
Anti-IgE and Biologic Approaches for the Treatment of Asthma

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Abstract

Current asthma treatments are effective for the majority of patients with mild-to-moderate disease. However, in those with more severe refractory asthma, agents other than inhaled corticosteroids and beta-agonists are needed both to better manage this group of patients and to avoid the side effects of high-dose corticosteroids and the social and personal hardship endured. Several biological pathways have been targeted over the last 20 years, and this research has resulted in pharmacological approaches to attempt to better treat patients with severe refractory asthma. The flagship of the biologics, the anti-IgE monoclonal antibody, omalizumab, has proven efficacious in selected subgroups of asthma patients. Tailoring asthma treatments to suit specific subtypes of asthma patients is in

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keeping with ideals of personalized medicine. Research in the complex interplay of allergens, epithelial host defenses, cytokines, and innate and adaptive immunity interactions has allowed better understanding of the mechanics of allergy and inflammation in asthma. As a result, new biologic treatments have been developed that target several different phenotypes and endotypes in asthma. As knowledge of the efficacy of these biological agents in asthma emerges, as well as the type of patients in whom they are most beneficial, the movement toward personalized asthma treatment will follow.

Keywords

Anti-IgE • Biologics • Monoclonal antibodies • Severe refractory asthma

1 Introduction

The pathophysiological mechanisms underlying asthma are characterized by discordant responses among various cell types, including airway epithelial and smooth muscle cells, mesenchymal cells, and the hematopoietic cells of the adaptive and innate immune systems. The interplay among these cell groups involves a complex pattern of cytokine-driven processes, resulting in cell migration and recruitment, pro-inflammation, and antiapoptotic and proliferative states.

The significant majority of patients with a diagnosis of asthma respond well to conventional inhaled treatments. However, about 5% of asthmatics have severe refractory asthma that fails to achieve an adequate response to both high-dose inhaled corticosteroids (ICS) and systemic corticosteroid treatment. These patients also account for 50% of the health expenditure on asthma (Turner et al. 2011), and the more severe the disease, the greater the healthcare cost (Jacob et al. 2016). The pathophysiology of severe refractory asthma differs from milder asthma with more persistent chronic airway inflammation, steroid resistance, and airway remodeling (Bergeron et al. 2006). Asthma treatments used in the severe asthma population, especially systemic corticosteroids and xanthine derivatives, have well-established side effect profiles (Pandya et al. 2014). Other therapies with better or equivocal clinical efficacy and fewer side effects are required.

Asthma is not a single disease entity and represents a heterogeneous mix of several overlapping phenotypes, characterized by varying cellular immunogenic and inflammatory pathways (Anderson 2008). Atopic asthma is an immune-inflammatory response mediated by T-helper-type (Th2) lymphocytes. The atopic asthma phenotype arises from a complex interplay between the innate and adaptive immune system (Woodruff et al. 2009). Multiple allergens such as pollens, house-dust mite, and animal dander have proteolytic properties. Trace amounts of bacterial constituents such as lipopolysaccharide (LPS) and viral RNA or DNA cause activation of the innate and adaptive immune system. These pathways of the innate and adaptive immune system are stimulated by these environmental and/or endogenous pathogen-associated molecular patterns (PAMPs) and danger-associated

molecular patterns (DAMPs) that influence the activation and trafficking of dendritic cells (DCs), cytokine production, and the hematopoietic system (Holgate 2012).

Airway epithelium exposed to inhaled allergens can prime Toll-like receptor (TLR) class of pattern recognition receptors involved in innate immunity. Activation of TLR generates the production of innate epithelial-derived cytokines such as thymic stromal lymphopoietin (TSLP) and interleukin-25 (IL-25) and interleukin-33 (IL-33) (known collectively as the epithelial alarmins) which elicit the development of Th2 adaptive responses (Gregory and Lloyd 2011). The “phenotyping” of asthma by its distinct functional and pathophysiological contrivances has allowed a more targeted and personalized approach to treat asthma. T-helper (Th) cell subsets secrete specific cytokines, which, in turn, influence the development and perpetuation of systemic inflammation. Th cells and their cytokines can be categorized into two general subsets, termed Th1 and Th2. The cytokines generated by Th1 or Th2 cells inhibit the cellular function of the other phenotype. As examples, interferon gamma and interleukin (IL)-12, which are produced by Th1 cells, inhibit Th2 cell proliferation, and IL-4 and IL-10, which are produced by Th2 cells, inhibit Th1 cytokine production. The concept that specific types of immune response are dominated exclusively by Th1 or Th2 response is now recognized as too simplistic to explain allergic or inflammatory disease in their entirety. However, many biologic therapies have been developed for the purpose of targeting the production of either Th1 or Th2 pro-inflammatory cytokines. Novel therapies have been developed that are more specific to targeting these phenotypes. The content of this chapter will discuss biologic therapies. Biologics, also known as “biologicals,” are by definition an array of protein-based therapies (Cook and Bochner 2010). The majority of biologics studied or used in asthma have been monoclonal antibodies (Table 1).

