

The potential of biologics for the treatment of asthma

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Abstract | Recent advances in the knowledge of asthma pathobiology suggest that biological therapies that target cytokines can be potentially useful for the treatment of this complex and heterogeneous airway disease. The use of biologics in asthma has been established with the approval of the humanized monoclonal immunoglobulin E-targeted antibody omalizumab (Xolair; Genentech/Novartis) as an add-on treatment for inadequately controlled disease. Furthermore, evidence is accumulating in support of the efficacy of other biologics, such as interleukin-5 (IL-5)- and IL-13-specific drugs. Therefore, these new developments are changing the scenario of asthma therapies, especially with regard to more severe disease. The variability among patients' individual therapeutic responses highlights that it will be necessary to characterize the different asthma subtypes so that phenotype-targeted treatments based on the use of biologics can be implemented.

Corticosteroids

A class of steroid hormones that generally inhibit inflammation and immunity.

Asthma is a chronic disease of the airways, and is characterized by inflammatory, structural and functional changes that are responsible for bronchial hyperresponsiveness and limitations in airflow (the latter is usually reversible)^{1,2}. Although asthma symptoms can be controlled in the majority of patients using current standard therapies — which, according to the Global Initiative for Asthma (GINA) guidelines, are mainly based on combinations of inhaled corticosteroids, β_2 -adrenergic receptor agonists and eventually oral leukotriene inhibitors^{3–5} — in around 5–10% of people with asthma the disease remains symptomatic and inadequately controlled.

In these patients, asthma symptoms can be worsened by concomitant comorbidities including rhinitis, sinusitis, gastroesophageal reflux, obesity and obstructive sleep apnoea⁶. Therefore, although patients with uncontrolled asthma are a minority of the total asthmatic population, they have a high risk of serious morbidity and mortality, and use the largest share of economic resources and health-care services, including emergency visits, hospitalizations and additional consumption of drugs for recurrent exacerbations⁷. Moreover, severe asthma results in frequent absences from school and work, and patients with difficult-to-treat disease are often prone to anxiety and depression⁸. Therefore, additional therapeutic approaches are urgently required for those individuals who have poorly controlled asthma.

It is now widely accepted that asthma is a heterogeneous disease that includes several different phenotypes, each of which is defined by distinct clinical, functional

and pathobiological patterns². These aspects, together with the evaluation of patient responses to treatment, contribute to the characterization of increasing degrees of disease severity, including mild, moderate and severe disease. The most common phenotypic classification divides asthma into allergic and non-allergic forms. Allergic asthma encompasses all levels of disease severity; according to current data, about 50–80% of patients with severe asthma have allergic asthma^{9,10}. In addition to the well-recognized role of immunoglobulin E (IgE) in the pathogenesis of allergic asthma¹¹, a large body of evidence suggests that many cytokines released by immune-inflammatory cells and airway structural cells contribute substantially to the manifestation of several disease phenotypes¹². Within this context, severe asthma is characterized by unusual features such as difficult-to-treat bronchial inflammation and marked airway remodelling, which are predominately due to an intricate network of interacting cytokines and chemokines¹³. This makes the disease pathobiology of severe asthma much more complex than the mild and moderate subtypes.

Indeed, basic and clinical research has identified several potentially suitable cytokine targets for asthma therapies¹⁴. Such findings highlight the potential importance of biological treatments directed against IgE and pro-inflammatory cytokines, including monoclonal antibodies and small-molecule inhibitors. In particular, biologics may represent useful adjunct therapies, especially for patients with more severe asthma that is not fully responsive to conventional treatments¹⁵.

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Eosinophilia

The accumulation of eosinophils (white blood cells that produce cytokines, cationic proteins and reactive oxygen species) in tissue or blood.

Dyspnoea

Shortness of breath that causes discomfort.

Airway hyperresponsiveness

An exaggerated contractile response of airway smooth muscle that has been exposed to potentially bronchoconstrictive stimuli.

Antigen presentation

An immunological event that is mediated by antigen-presenting cells. These cells internalize and process antigens, then display antigenic peptidic fragments on their surface, together with co-stimulatory molecules that are required for the activation of the cognate lymphocytes.

T_H1 polarization

Interleukin 12 (IL-12)-driven expansion of T helper 1 (T_H1) cells, which produce large amounts of T_H1 cytokines (such as interferon- γ and IL-2), activate macrophages and are essential for the defence against intracellular pathogens.

