	Double-blind, randomized, placebo-controlled	Mild asthma Nonsmokers Positive skin-prick test	30 days	24 (16 mepolizumab; 8 placebo)	Significant decrease in blood and sputum eosinophils No difference between groups on histamine challenge	[75]
Flood-Page <i>et al.</i>	Double-blind, randomized, placebo-controlled	Mild asthma (airway hyper-responsiveness) Ages 18–55 years Nonsmokers	20 weeks	24 (11 mepolizumab; 13 placebo)	55% reduction in airway eosinophils 52% reduction in bone marrow eosinophils but no change in clinical outcomes	[77]
Flood-Page <i>et al.</i>	Double-blind, multicenter, randomized, placebo-controlled	Moderate-to-persistent asthma despite ICS use		362	Decrease in blood and sputum eosinophils but no change in peak flow or other clinical outcomes	[78]

ICS: Inhaled corticosteroid.

necessary to abrogate the airway tissue eosinophilia required to attenuate AHR. Flood-Page and colleagues have more recently published data from 362 patients with moderate-to-persistent asthma (defined as those who remained symptomatic despite ICS treatment) in which patients were randomized to receive one of three infusion doses of mepolizumab or placebo. The results of this study showed no significant differences between mepolizumab treatment and placebo in morning peak flow rates, FEV₁ measurements, the use of inhaled β_2 -agonist rescue medications, asthma-symptom scores or asthma exacerbation rates [78]. While these results suggest a limited role for mepolizumab therapy in the treatment of asthma, it is important to note there is a RCT that has recently been concluded at McMaster University (Hamilton, Canada) that has examined the effects of high-dose mepolizumab therapy on asthma exacerbation rates, sputum eosinophilia and prednisone-sparing effects [202]. The results of this study, which are expected to be published within the coming months, are awaited with interest.

IL-4 antagonist therapy

IL-4 is a key cytokine in the early stages of the immune-mediated inflammatory cascade following immunogen activation due to its regulatory role in facilitating B-cell isotype switching from IgM to IgE production. IL-4 is also involved in eosinophil chemotaxis and in the development of effector T-cell responses from undifferentiated T-helper cells to memory-specific Th2 cells [79,80]. Once activated, Th2 cells are primed to secrete cytokines, such as IL-3, IL-4, IL-5, IL-13 and granulocyte-macrophage colony-stimulating factor, which initiate and perpetuate the immune-mediated inflammatory airway responses that are characteristic of allergic asthma and are thought to underpin the pathophysiology of the variable airway dysfunction that is the hallmark of the condition [81–83].

Inhibiting the biological actions of IL-4 in asthma should, at least theoretically, result in a decrease in airway inflammation, and, thus, would appear to be a promising therapeutic target in the treatment of asthma and related diseases [84]. In addition, blockade of IL-4 offers the potential additional benefit for attenuating IgE production and the subsequent IgE-mediated airway inflammation [85]. To this end, a humanized anti-IL-4 mAb (pasolizumab) that binds IL-4 and prevents the interaction of IL-4 with the α -chain of the IL-4 receptor (IL-4R) was developed and tested on cynomolgus monkeys. Although it was found to be well tolerated, the compound had no measurable effect on serum IgE levels [85]. Based on these disappointing clinical results, the proposed human clinical trials that were planned with this anti-IL-4 mAb have been discontinued [86]. However, another human mAb reported to block the action of IL-4 and IL-13 is currently under development by Amgen (CA, USA; AMG-317), with a Phase II clinical trial in participants with moderate-to-severe asthma currently underway [203].

A second approach of IL-4 antagonism has been to generate a soluble recombinant human IL-4 receptor (consisting of the extracellular portion of human IL-4R- α) to bind and inactivate free circulating IL-4 [87,88]. It was hoped that this might result in some clinical benefit in the treatment of asthma and related

diseases, and two initial studies indicated that treatment with the compound altrakincept prevented worsening of asthma symptoms and the fall in FEV₁ that was observed in the placebo-treated control group (TABLE 4) [87,88]. However, despite these initial promising results, a subsequent Phase III RCT has failed to confirm these earlier observations.