2 Anti-IgE Biologic Treatments

The anti-IgE monoclonal antibody omalizumab was the first biologic treatment approved for the treatment of allergic asthma. In 1921, Prausnitz and Küstner described the passive transfer of an allergen-specific response in the skin with serum (Frankland 2004). Forty-five years later, Ishizaka and Ishizaka and Johansson and Bennich identified the protein as being immunoglobulin IgE (Ishizaka et al. 1967). Further studies determined the mechanism of cell activation through cross-linkage of cell-bound IgE on mast cells and basophils. This cross-linkage was shown to involve dimerization of the high-affinity IgE receptor, FcεR1. Cross-linkage leads to noncytotoxic degranulation and production of newly formed lipid mediators. These lipid mediators are responsible for the allergic responses. The discovery that both mast cells and basophils can also release preformed and newly generated cytokines, chemokines, and growth factors helps explain how the acute allergic response transits into late phase and the more chronic responses associated with leukocyte recruitment and activation of tissue remodeling

Table 1 Biologicals evaluated for the treatment of asthma

Target cytokine/ receptor or antibody	Drug name	Mechanism of action	Developmental status	Reference
IgE	Omalizumab	Blocks IgE to FcεRI–Ig fusion protein and membrane FcεRI	Approved by the FDA and EMA	Johansson et al. (2002)
	Ligelizumab	Binds Cε3 domain of IgE	Phase IIa trial 2014 completed	Arm et al. (2014)
	CMAB007	Blocks IgE to FcεRI–Ig fusion protein and membrane FcεRI	Phase I study 2012 completed	Zhou et al. (2012)
IL-4/IL-13	Dupilumab	Binds IL-4Rα inhibiting both IL-4 and IL-13 signaling	Phase I study 2014 completed	Wenzel et al. (2013)
	AMG 317	AMG 317 is a fully human monoclonal IgG2 antibody to IL-4R	Phase II study 2010 completed ^a	Corren et al. (2010)
	Pitrakinra	Recombinant human IL-4 variant that inhibits both IL-4R and IL-13R	Phase IIb study 2011 completed ^a	Wenzel et al. (2007)
	Altrakincept	Soluble recombinant human IL-4R	Phase III trial completed ^a	Borish et al. (1999)
	Pascolizumab	Humanized mAb blocking IL-4	Phase II trial completed ^a	Hart et al. (2002)
IL-13	Lebrikizumab	IgG4-humanized monoclonal antibody that binds IL-13	Phase IIb trial completed. Ongoing trials	Scheerens et al. (2014)
	Tralokinumab	Human monoclonal antibody that neutralizes IL-13	Phase III trial ongoing	Piper et al. (2013)
IL-5	Mepolizumab	Anti-IL-5 humanized IgG1 monoclonal antibody	EBM approval 2014	Nair et al. (2009)
	Reslizumab	Humanized monoclonal anti-IL-5 antibody	Phase III trial completed	Lim and Nair (2015)
	Benralizumab	Humanized, afucosylated monoclonal antibody against IL-5Rα	Phase IIb trial completed	Nowak et al. (2015)
IL-9	MEDI-528	Humanized IgG1 monoclonal antibody that binds to IL-9	Phase IIb completed ^a	White et al. (2009)
IL-17 and IL-23	Brodalumab	Human IL-17RA-specific monoclonal antibody	Phase II ongoing 2015	Busse et al. (2013)

(continued)

Table 1 (continued)

Target cytokine/ receptor or antibody	Drug name	Mechanism of action	Developmental status	Reference
	Secukinumab	Monoclonal antibody that neutralizes IL-17A	Phase II, ongoing 2015	Wakashin et al. (2008)
TSLP	AMG 157	Human anti-TSLP monoclonal antibody that binds TSLP	Phase I completed	Gauvreau et al. (2014)
TNF	Infliximab	Human–murine chimeric anti-TNF α monoclonal antibody	Phase II completed ^a	Erin et al. (2006)
	Etanercept	Soluble TNF α receptor fusion protein	Phase II completed ^a	Holgate et al. (2011)
	Golimumab	Fully human TNF α -blocking antibody	Phase II trial (withdrawn) ^a	Wenzel et al. (2009)
CCR4	Mogamulizumab	Defucosylated	Phase I trial 2011 ^a	Subramaniam et al. (2012)
C5	Eculizumab	Humanized monoclonal antibody cleaves and deactivates C5	Phase II 2008 completed ^a	Smith et al. (2012)

EMA European Medicines Agency

^aNo further studies ongoing or yet planned (www.clinicaltrials.gov)

pathways. Fc ϵ R1 has also been found on antigen-presenting dendritic cells (DCs) where they function to facilitate the uptake and processing of allergens that augment sensitization (Sharquie et al. 2013).

Blocking IgE, without causing membrane-bound cross-linkage, had been a target for asthma drug development for many years (Holgate 2014). During the allergic immune response, allergen-specific B cells are stimulated by IL-4 and IL-13, provided by Th2 cells, and begin IgE production. IgE antibodies act by arming cells bearing either high-affinity IgE receptors (Fc ϵ RI) such as mast cells, basophils, and dendritic cells or low-affinity IgE receptors (Fc ϵ RII, CD23) such as B cells, monocytes, and other inflammatory cells (Heusser and Jardieu 1997). A breakthrough came about when an α Fc ϵ RI-IgG fusion protein was shown to inhibit passive cutaneous anaphylactic responses (Haak-Frendscho et al. 1993). A further discovery was the identification that the C ϵ 3 region of the Fc fragment of IgE binds very selectively to a particular component of the α -chain of the tetrameric Fc ϵ R1 (α 1 β 1 γ 2); C epsilon4 is not required for binding (Baird et al. 1986). This feature of IgE allows the C ϵ 3 region to be targeted and blocked without causing cross-linking of IgE bound to its high-affinity receptor. A chimeric IgG monoclonal antibody that was active in humans was produced in mice. This antibody was shown to be

effective in reducing circulating IgE and most importantly without causing an anaphylactic response (Corne and Holgate 1997). Omalizumab was created by humanizing a non-anaphylactogenic IgG1 antihuman IgE mAb that contained only the antigen-binding site as mouse sequences. Omalizumab is a recombinant humanized monoclonal antibody, which specifically binds to the Cepsilon3 domain of IgE that is the site of high-affinity IgE receptor. It is therefore able to reduce free IgE levels and avoid binding IgE to Fcε RI (this binding induces consequent cross-linking of IgE triggering degranulation and synthesis of new-generated chemical mediators) (Easthope and Jarvis 2001).