T_H2-adaptive responses

A type of adaptive immunity mediated by T helper 2 (T_H2) cells; a T_H cell subset that produces T_H2 cytokines (for example, interleukin 4 (IL-4), IL-5 and IL-13), which are involved in atopic immune responses.

Immunoglobulin class switching

Interleukin-4 (IL-4)- and IL-13-mediated induction of B cells to perform immunoglobulin class recombination, resulting in prevalent production of immunoglobulin E.

CD4⁺ T cell

A subset of helper T lymphocytes expressing the cell surface glycoprotein called CD4.

Neutrophilic inflammation

A type of inflammation that is mediated by the recruitment and activation of neutrophils (white blood cells that produce pro-inflammatory cytokines, proteases and reactive oxygen species).

Current data suggest that many patients with allergic asthma, especially those with moderate-to-severe and exacerbation-prone disease, can greatly benefit from add-on treatment with the IgE-targeted monoclonal antibody omalizumab (Xolair; Genentech/Novartis)¹⁶. Moreover, variable responses to experimental cytokine-directed therapies have been observed among patients, probably because of the substantial differences that exist among distinct cytokine-based asthma phenotypes¹⁷. For example, as discussed below, individual patient responses to specific anti-cytokine treatments can be affected by single nucleotide polymorphisms in the gene encoding interleukin-4 receptor (IL-4R), or by IL-13 bioactivity, as well as by the susceptibility to develop an IL-5-induced, corticosteroid-refractory eosinophilia. This implies that biological drugs need to address the molecular targets that are relevant to each phenotypic subgroup of asthma. In this regard, the aim of this article is to outline, after recalling the most recent advances in asthma pathobiology, currently used and newly developing biological therapies for severe asthma. In particular, we first highlight biologics targeting IgE and then focus on the targeting of pro-inflammatory cytokines.

Pathobiology of asthma

Asthma is characterized by different patterns of cytokine-based airway inflammation involving immune and/or inflammatory cell types such as T and B lymphocytes, mast cells, eosinophils, basophils, neutrophils and dendritic cells, as well as structural cell types including epithelial and mesenchymal cells (FIG. 1). This widespread respiratory disease, which originates from numerous interactions between genetic factors and environmental agents such as allergens, respiratory viruses and airborne pollutants¹⁸, is characterized by recurrent episodes of dyspnoea, wheezing, chest tightness and coughing, and is usually associated with reversible limitations in airflow and an exaggerated bronchoconstrictive response to several different stimuli (known as airway hyperresponsiveness)³. Allergic asthma is the predominant disease manifestation during early life and young adulthood, whereas the non-allergic form is more frequent in older patients and is thus often referred to as the late-onset subtype of asthma¹⁹. Both of these asthma subtypes are present within the minority (5–10%)²⁰ of patients who have severe, uncontrolled disease.

Chronic airway inflammation. The main pathological feature of most types of asthma is chronic inflammation, which is frequently associated with structural changes to the airway wall, referred to as tissue remodelling. Allergic asthma is widely believed to be triggered by an immune-inflammatory response driven by T helper type 2 (T_H2) lymphocytes²⁰. This T_H2-driven subphenotype of asthma arises from a complex interplay between the innate and adaptive branches of the immune system, and encompasses all levels of disease severity, and is thus also very relevant in severe asthma^{21–23}. Indeed, although the ‘T_H2-high’ pattern of airway inflammation has been characterized using molecular biomarkers in patients with mild-to-moderate asthma²⁴, a ‘T_H2 status’ can

also be determined in individuals with severe disease, as shown by a recent clinical trial of T_H2 cell-specific biological therapies²⁵.