A third approach, involving IL-4 antagonism as a potential treatment for asthma and other related allergic diseases, is to use a variant form of IL-4 called pitrakinra. This IL-4 cytokine has two functional mutations, which result in a molecule that potently inhibits binding of IL-4 and IL-13 to the IL-4R- α complex on the cell-surface membrane. Early studies in cynomolgus monkeys treated with this compound by either subcutaneous injection or nebulization confirmed that this treatment protected monkeys from allergen-induced airway eosinophilia and AHR [89]. To date, there have been two independent randomized, double-blind, placebo-controlled Phase IIa clinical trials in patients with atopic asthma treated with either pitrakinra or placebo [89]. In the first trial, patients were randomized to receive either pitrakinra or placebo by once-daily subcutaneous injection. In the second trial, patients were randomized to receive either pitrakinra or placebo by twice-daily nebulization. Inhaled allergen challenge was carried out before treatment and after 4 weeks of treatment. The primary study end point for the first study was maximum percentage decrease in FEV₁ over a 4–10-h period following allergen challenge, whereas in the second study, the end point was the average percentage decrease in FEV₁ over a 4–10-h period following allergen challenge. The first trial found a small but not significant attenuation of the allergen-induced FEV₁ decrease in favor of pitrakinra, and there were fewer asthma-related adverse events in the pitrakinra-treated group compared with the placebo-treated group. The second trial showed a more substantial effect of twice-daily nebulized pitrakinra in significantly protecting against an allergen-induced late asthmatic response ($p = 0.0001$).

Overall, the results of therapies targeting IL-4 have been disappointing to date, which might reflect redundancies with IL-13 in the immune-mediated inflammatory pathways. The available clinical evidence suggests that there is no role for the use of either the anti-IL-4 mAb pasolizumab or the soluble recombinant human IL-4 receptor altrakincept for the treatment of asthma. However, following the initial beneficial results observed with twice-daily nebulized pitrakinra in attenuating allergen-induced airway responses [89], further clinical studies into the clinical efficacy of this compound are warranted [90].

IL-13 antagonist therapy

IL-13 regulates inflammatory immune responses and is believed to play a key role in the pathogenesis of asthma. It is known to drive epithelial cell maturation and mucus production, promote the generation of extracellular matrix proteins and increase the contractility of airway smooth muscle cells [91]. It is produced by Th2 cells as well as by natural killer T cells, mast cells and basophils, and is similar in activity to IL-4 [92,93]. The activity of the cytokine is mediated through binding to two different cytokine receptor chains that form a heterodimer expressed on IL-13-responsive cells: the

Table 4. Selected IL-4 studies in patients with asthma (altrakincept [soluble IL-4 receptor] and pitrakinra [IL-4 variant]).

Author	Study type	Patient population	Duration of study	Patients (n)	Major study results	Ref.
Borish <i>et al.</i>	Double-blind, placebo-controlled, Phase I/II of altrakincept	Moderate asthma for over 1 year (with ICS use) Age over 18 years, positive skin-prick test	29 days	25 (8 in placebo group; 8 in 500 µg nebulizer-dose group; 9 in 1500 µg nebulizer-dose group)	Statistically significant improvement in the FEV ₁ for the higher concentration group Improved asthma symptom scores	[87]
Borish <i>et al.</i>	Double-blind, randomized, placebo-controlled dose-finding study for altrakincept	Poorly controlled asthma requiring ICS	12 weeks	62 (16 in placebo group; 15 in 0.75-mg group; 16 in 1.5-mg group; 15 in 3-mg group)	Placebo group had decrease in FEV ₁ , not observed in 3-mg group and better asthma symptom scores were observed in 3-mg group	[88]
Wenzel <i>et al.</i>	Double-blind, randomized, placebo-controlled, parallel group, Phase IIa	Atopic asthma (measured by reactive airways), Age over 18 years	1 month	56 (12 in sc. placebo group; 12 in sc. pitrakinra group; 16 in nebulizer placebo group; 16 in nebulizer pitrakinra group)	For sc. administration, no significant change was observed in FEV ₁ between the groups For nebulizer group, there was a statistically significant drop in FEV ₁ in placebo group compared with pitrakinra group	[89]

FEV₁: Forced expiratory volume in 1 s; ICS: Inhaled corticosteroid; sc.: Subcutaneous.