Omalizumab expresses a high degree of isotype specificity and can neutralize serum-free IgE without affecting other antibody classes. Omalizumab is administered via a subcutaneous injection. Its bioavailability is 62% with an estimated half-life elimination of 26 days. The time to peak levels is 7–8 days. Excretion is primarily via hepatic degradation and intact IgG may be secreted in the bile (Hayashi et al. 2007; Lowe et al. 2009).

Initial studies with omalizumab showed attenuation of allergen-induced asthmatic responses (Fahy et al. 1997), followed by studies demonstrating improvements in asthma symptoms and health-related quality of life (Busse 2001) and a significant reduction in the frequency of asthma exacerbations in allergic asthmatic patients treated with omalizumab (Sorkness et al. 2013). Furthermore, omalizumab was also used in the treatment of children with allergic asthma, demonstrating improvements in health-related quality of life as well as a significant dosage reduction of ICS (Busse et al. 2011). Omalizumab given to patients with allergic rhinitis resulted in a rapid, but importantly dose dependent, suppression of circulating serum-free IgE levels. Omalizumab significantly improves health-related quality of life and nasal symptoms in patients with seasonal allergic rhinitis (Stokes and Casale 2015). Omalizumab has been evaluated in patients with severe refractory asthma. The INNOVATE study demonstrated that treatment with omalizumab significantly reduced the rate of severe asthma exacerbations by 50% and the rate of total emergency visits for asthma. A greater proportion of patients receiving omalizumab achieved a clinically meaningful Asthma Quality of Life Questionnaire (AQLQ) score (greater than a 0.5-point) improvement from baseline compared with placebo recipients (Humbert et al. 2005).

Omalizumab has been shown to have a beneficial effect on airway remodeling in asthmatic patients. One study investigated the effect of long-term anti-IgE on the thickening of the reticular basement membrane (RBM) and eosinophil infiltration in bronchial biopsies from patients with severe persistent allergic asthma before and after 12 months of treatment with omalizumab. The study showed that a substantial proportion of severe asthmatics had reduced the levels of airway eosinophil and RBM thickness after treatment with omalizumab (Riccio et al. 2012). In another study omalizumab demonstrated reduced airway wall thickness and airway inflammation that correlated to a lowering of the sputum eosinophil count and increase in the FEV₁ (Hoshino and Ohtawa 2012).

The optimal duration of treatment of omalizumab is not known. One study suggested a reemergence of asthma symptoms with increasing serum-free IgE

levels when omalizumab is stopped (Slavin et al. 2009). Another study followed 18 patients for 3 years after withdrawal of omalizumab reported improved or unchanged asthma compared with ongoing omalizumab treatment (Nopp et al. 2010).

The safety profile of omalizumab has been extensively studied. Pooled data from 7,500 subjects from all randomized trials indicate an overall incidence of adverse events with omalizumab similar to that in the placebo or control groups (Corren et al. 2009). No cases of serum sickness were reported. None of the omalizumab recipients developed measurable anti-omalizumab antibodies (Corren et al. 2009). Anaphylaxis was reported in 124 patients treated with omalizumab from June 2003 to Dec 2006, equating to a rate of 2 per 1,000 patients treated. Some of these cases of anaphylaxis occurred more than 2 h after omalizumab administration (Limb et al. 2007).

Anti-parasite IgE has been associated with immunity against a range of helminth infections, and IgE and its receptors have evolved to help defend from parasite infection (Fitzsimmons et al. 2014). A 52-week, randomized, double-blind, parallel-group, placebo-controlled study in patients deemed at high risk of geohelminth infection was found to have a slightly, but not significantly, higher number of patients with one or more geohelminth infections during treatment in the omalizumab group (odds ratio 1.47, 95% CI 0.74–2.95) (Cruz et al. 2007). Clinically, the severity of infection and response to anthelmintic treatment was unaltered by omalizumab therapy (Cruz et al. 2007).

Initial comparative analysis from clinical trials revealed a worryingly higher incidence of malignancy in the omalizumab group (0.5%) when compared to the placebo group (0.2%) (Busse et al. 2012). However, when compared to the incidence of malignancy in the general population, the incidence of malignancy in the omalizumab group was found to be nonsignificant (Corren et al. 2009). The incidence of primary malignancy per 1,000 patient years was 4.14 in the omalizumab group compared with 4.45 in the placebo group (Busse et al. 2012). To establish the potential risk for malignancy during routine clinical practice, a prospective post-marketing safety evaluation was requested by the FDA (Long et al. 2014). Both the omalizumab and the non-omalizumab cohorts were followed for approximately 5 years. Malignancy rates were similar in the omalizumab and non-omalizumab cohorts, with a hazard ratio, adjusted for confounders and risk factors, of 1.09 (95% CI, 0.87–1.38) for all malignancies and 1.15 (95% CI, 0.83–1.59) for all malignancies excluding nonmelanoma skin cancer (Long et al. 2014). It is worthwhile noting that the protocol excluded persons with any increased risk of malignancy or cancer history (Long et al. 2014). The FDA has advised that they are continuing to monitor as data emerges on omalizumab.