The cytokine milieu determines the type of antigen presentation-dependent differentiation of T lymphocytes. In particular, IL-12 produced by dendritic cells promotes T_H1 polarization, whereas commitment towards the T_H2 lineage is driven by IL-4, which is probably released from mast cells, T cells, eosinophils and basophils²⁶. Moreover, the innate cytokine thymic stromal lymphopoietin (TSLP) is secreted in large amounts by bronchial epithelial cells and mast cells of patients with asthma²⁷, thus eliciting the development of T_H2-adaptive responses²⁸ and inducing dendritic cells to release the chemokines CC motif chemokine 17 (CCL17) and CCL22, which recruit T_H2 cells upon binding to their receptor: CC chemokine receptor 4 (CCR4)¹². As a consequence, T_H2 cells that express CCR4 synthesize large amounts of cytokines encoded by the gene cluster located on the long arm of chromosome 5, including IL-3, IL-4, IL-5, IL-6, IL-9, IL-13 and granulocyte-macrophage colony-stimulating factor (GM-CSF)²⁹. These cytokines and growth factors stimulate the maturation and recruitment of other immune cells involved in the allergic cascade, such as eosinophils and mast cells. In particular, eosinophil differentiation in the bone marrow is promoted by IL-5 (REF. 30), whose action is synergized by eosinophil-recruiting chemokines such as eotaxin and CCL5 (also known as RANTES (regulated on activation normal T cell expressed and secreted)), which are released by both inflammatory and airway-resident cells^{30,31}. IL-4 and IL-13 act on B lymphocytes by driving immunoglobulin class switching towards the production of IgE^{32,33}. IL-9, which is secreted by a further subset of T lymphocytes (T_H9 cells) that are derived from T_H2 cells, attracts mast cells and triggers their differentiation³⁴; however, the unequivocal demonstration of the existence and biological relevance of T_H9 cells *in vivo* has come only from studies performed in mice³⁵.

Therefore, these cytokines are important potential targets of biological treatments, which are especially needed for patients who do not respond fully to high doses of inhaled corticosteroids. Indeed, a better understanding of the pathogenic role of such cytokines, particularly in difficult-to-treat asthma, could stimulate the development and application of anticytokine therapies. Such therapies would help to achieve personalized treatments that are tailored for patients who express high airway levels of specific cytokines implicated in the pathobiology of different subphenotypes of severe allergic asthma.

T_H2 lymphocytes are probably the main CD4⁺ T cell subpopulation involved in the development of the phenotype of inflammatory asthma — referred as eosinophilic asthma²⁹. Neutrophilic inflammation of the airways, which is often associated with the most severe clinical phenotypes of asthma, is induced by other subsets of T_H cells³⁶. In particular, a specific lineage of CD4⁺ effector T lymphocytes, which express IL-17 and so are called T_H17 cells, appears to have a pivotal role in bronchial neutrophilia. Indeed, lung tissue sections from patients with asthma show overexpression of the IL-17 family members IL-17A and IL-17F, and levels of