IL-13R- α 1 and the IL-4R- α chains [94]. This receptor is activated by both IL-13 and by IL-4 [95]. In mouse models, administration of IL-13 to the lungs or targeted overexpression of IL-13 in lung tissue triggers asthma-like phenotypes, including airway eosinophilia, mucus-cell metaplasia, airway fibrosis and AHR [96–100].

In asthmatic individuals, IL-13 is present at increased levels in bronchial tissue and in induced sputum [101], and in nasal lavage fluid [102]. In addition, both IL-13 mRNA and protein levels are further increased in bronchoalveolar lavage (BAL) fluid in asthmatic subjects following allergen challenge [103,104]. The gene for IL-13 is located on chromosome 5q31 and this region has been linked to asthma and related phenotypes [105–107]. In further support of the role of IL-13 in asthma, genetic polymorphisms of IL-13 have been linked with increased risk of atopic disease. Specifically, the R110Q variant of IL-13 has been linked to high serum IgE titers, as well as to increased occurrence of allergy and atopic dermatitis [108,109].

Human clinical studies examining the role of IL-13 antagonists in the treatment of asthma and related allergic diseases are in the early stages of development. The human anti-IL-13 IgG4 monoclonal antibody CAT-354 has been tested in a murine model of IL-13 mediated airway and esophageal inflammation. Results in this study show that if mice are pretreated with the anti-IL-13 antibody before exposure to IL-13, there is a significant reduction in airway eosinophilia and airway reactivity [110]. The CAT-354 mAb is currently being evaluated in a double-blind placebo controlled study in patients with refractory asthma whose symptoms remain uncontrolled despite optimal treatment [204]. A second

monoclonal antibody against IL-13, termed QAX576 is also being examined in Phase I and II clinical studies to assess the safety, tolerability, pharmacokinetics and dynamics, and clinical efficacy of various intravenous dosages of QAX576 in patients with moderate allergic asthma [205].

IL-9 antagonist therapy

IL-9 is a cytokine produced by CD4⁺ T cells (mainly of the Th2 subtype) [111,112]. It is also expressed by human eosinophils and mast cells [113]. IL-9 stimulates the proliferation of activated T cells, promotes the proliferation and differentiation of mast cells and increases production of IgE by B cells [114]. IL-9 has been linked to the pathogenesis of asthma in multiple ways. Mapping studies in both mice and humans have shown a linkage between AHR regulation and the IL-9 gene locus [115,116]. In addition, the introduction of IL-9 into the lungs of mice triggers lung eosinophilia [117]. In clinical studies, patients with allergic asthma have been shown to have increased levels of both the IL-9 protein and its receptor in BAL fluid [113]. Experiments in which the actions of IL-9 have been blocked in mice have confirmed that intravenous administration of an anti-IL-9 mAb (compared with a isotype control mAb), followed by allergen exposure, resulted in decreased expression of IL-4 and IL-13 in BAL fluid, decreased airway eosinophilia and decreased AHR [118].

There are currently a number of human clinical trials underway to assess the effects of a humanized anti-IL-9 mAb in the treatment of asthma. This compound, known as MEDI-528,

has been examined in an open-label Phase I trial in healthy volunteers, as well as in a Phase IIa multicenter, randomized trial of patients with atopic asthma. In this latter trial, the primary study end point was mean maximum decline of FEV₁, while the secondary end point was safety [206,207]. The results from these trials have not yet been published, although the pharmaceutical company involved in the manufacture of MEDI-528 is currently undertaking two further Phase II clinical trials to further assess the clinical efficacy of MEDI-528 in the treatment of asthma [208,209]. This suggests that initial results from the earlier Phase I and IIa trials are likely to have been encouraging.

IL-2 antagonist therapy

The soluble IL-2 receptor is a marker of T-cell activation and is upregulated in the serum of children during the time of acute asthma exacerbations [119]. This upregulation may be proportional to exacerbation severity. Humanized mAbs directed against the soluble IL-2 receptor (e.g., daclizumab [Zenapax®]) have been approved for use in therapy to prevent renal allograft rejection. There are currently several small studies underway to evaluate the potential efficacy and safety of this compound in asthma and other immune-mediated diseases but results from these studies have not yet been published [120,210].