Omalizumab does cross the placental barrier and this suggests that the potential for fetal harm exists. Omalizumab is therefore not recommended for use during pregnancy unless absolutely necessary. The omalizumab pregnancy registry was a prospective observational study of pregnant women exposed to one or more doses of omalizumab within 8 weeks prior to conception or at any time during pregnancy. The prevalence of major congenital defects was no higher than that reported in the

general population with asthma. In addition, omalizumab does not appear to increase the risk of preterm birth or small for gestational age infants beyond that seen in the general asthma population (Namazy et al. 2015).

Maximizing control of free unbound IgE has been suggested to offer superior asthma symptom control (Lowe et al. 2009). Ligelizumab is another humanized IgG1 monoclonal antibody that binds with very high affinity to the Cε3 domain of IgE. Ligelizumab may achieve superior IgE suppression than omalizumab and lead to better clinical outcomes. Two small trials have been conducted with ligelizumab; one administered ligelizumab intravenously and the other subcutaneously (Arm et al. 2014). Ligelizumab demonstrated higher-affinity binding for human IgE compared with omalizumab, with an equilibrium KD of 139 pm vs. 6.98 nm, respectively. These trials concluded that treatment with ligelizumab provided greater and longer suppression of free IgE and IgE on the surface of circulating basophils and markedly superior suppression of skin prick test responses to allergen compared to omalizumab. Another humanized anti-IgE monoclonal antibody, CMAB007, was studied in a phase I, single-arm, open-label study. No anti-CMAB007 antibodies were detected after dosing in any subject. Subcutaneous administration of CMAB007 was well tolerated and seemed to be effective in reducing free IgE in healthy Chinese volunteers. Further trials have yet to be conducted (Zhou et al. 2012).

Omalizumab was approved by the US Food and Drug Administration (FDA) in 2003 for persons 12 years of age and older with moderate-to-severe persistent allergic asthma whose symptoms are inadequately controlled with ICS (Hussar 2004). In the European Union, omalizumab is approved for the treatment of patients 6 years and older with severe persistent allergic asthma inadequately controlled with ICS and LABA.

3 Anti-IL-4/IL-13 Biologics

IL-4, a multifunctional pleiotropic cytokine, was discovered in the mid-1980s (REF). This mediator is produced mainly by activated T cells but also by mast cells, basophils, and eosinophils (Nelms et al. 1999). There is significant functional homogeneity between IL-4 and IL-13 as they play their biological roles by activating a heterodimeric receptor complex consisting of the IL-4 receptor α-subunit (IL-4Rα) and the IL-13 receptor α1-subunit (IL-13Rα1) (Oh et al. 2010).

Functionally, IL-4 is best known for defining the Th2 phenotype of lymphocytes and for regulating cell proliferation, apoptosis, and expression of numerous genes in various cell types, including lymphocytes, macrophages, and fibroblasts, as well as epithelial and endothelial cells (Kelly-Welch et al. 2003). Dupilumab is a fully human monoclonal antibody to the IL-4Rα that inhibits both IL-4 and IL-13 signaling. In a proof of principal study, patients with persistent, moderate-to-severe asthma and an elevated blood or sputum eosinophilia, who used medium-dose to high-dose ICS and LABAs, dupilumab was associated with fewer episodes of loss of asthma control when LABAs and inhaled glucocorticoids were withdrawn, with

improved lung function and reduced levels of Th2-associated inflammatory markers (Wenzel et al. 2013).

Several monoclonal antibodies that have targeted IL-4, or its receptor, have not demonstrated clinical benefit in asthma. AMG 317 is a fully human monoclonal IgG2 antibody to IL-4R. In a phase II, randomized, double-blind, placebo-controlled study, patients received weekly subcutaneous injections of placebo or AMG 317. The primary endpoint was changed from baseline at week 12 in Asthma Control Questionnaire (ACQ) symptom score. AMG 317 did not demonstrate clinical efficacy across the overall group of patients. Clinically, significant improvements were observed in several outcome measures in patients with higher baseline ACQ scores (Corren et al. 2010). Pitrakinra is a recombinant human IL-4 variant that is a potent inhibitor of both the IL-4 and IL-13 receptors (Antoniu 2010). A large clinical trial of 534 patients with moderate-to-severe asthma, who were inadequately controlled on the combination of ICS and LABA, found that the addition of inhaled pitrakinra failed to show clinical benefit compared with placebo (Wenzel et al. 2007). Altrakincept is a soluble recombinant human IL-4R, which inactivates IL-4 without mediating cellular activation. In a small double-blind, placebo-controlled trial, patients with moderate asthma requiring inhaled corticosteroids were randomly assigned to receive a single nebulized dose of altrakincept. Patients in the highest dosing arm required significantly less LABA rescue use. The anti-inflammatory effects were further demonstrated by significantly reduced exhaled nitric oxide (Borish et al. 1999). Finally, pascolizumab is a humanized anti-IL-4 monoclonal antibody that also failed to demonstrate clinical benefit (Hart et al. 2002).