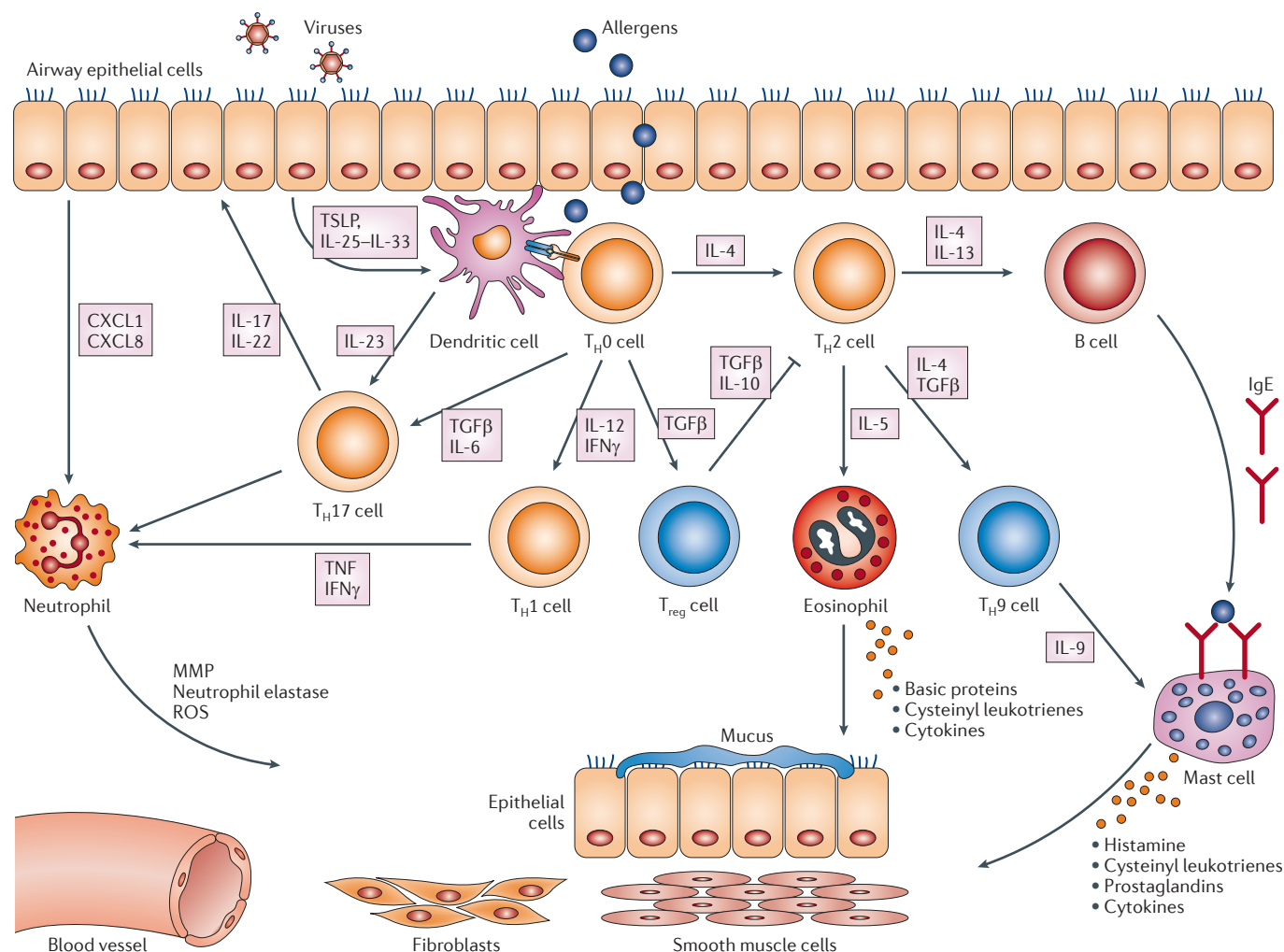


Figure 1 | Pathobiology of asthma. Asthma originates from complex interactions between genetic factors and environmental agents such as aeroallergens and respiratory viruses. In particular, within the airway lumen, allergens can be taken up by dendritic cells, which process antigenic molecules and present them to naive T helper (T_H) cells. The consequent activation of allergen-specific T_H 2 cells is responsible for the production of interleukin-4 (IL-4) and IL-13, which promote B cell-operated synthesis of immunoglobulin E (IgE) antibodies. Moreover, T_H 2 cells release IL-5, which induces eosinophil maturation and survival. These events are facilitated by a functional defect in IL-10- and transforming growth factor- β (TGF β)-producing T regulatory (T_{reg}) cells, which normally exert an immunosuppressive effect on T_H 2 cell-mediated responses. In addition to T_H 2 cells, IL-9-releasing T_H 9 cells can become activated, thus leading to the growth and recruitment of mast cells, which — following IgE-dependent degranulation — release both preformed and newly synthesized mediators. Other important T lymphocytes contributing to asthma pathobiology are T_H 17 cells, which produce IL-17A and IL-17F, which in turn cause neutrophil recruitment and expansion. Furthermore, IL-12-dependent, interferon- γ (IFN γ)-releasing T_H 1 cells can become activated, especially as a result of airway infections sustained by respiratory viruses. Finally, many mediators, cytokines and growth factors produced by several different cells involved in chronic asthmatic inflammation may also affect the functions and proliferation rates of airway structural-type cells, including epithelial cells, fibroblasts, smooth muscle cells and endothelial vascular cells. CXCL1, chemokine CXC motif ligand 1; MMP, matrix metalloproteinase; ROS, reactive oxygen species; TNF α , tumour necrosis factor- α ; TSLP, thymic stromal lymphopoietin.

expression correlate with disease severity, especially in individuals with neutrophilic steroid-resistant disease¹³. In mice, differentiation of T_H 17 lymphocytes from uncommitted cell precursors requires IL-6 and transforming growth factor- β (TGF β), and IL-17 expression is further enhanced by IL-23. IL-17A and/or IL-17F stimulate structural cellular components of the airway such as bronchial epithelial cells and subepithelial fibroblasts

to secrete powerful neutrophil chemoattractants such as chemokine CXC motif ligand 1 (CXCL1) and IL-8 (also known as CXCL8)¹². T_H 17 cells may also contribute to the pathogenesis of allergic asthma, thus worsening its severity³⁷.