Expert commentary

The development of biological compounds represents an exciting advance in the management of asthma and related immune-mediated inflammatory diseases. These compounds offer an advantage over currently available glucocorticosteroid therapies by specifically targeting components of the immunologic cascade, allowing patients to either reduce or discontinue their glucocorticosteroid treatment, thereby minimizing steroid-related adverse effects. Another potential advantage of biological compounds is that they may provide additional anti-inflammatory benefits, over and above those provided by glucocorticosteroid therapy, for patients who continue to have evidence of refractory asthma. In this context, the use of anti-TNF- α antagonists may be effective as a treatment for those asthma patients with predominantly neutrophilic airway inflammation, which appears to be relatively resistant to glucocorticosteroid treatment; however, clinical studies examining the use of these agents in specific inflammatory asthma phenotypes have not yet been published, and there are several unanswered questions regarding the appropriate use of biological compounds for the treatment of asthma and related diseases.

The anti-IgE mAb omalizumab is already used for the treatment of allergic asthma, where it has been shown to decrease asthma-related exacerbations and reduce the dose of maintenance glucocorticosteroid treatment required. Consequently, recent updates to asthma guidelines recommend omalizumab as an alternative treatment option to oral prednisone for patients who continue to experience asthma symptoms despite being on high-dose ICS and inhaled long-acting β_2 -agonists or leukotriene modifiers [121,122]. It is important to note that, while

several studies have evaluated omalizumab in the treatment of certain allergic diseases other than asthma, such as allergic rhinosinusitis, the compound is currently only approved for the treatment of moderate-to-severe persistent atopic asthma, and its potential role for other allergic diseases has yet to be clearly defined.

A number of other biological therapies are currently in various stages of clinical development and several are undergoing evaluation in human clinical trials. While none of these are currently approved for the treatment of asthma or other related diseases, Phase III studies with the anti-IL-5 mAb, mepolizumab, and with anti-TNF- α mAbs, suggest that these compounds offer the prospect that at least these two approaches may be useful treatment options for those individuals with asthma who are refractory to high-dose glucocorticosteroid treatment.

Five-year view

Clinical studies to date have largely evaluated the safety and efficacy of various biological compounds for the treatment of asthma. It is probable that there will be a proliferation of studies over the next 5 years in which existing biological compounds are evaluated in related allergic diseases (e.g., allergic rhinitis, acute bronchopulmonary aspergillosis, atopic dermatitis and food allergies). Future studies will probably also address specific questions, including whether compounds such as omalizumab are able to reverse or attenuate aspects of airway remodeling and whether certain compounds, such as anti-TNF- α antagonists, are likely to be more effective in inflammatory asthma phenotypes, for example, in those patients with predominantly neutrophilic airway inflammation. Future studies may also consider whether combinations of biological compounds, each targeting a specific aspect of the inflammatory cascade, are more efficacious than either agent alone. It should be anticipated that biological compounds will become increasingly established as an appropriate option for those asthma patients whose symptoms are either refractory to glucocorticosteroid treatment or those who simply cannot tolerate the adverse effects associated with glucocorticosteroid treatment.

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Key issues

- Immune-mediated airway inflammation is a characteristic feature of asthma.
- Currently, inhaled corticosteroid treatment is considered to be the cornerstone of asthma management but a proportion of asthmatic patients are refractory to this therapy.
- Prednisone is associated with steroid-induced systemic adverse effects and there is a pressing need to develop alternative anti-inflammatory therapies for patients with refractory asthma.
- The anti-IgE monoclonal antibody (mAb) omalizumab has been approved for the treatment of allergic asthma and has been shown to decrease the incidence of exacerbations and hospitalizations and to improve asthma-related quality-of-life scores.
- Clinical studies using two antihuman IL-5 mAbs have confirmed that these agents result in a profound decrease of peripheral blood eosinophilia but that, at the doses administered, they did not abrogate tissue eosinophilia and did not improve airway hyperresponsiveness.
- A recently completed, but as-yet-unpublished, study using the anti-IL-5 mAb mepolizumab suggests that this compound may be associated with a significant reduction in asthma exacerbations.
- The results of several clinical trials evaluating anti-TNF- α mAbs for the treatment of asthma have been inconsistent. However, anti-TNF- α therapy may be an appropriate treatment option for patients with predominantly neutrophilic airway inflammation.
- Further clinical studies are required to properly determine the roles of anti-IL-13, anti-IL-4 and anti-IL-2-receptor therapies in asthma.
- Identifying patients likely to respond to biologic treatments will be important in order to help justify the high cost of these treatments.

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