IL-13 increases goblet cell differentiation, promotes eosinophil migration to the airway, activates fibroblasts enabling their transformation into myofibroblasts, and switches B-cell antibody production from IgM to IgE (Corren 2013). IL-13R is a heterodimer complex consisting of IL-13R α 1 and IL-4R α , expressed on B cells, monocytes and macrophages, dendritic cells, eosinophils, basophils, fibroblasts, endothelial cells, airway epithelial cells, and airway smooth muscle cells (Skowron-zwarg et al. 2007). Several biologics have been developed targeting either IL-13 or its receptor IL-13R. Lebrikizumab is an IgG4-humanized monoclonal antibody that binds IL-13 with high affinity at an epitope that strongly overlaps with the binding site of IL-4 α and inhibits its activity. In a randomized, double-blind study, poorly controlled asthmatics on high-dose ICS received lebrikizumab or placebo. The primary endpoint of FEV₁ significantly increased by 9.8% from baseline compared with 4.3% for placebo (Corren et al. 2010). Secondary endpoints, including ACQ5 and rates of asthma exacerbations, were not significantly reduced by active treatment, although the study was not powered to detect differences in asthma exacerbations. The effect of lebrikizumab on lung function was greatest in patients with high serum periostin levels. In another study, asthmatic subjects, not on ICS, were treated with either lebrikizumab or placebo. Both primary (post-bronchodilator FEV₁ at 12 weeks) and secondary endpoints were not meeting (Noonan et al. 2013).

Tralokinumab is another human anti-IL-13 monoclonal antibody that was studied in a trial involving subjects with uncontrolled asthma. The primary endpoint in this study, ACQ6, was not affected by treatment with tralokinumab compared with placebo. However, changes in FEV1 at week 13 in patients with detectable sputum IL-13 demonstrated a trend toward improvement with tralokinumab (Piper et al. 2013). IMA-638 and IMA-026 are fully humanized IgG1 antibodies that bind to different epitopes on IL-13 and neutralize its bioactivity. These antibodies were studied in subjects with mild, atopic asthma challenged with inhaled allergen. The antibody that prevented binding of IL-13 to IL4R α , but not that which prevented binding to IL-13R α 1, significantly attenuated allergen-induced early and late asthmatic responses (Gauvreau et al. 2014). There was no effect of either antibody on allergen-induced airway hyperresponsiveness or sputum eosinophils.

4 Anti-IL-5 Biologics

Interleukin-5 (IL-5) has a central role in the maturation, recruitment, activation, and enhanced survival of eosinophils. Many studies of allergen-induced airway responses in mice suggested IL-5 plays an important role in allergen-induced airway eosinophilia and airway hyperresponsiveness. Several clinical trials aimed at neutralizing circulating IL-5 or targeting the IL-5R α (Lim and Nair 2015; Mukherjee et al. 2014; Nowak et al. 2015).

The flagship of this class of biologics is the anti-IL-5 humanized IgG1 monoclonal antibody mepolizumab (Pavord et al. 2012). Mepolizumab has a high affinity for binding free IL-5. An early clinical trial in nonselected patients with moderate-to-severe asthma did not demonstrate mepolizumab to be effective in improving asthma symptoms or lung function (Flood-Page et al. 2003). However, two studies with carefully phenotyped patients, with persistently elevated eosinophil counts in blood and sputum and who also had history of frequent exacerbations, demonstrated a significant decrease in the exacerbation frequencies and improvement in asthma control. One of these studies also showed an oral steroid-sparing effect in the mepolizumab arm (Nair et al. 2009). Lower doses of mepolizumab (75 and 100 mg) were chosen in the MENSA study where significant reductions in exacerbation rates (47–53%) compared to placebo were seen (Ortega et al. 2014) (Fig. 1). In the SIRIUS study, mepolizumab 100 mg was shown to have a corticosteroid-sparing effect in the order of 50% (Bel et al. 2014). Mepolizumab has been shown to be clinically beneficial in asthma patients with moderate-to-severe asthma with high sputum and/or blood eosinophil counts and has recently been approved for use in severe eosinophilic asthma patients by the European Medicines Agency.

Reslizumab is another humanized monoclonal anti-IL-5 antibody (IgG4/k) that exhibits an extremely long duration of action in mice, monkeys, and guinea pigs (Egan et al. 1999). In an initial clinical trial, reslizumab caused a reduction in sputum eosinophils and a significant improvement in lung function in patients diagnosed with severe refractory eosinophilic asthma (Castro et al. 2011). Two

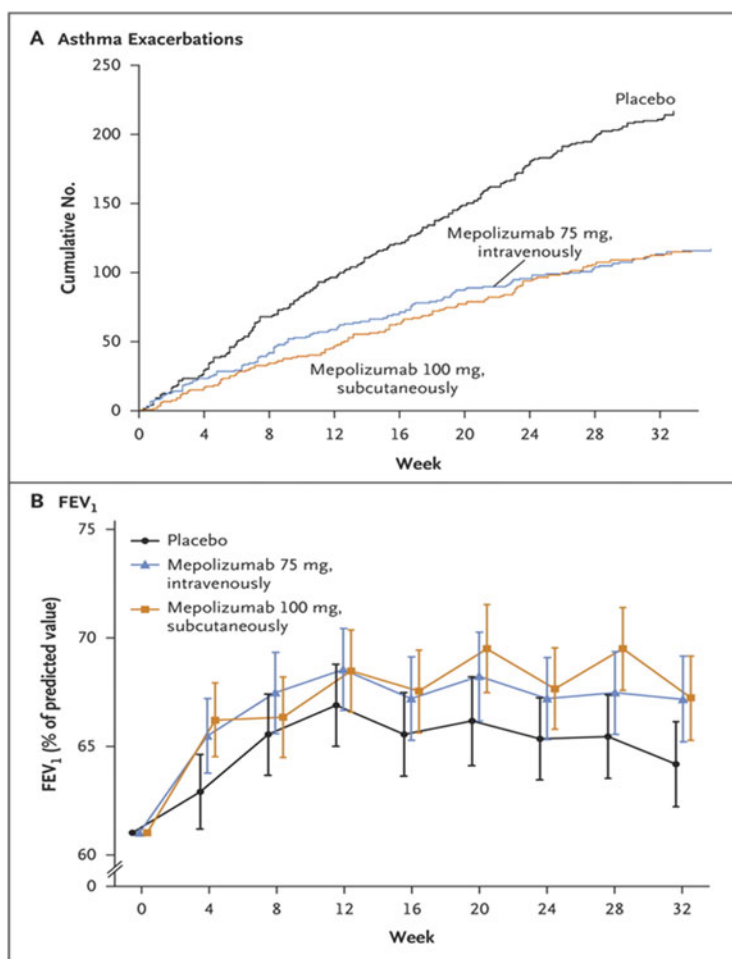


Fig. 1 Effect of mepolizumab treatment or placebo on asthma exacerbations and the forced expiratory volume in 1 second (FEV₁) at 32 weeks of treatment (Reproduced with permission from Ortega et al. 2014)