Therefore, it is reasonable to speculate that airway eosinophilia — which is predominantly mediated by T_H 2 cells — is responsible for mild and moderate allergic

IL-17
Interleukin-17; a T helper 17 (T_H 17) cell-derived cytokine that induces neutrophil recruitment.

asthma, whereas concomitant activation of both T_H2 cells and T_H17 cells is associated with a mixed eosinophilic and/or neutrophilic inflammatory phenotype that underlies more severe forms of the disease.

Another cytokine that is implicated in the pathogenesis of severe neutrophilic asthma is tumour necrosis factor- α (TNF α), which is produced by CD4⁺ T lymphocytes, monocytes and/or macrophages as well as several other cell types and exerts pleiotropic effects on inflammatory and structural airway cells^{38,39}. Combined patterns of both neutrophilic and eosinophilic airway infiltrates may occur in recurrent acute relapses of asthma, which characterize the so-called exacerbation-prone subphenotype of severe disease⁴⁰. These exacerbations can be caused by exposure to allergens, as well as respiratory viruses, whose effects are facilitated by deficient epithelial synthesis of antiviral cytokines such as interferon- β (IFN β) and IFN λ within the airways of patients with asthma⁴¹.

The development of both the T_H2 cell and T_H17 cell arms of T cell-mediated adaptive immunity is facilitated by an impairment of specific immunomodulatory cells known as regulatory T (T_{Reg}) cells. Defective functioning of T_{Reg} cells can occur in all forms of asthma^{42,43} but probably contributes most substantially to the majority of severe subtypes⁴⁴. Therefore, it is reasonable to suggest that patients with severe and uncontrolled disease could benefit from combined biological therapies that address multiple targets, including T_H2- and T_H17-derived cytokines.

Airway remodelling. In asthma, chronic inflammation is frequently associated with dynamic structural changes that affect all layers of the airway wall, including proximal and distal airways. Such tissue remodelling occurs in both allergic and non-allergic asthma⁴⁵, and includes epithelial shedding, goblet cell and mucous gland hyperplasia as well as enhanced deposition of extracellular matrix proteins, which leads to subepithelial fibrosis. In addition, there is increased angiogenesis and proliferation of airway smooth muscle cells¹ (FIG. 1), which acquire a highly secretory and contractile phenotype^{46,47}. A particular hallmark of airway remodelling is thickening of the epithelial reticular basement membrane (RBM) as a result of subepithelial fibrosis, which occurs quite early in the course of asthma and is more prominent in patients with severe disease⁴⁸. Moreover, an increased thickness of the RBM appears to be more closely associated with the eosinophilic phenotype of airway inflammation⁴⁹. Remodelling in asthma also includes increases in the mass of airway smooth muscle, caused by cellular hypertrophy and hyperplasia⁵⁰. Thickening of airway smooth muscle, which is particularly evident in individuals who died from fatal asthma, seems to be directly related to disease duration and severity⁵¹. The vascular area is also enlarged in airways of patients with asthma⁵². Indeed, an increased number of vessels can be detected in airway biopsy samples of patients with asthma, in whom the bronchial walls are characterized by high levels of immunoreactivity for vascular endothelial growth factor (VEGF)⁵³.

It is currently believed that airway remodelling in asthma is largely due to complex interactions between the bronchial epithelium and the underlying mesenchyme⁵⁴, resulting from a reactivation of the developmental epithelial–mesenchymal trophic unit that is responsible for lung morphogenesis during fetal life. TGF β has a crucial role during reactivation of the epithelial–mesenchymal trophic unit^{55,56}. Levels of this fibrogenic growth factor are upregulated in airways of patients with asthma because of its increased release from immune-inflammatory cells as well as damaged epithelial cells and activated mesenchymal cells⁵⁷. Other growth factors and cytokines that contribute to airway remodelling in asthma include VEGF, IL-13 and IL-9 (REF. 58).

Overall, airway remodelling results in the thickening of bronchial and bronchiolar walls, leading to reduced airway calibre and fixed (that is, not reversible) airflow limitations that are correlated with a progressive decline in respiratory function⁵⁹. These features are more prominent in patients with severe asthma, which can often be characterized by barely reversible bronchial obstruction and progressively worsening pulmonary dysfunction²².

Therapeutic implications of pathobiological advances.