duplicate, multicenter, double-blind, parallel-group, randomized, placebo-controlled trials have compared reslizumab to placebo (Castro et al. 2015). The inclusion criteria were patients with asthma inadequately controlled by medium-to-high doses of inhaled corticosteroid and with a blood eosinophil count of $>400/\mu\text{L}$ and one or more asthma exacerbations in the previous year. In both studies, patients receiving reslizumab had a significant approximately 50% reduction in the frequency of asthma exacerbations compared with those receiving placebo. Adverse events were similar in both the treatment and placebo groups. In the reslizumab arms, 0.4% of subjects suffered anaphylaxis that responded to conventional treatment (Castro et al. 2015).

IL5R α is expressed by both mature eosinophils and eosinophil-lineage progenitor cells (Rothenberg and Hogan 2006). Benralizumab is a humanized, afucosylated monoclonal antibody. Being afucosylated, benralizumab induces apoptosis in its target cells by enhanced antibody-mediated cellular toxicity. This feature may confer benralizumab with increased efficiency to reduce eosinophil numbers in comparison to the other anti-IL-5 biologics (Ghazi et al. 2012). In a small initial, double-blind, placebo-controlled study, a single-dose intravenous and multiple-dose subcutaneous benralizumab reduced eosinophil counts in airway mucosa and sputum. Benralizumab also suppressed eosinophil counts in the bone marrow and peripheral blood (Laviolette et al. 2013). In another trial, 20–100 mg of subcutaneous benralizumab exhibited significant improvement in annual exacerbation rates, lung function, and asthma scores, with greater benefits seen in patients with blood eosinophil levels >400 cells/ μ l (Castro et al. 2014).

5 Anti-IL-9 Biologics

IL-9 was first described in the mouse as a T cell and mast cell growth factor (Hultner et al. 1990). The function of IL-9 in asthma and allergy has been investigated by research into its pleiotropic activities on cell types associated with allergic diseases including Th2 lymphocytes, mast cells, B cells, eosinophils, and airway epithelial cells (Xing et al. 2011). In the largest human clinical study of anti-IL-9 to date, 327 subjects were randomized to subcutaneous placebo or MEDI-528 in addition to their usual asthma medications. The authors concluded that the addition of MEDI-528 to existing asthma controller medications was not associated with any improvement in asthma control, asthma exacerbation rates, or FEV₁ (Oh et al. 2010).

6 Anti-IL-17 and Anti-IL-23 Biologics

IL-17A, IL-17F, and IL-23 (IL-23 is a growth factor and inducer of pro-inflammatory Th17 cells, which secrete IL-17 (Aggarwal et al. 2003) can upregulate the antigen-induced recruitment of both neutrophils and eosinophils into the airways (Nakajima and Hirose 2010). In vitro, IL-17A and IL-17F have been shown to augment glucocorticoid receptor-beta expression in bronchial epithelial cells that correlates with decreased cellular response to dexamethasone (Vazquez-Tello et al. 2010). IL-17 cytokines may have a role in steroid-resistant asthma (Vazquez-Tello et al. 2013). They have been shown to be upregulated in bronchial biopsy samples obtained from patients with severe asthma (Al-Ramli et al. 2009). In a randomized placebo-controlled trial of patients with severe asthma that was not adequately controlled by ICS and LABAs, a human IL-17RA-specific monoclonal antibody, brodalumab, failed to show clinical benefit for the overall study population (Busse et al. 2013). Secukinumab, an anti-IL-17 monoclonal antibody that selectively neutralizes IL-17A, is currently being studied in patients

with asthma. Another treatment approach is the use of biologics directed against the IL-17-regulating cytokine IL-23 targeting inhibition of IL-17-dependent recruitment of neutrophils, eosinophils, and lymphocytes into the airways of sensitized mice (Wakashin et al. 2008).

7 Anti-IL-25 Biologics

IL-25, an epithelial-derived cytokine, also known as IL-17E, is a member of the IL-17 cytokine family and is expressed by Th2 cells and activated mast cells, basophils, and eosinophils (Corrigan et al. 2011). The systemic administration of IL-25 in mice causes eosinophilia via the production of IL-5 and enhances the production of other Th2 cytokines such as IL-4, IL-13, and eotaxin (Fort et al. 2001; Tamachi et al. 2006). IL-25 acts principally on its own receptor called IL-25R, a 56-kd single transmembrane protein composed of two subunits, IL-17RA and IL-17RB. IL-25R expression is found on T cells, eosinophils, mast cells, and endothelial cells and is upregulated in response to allergen challenge (Corrigan et al. 2011). Increased plasma levels of IL-25 are seen in allergic asthmatic subjects, and the expression of IL-17RB is higher on eosinophils compared to non-asthmatic controls (Tang et al. 2014). Blocking IL-25 in a mouse model of allergic asthma caused a significant reduction in BAL fluid levels of IL-5 and IL-13, serum IgE, and eosinophils and prevented AHR (Ballantyne et al. 2007). There are no studies yet reported of the effects of blocking IL-25 in asthmatic subjects.

8 Anti-IL-33 Biologics

IL-33 is an IL-1-like pro-inflammatory epithelial-derived cytokine (Haraldsen et al. 2009). In its full form, it is biologically active; however, its bioactivity can be increased tenfold following processing by inflammatory proteases such as neutrophil elastase and cathepsin G, whereas processing by caspases inactivates IL-33 (Lefrancais et al. 2012). IL-33 drives production of Th2-associated cytokines, including IL-4, IL-5, and IL-13, by various hematopoietic cells (Yagami et al. 2010). The receptor for IL-33, ST2L, is an IL-1 receptor-related protein expressed on mast cells and, to a lesser extent, on macrophages, hematopoietic stem cells, NKT cells, eosinophils, basophils, innate lymphoid cells (ILC2), and fibroblasts (Neill et al. 2010; Pecaric-Petkovic et al. 2009). Another form of ST2 exists as a soluble receptor, sST2 that acts a decoy ligand for IL-33 (Ho et al. 2013). IL-33 is expressed at higher levels in asthmatic subjects particularly those with severe asthma (Prefontaine et al. 2009). The IL-33/ST2 axis is involved in Th2/IL-31 and Th17 immune response during the progression of allergic airway disease (Jacob et al. 2016). IL-33 also seems to modulate hematopoietic progenitor cells in patients with allergic asthma (Smith et al. 2015). These features make the inhibiting or blocking of the IL-33/ST2 axis an attractive option in pharmacological management of asthma. Anti-IL-33 monoclonal antibody treatments have been reported to

inhibit allergen-induced eosinophilic airway inflammation, mucus hypersecretion, and Th2 cytokine production in mice (Mizutani et al. 2013). Both the membrane-bound ST2 receptor and the soluble ST2 receptor could also both be feasible targets for biologic asthma therapy (Nabe 2014). A study found that in a murine model of allergic asthma, sST2 exerts a negative regulation on OVA-mediated allergic airway inflammation (Lee et al. 2014). While neutralizing the IL-33/ST2R axis in allergic asthma has significant potential clinical benefits in asthma subject, caution needs to be paid to other biological roles IL-33 has and where its inhibition may be harmful (Schiering et al. 2014).

9 Anti-TSLP Biologics

Thymic stromal lymphopoietin (TSLP), an epithelial cell-derived cytokine, is generated in response to pro-inflammatory stimuli and drives allergic inflammatory diseases such as asthma (Ying et al. 2005, 2008). TSLP is thought to cause airway and blood eosinophilia among patients with allergic asthma by activating airway dendritic cells and mast cells and by increasing the numbers of Th2 cells, resulting in the production of pro-inflammatory cytokines, including interleukin-5 and interleukin-13 (Zhou et al. 2005). Given the plethora of cytokines and cellular dynamics, TSLP is an attractive target to inhibit to blunt the allergen-induced airway response in asthma (Ziegler 2012). AMG 157 is a fully human anti-TSLP monoclonal immunoglobulin G2 λ that specifically binds human TSLP and prevents interaction with its receptor (Gauvreau et al. 2014). In a double-blind, placebo-controlled study, 31 patients with mild allergic asthma were randomly assigned to receive three monthly doses of AMG 157 or placebo intravenously. AMG 157 reduced allergen-induced bronchoconstriction and indexes of airway inflammation before and after allergen challenge. AMG 157 attenuated allergen-induced early and late asthmatic responses. In addition, in patients receiving AMG 157, there was a significant decrease in levels of blood and sputum eosinophils before and after the allergen challenge (Fig. 2) (Gauvreau et al. 2014).

10 Anti-TNF Biologics

Tumor necrosis factor alpha (TNF α) is the most widely studied pleiotropic cytokine of the TNF superfamily. In pathophysiological conditions, generation of TNF α at high levels leads to the development of inflammatory responses that are hallmarks of many diseases (Mukhopadhyay et al. 2006). TNF α is stimulated by multiple factors including IL-1, bacterial endotoxins, TNF α itself, platelet-derived growth factor (PDGF), and oncostatin M. In epithelial, endothelial, and fibroblastic cells, secretion of TNF α is induced by IL-17 (Mukhopadhyay et al. 2006).

TNF α has been implicated in the pathogenesis of asthma (Berry et al. 2006; Cembrzynska-Nowak et al. 1993; Thomas and Heywood 2002). Several anti-TNF α biologics have been studied in asthma. In a double-blind, placebo-controlled,