Recent advances in the understanding of asthma pathobiology, especially a better understanding of the cellular and molecular mechanisms that underlie uncontrolled asthma, may have important prospective therapeutic implications. In addition to the already evident role of IgE in the pathogenesis of allergic asthma (including the most severe phenotypes), it is possible to outline the complex cascades of pro-inflammatory mediators involved in difficult-to-treat disease²². Such an improved awareness of the inflammatory and immune events responsible for severe asthma is unravelling potential targets for the development and implementation of new biological therapies. So far, the only biological drug approved for the treatment of severe asthma is the IgE-blocking monoclonal antibody omalizumab. Other biologics, which are currently under different stages of investigation, include molecules directed against IL-5, IL-4, IL-13, IL-9, GM-CSF and TNF α . Moreover, IL-17 and IL-23, as well as the innate cytokines TSLP, IL-25, IL-33 and IL-27, are additional interesting targets for future asthma therapies.

All of these therapeutic approaches are discussed below; TABLE 1 and FIG. 2 provide a summary of the main biological drugs targeting IgE and the pro-inflammatory cytokines that have potential for the treatment of asthma.

IgE-targeted antibodies

The approval of the humanized monoclonal IgE-targeted antibody omalizumab established that biologics can be used to treat asthma. Although IgE was considered to be a suitable target for the development of anti-allergy treatments following its discovery in 1967, it took almost 40 years to translate this basic research finding into a therapeutic application^{60,61}. Omalizumab was first approved in Australia in 2002, which was followed by approvals in other countries for patients who have inadequately controlled disease despite receiving daily

Adaptive immunity

The ability of the immune system to recognize and remember a specific pathogen, causing the host to induce a strong immune response every time that pathogen is encountered.

Epithelial shedding

Epithelial detachment from the basement membrane, which results in the loss of ciliated cells from the layer of epithelial cells in the airway.

Goblet cell

A modified columnar epithelial cell that produces and secretes mucus.

Epithelial reticular basement membrane

(RBM). A histological structure that is underneath epithelial cells in the airway.

Mesenchyme

Embryological tissue from which all types of connective tissue are derived.

Table 1 | **Biological drugs in asthma treatment**

Drug	Mechanism of action	Effects	Development*
Omalizumab	Binds free IgE	Reduces exacerbations, improves symptoms and quality of life	FDA- and EMA-approved
Mepolizumab	Blocks IL-5	Decreases the number of eosinophils and frequency of exacerbations, as well as prednisone consumption	Phase II/III
Reslizumab	Blocks IL-5	Decreases the number of sputum eosinophils and enhances FEV1	Phase II
Benralizumab	Inhibits binding of IL-5 to IL-5R α	Depletes the number of peripheral blood eosinophils	Phase I/II
Pascolizumab	Blocks IL-4	No significant clinical efficacy	Phase II
Altrakinecept	Soluble IL-4R	No significant clinical efficacy	Phase II
Pitrakinra	Inhibits binding of IL-4 and/or IL-13 to IL-4R α	May prevent a decrease in FEV1 after allergen challenge	Phase II
Tralokinumab	Blocks IL-13	Reduces airway eosinophilia	Phase I/II
Anrukizumab	Blocks IL-13	Inhibits allergen-induced late-phase asthmatic responses	Phase II
Lebrikizumab	Blocks IL-13	Enhances FEV1 in patients with high serum levels of periostin	Phase II
MEDI-528	Blocks IL-9	Reduces airway inflammation and hyper-responsiveness in mice	Phase II
MT203	Blocks GM-CSF	Decreases survival and activation of eosinophils	Phase II
Secukinumab	Blocks IL-17	Data not yet available	Phase II; NCT01478360
Golimumab	Blocks TNF α	May increase the risk of infections and malignancies	Suspended
Infliximab	Blocks TNF α	Reduces PEF oscillations and asthma exacerbations	Phase II
Etanercept	Soluble TNF α receptor	Conflicting data; see main text	Phase II

*Unless given in the table, details of ClinicalTrials.gov identifiers or publications are given in the main text. EMA, European Medicines Agency; FDA, US Food and Drug Administration; FEV1, forced expiratory volume in 1 second; GM-CSF, granulocyte-macrophage colony-stimulating factor; IgE, immunoglobulin E; IL-4, interleukin-4; IL-4R α , IL-4 receptor- α ; PEF, peak expiratory flow; TNF α , tumour necrosis factor- α .

Long-acting β_2 -adrenergic receptor agonists

A class of inhaled drugs that act by providing a prolonged bronchodilation, which is mediated by the stimulation of β_2 -adrenergic receptors expressed by airway smooth muscle cells.

Complementarity-determining region

A specific region of an antibody that consists of a highly variable amino acid sequence and confers antigen-binding specificity.

Fc ϵ receptor I

A high-affinity receptor for immunoglobulin E that is expressed by mast cells, basophils and a variety of other cell types, and is essential for several biological functions of immunoglobulin E.

Early-phase asthmatic responses

Bronchoconstrictive reactions that occur within minutes of the airway being exposed to allergens.

Late-phase asthmatic responses

Bronchoconstrictive reactions that occur several hours after allergens have been inhaled.

C ϵ 3 domain

The third domain of the constant region of immunoglobulin E that contains the Fc ϵ receptor I (Fc ϵ RI)-binding function.

high doses of inhaled corticosteroids and long-acting β_2 -adrenergic receptor agonists.

Omalizumab is a recombinant humanized antibody consisting of a human IgG framework that embeds the complementarity-determining region obtained from an anti-IgE antibody raised in mice⁶².

Mechanism of action of omalizumab. Atopy — the propensity to develop exaggerated IgE responses to common environmental allergens — has a dominant role in the pathological features and clinical manifestations of allergic asthma. In sensitized individuals, adjacent allergenic epitopes on the surface of mast cells induce the crosslinkage of two or more Fc ϵ receptor I (Fc ϵ RI)-bound IgE molecules. Antigen-induced IgE bridging then promotes receptor aggregation and cell activation⁶³. This is followed by mast cell degranulation and the subsequent release of preformed granule-associated mediators (including histamine, tryptase, chymase and heparin). In addition, newly formed eicosanoids (such as cysteinyl leukotrienes C $_4$ -D $_4$ and prostaglandin D $_2$) are secreted, as well as several different cytokines, chemokines and growth factors (such as IL-3, IL-4, IL-5, IL-6, IL-8, IL-13, CCL5 and

GM-CSF). These mechanisms are responsible for both early-phase asthmatic responses and late-phase asthmatic responses that occur in patients with atopic asthma following allergen exposure⁶⁴.

Omalizumab selectively binds with high affinity to the C ϵ 3 domain of IgE⁶⁵, thus preventing the interactions of IgE with high-affinity Fc ϵ RI expressed by mast cells, basophils, eosinophils and dendritic cells. Blocking the binding of IgE to Fc ϵ RI on mast cells and basophils inhibits allergen-induced degranulation. Furthermore, omalizumab lowers free serum levels of IgE by 96–99%, and it may also be able to suppress new IgE production⁶⁶. Moreover, by inhibiting the binding of IgE to Fc ϵ RI expressed on dendritic cells⁶⁷, omalizumab can reduce the efficiency of antigen presentation to T lymphocytes.

Omalizumab effectively reduces allergic bronchial inflammation, but its effects on airway remodelling — which is a key feature of severe asthma — are less clear. It is notable that airway structural cells such as bronchial epithelial cells and airway smooth muscle cells express high-affinity cell surface IgE receptors^{68,69}. These IgE receptors can be involved in the production of growth factors that have a central role in airway remodelling