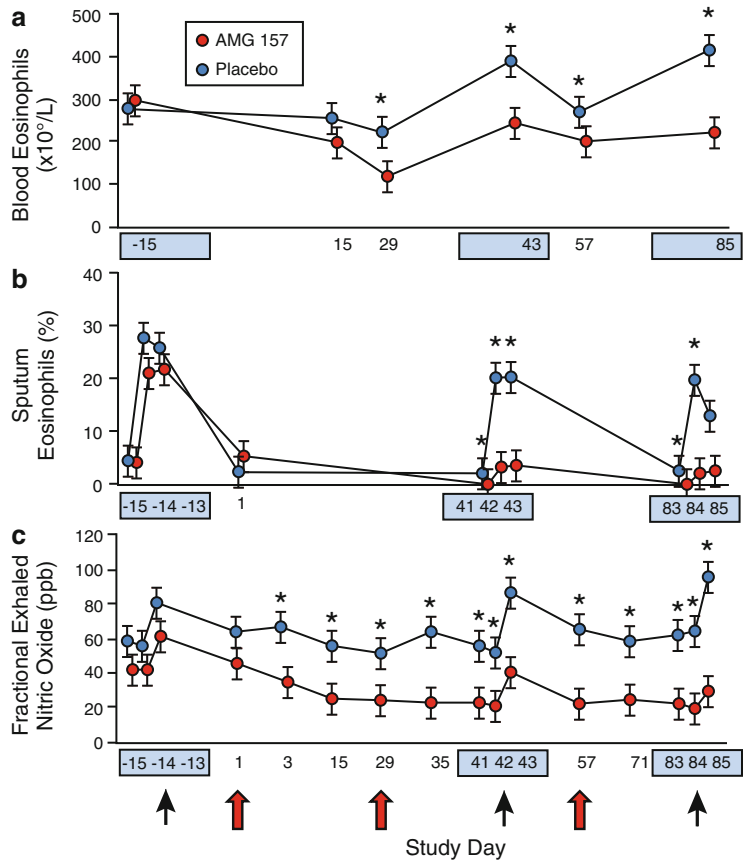


Fig. 2 Effect of treatment with an anti-TSLP hMab or placebo on blood and sputum eosinophils and FeNO before and after allergen inhalation challenge in mild allergic asthmatic subjects. *Red arrows* indicate treatment administered monthly for 3 months. *Black arrows* indicate allergen inhalation challenge before the treatment started (day 14) and after 6 weeks (day 42) and 12 weeks of treatment (day 84). (Drawn from data from Gauvreau et al. 2014)

parallel-group design study in 38 patients with moderate asthma treated with inhaled corticosteroids, but symptomatic during a run-in phase, the recombinant human–murine chimeric anti-TNF α monoclonal antibody infliximab caused a decrease in the number of patients with exacerbations (Erin et al. 2006). Two small trials using etanercept, a soluble TNF α receptor fusion protein administered to patients with severe asthma, demonstrated clinical benefits (Berry et al. 2006; Howarth et al. 2005). However, in a larger randomized, double-blind, placebo-controlled study involving 132 asthmatic subjects with moderate-to-severe persistent asthma over 12 weeks, etanercept was not shown to provide clinical benefit (Holgate et al. 2011). Golimumab (Simponi; Centocor Ortho Biotech), a fully human TNF α -blocking antibody, was assessed in a large multicenter, placebo-

controlled study in patients with uncontrolled severe asthma (Wenzel et al. 2009). No significant improvements in lung function and disease exacerbations were detected. Worryingly serious infectious and neoplastic events were reported, including active tuberculosis, pneumonia, sepsis, and several different malignancies (such as breast cancer, B-cell lymphoma, metastatic melanoma, cervical carcinoma, renal cell carcinoma, basal cell carcinoma, and colon cancer). These findings caused premature termination of the trial. A subgroup analysis of the patients enrolled in the trial identified that the drug possibly benefited older patients with late-onset asthma and a history of hospitalizations or emergency hospital visits during the year before screening and who also had lower baseline FEV1 levels and a post-bronchodilator FEV1 increase of greater than 12% (Wenzel et al. 2009). Overall, conflicting results have been obtained and serious concerns rose with regard to the safety of TNF α blockade in asthma.

11 Anti-granulocyte Monocyte Colony-Stimulating Factor (Anti-GM-CSF)

GM-CSF is a stimulatory growth factor overexpressed in the airway of asthmatic patients and plays a pivotal role in eosinophil recruitment and survival (Park et al. 1998). A human anti-GM-CSF monoclonal IgG1 antibody namilumab has been developed that decreased the survival and activation of peripheral human eosinophils (Krinner et al. 2007). A phase II, double-blind, placebo-controlled, and randomized clinical trial evaluated the safety, tolerability, and efficacy of a single dose (400 mg) of the GM-CSF-targeted monoclonal antibody KB003 in patients with moderate-to-severe asthma inadequately controlled by corticosteroids. It did not achieve significance and further clinical trials have been abandoned.

12 Conclusions

Asthma is a chronic respiratory disorder associated with type 2 airway inflammation as characterized by elevated levels of eosinophils, immunoglobulin E, and cytokines including interleukin (IL)-4, IL-5, IL-9, and IL-13 and tumor necrosis factor (TNF) alpha. However, mounting evidence has shown that considerable heterogeneity exists in human asthma in terms of the nature and intensity of airway inflammation. Novel biologic therapeutics are now emerging for severe asthma, some of the most promising of which are those monoclonal antibodies directed at selective targets. Those monoclonal antibodies act on very specific pathways. Some have shown to be effective, safe, and selective for the established asthma phenotypes, especially in patients with uncontrolled severe asthma, whereas other has proven to be unsafe and/or unsuccessful. The treatment of severe allergic asthma with anti-IgE monoclonal antibody (omalizumab) has been shown to be effective in a large number of patients, and new anti-IgE antibodies with improved pharmacodynamic properties are being investigated. Among developing therapies,

biologics designed to block certain pro-inflammatory cytokines, such as IL-5 (mepolizumab) and IL-13 (lebrikizumab), have a greater chance of being used. Perhaps, blocking more than one cytokine pathway (such as IL-4 and IL-13 with dupilumab) might confer increased efficacy of treatment, along with acceptable safety. It is becoming evident that significant clinical effects with anti-cytokine-based therapies are more likely in carefully selected patients that take asthma heterogeneity into account. It might also be more clinically effective if more than one mediator were to be targeted rather than a single mediator.

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