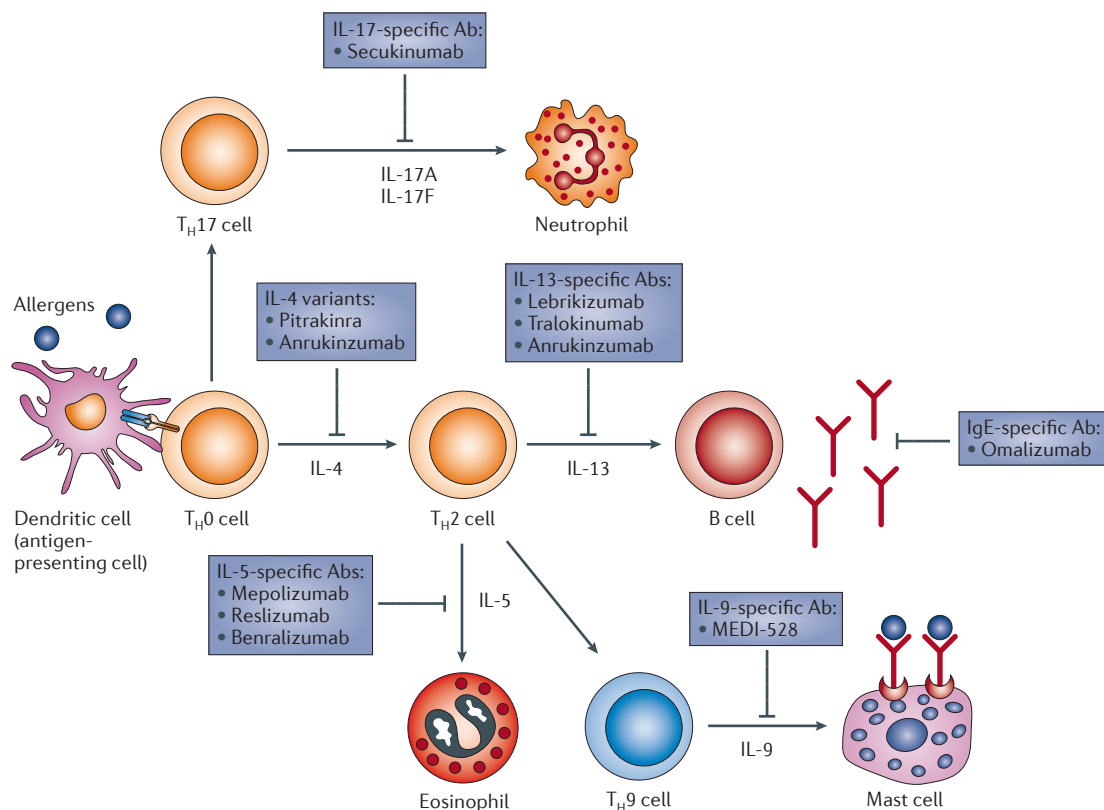


Figure 2 | Mechanism of action of biological therapies for asthma. The main targets of the currently used biologic (omalizumab) and other biologics that are currently under investigation as asthma therapies are depicted. See BOX 1 for a discussion of clinical trials and safety data for omalizumab. Ab, antibody; IgE, immunoglobulin E; IL-4, interleukin-4; T_H0, naive T helper.

events, which prominently occur in patients with the most severe disease phenotypes. Therefore, omalizumab could potentially interfere with the synthetic activity of the bronchial epithelium. Indeed, omalizumab decreases the production of TGF β in a cellular model of allergic asthma⁷⁰, suggesting that this drug could thus inhibit the fibrotic effects exerted by TGF β in airways affected by asthma. Furthermore, it has been reported that omalizumab can substantially decrease the concentration of endothelin 1 — a peptide involved in the pathogenesis of structural changes in the airways, such as subepithelial fibrosis and proliferation of bronchial smooth muscle cells — in the exhaled breath condensate of patients with severe persistent allergic asthma⁷¹. Moreover, omalizumab reduced airway wall thickness, mucous gland metaplasia and subepithelial fibrosis in mouse models of allergic asthma. Recent preliminary findings, obtained using computed tomography imaging in a limited number of patients with asthma, have shown that omalizumab reduces airway wall thickness and increases the bronchial luminal area⁷². These findings have been recently corroborated by histopathological observations of bronchial biopsy samples obtained from patients with severe persistent allergic asthma before and 12 months after treatment with omalizumab, showing a significant reduction in RBM thickness⁷³. See BOX 1 for a discussion of clinical trial and safety data for omalizumab.

IL-5-targeted antibodies

IL-5 has a crucial role in the growth, maturation and activation of eosinophils⁷⁴. Therefore, therapeutic strategies that target IL-5 may be effective in the treatment of eosinophilic asthma phenotypes that eventually become refractory to corticosteroids and omalizumab. In this regard, several preclinical studies have been carried out in experimental animal models of asthma. For example, pre-treatment with the IL-5-specific blocking antibody TRFK-5 inhibited eosinophil influx into the airways of allergen-sensitized mice⁷⁵. Furthermore, TRFK-5 suppressed infiltration of eosinophils into the airway and bronchial hyperresponsiveness in a non-human primate model of asthma⁷⁶. More recently, other antibodies such as mepolizumab, reslizumab and benralizumab have been developed and evaluated in clinical studies⁷⁷.

Some clinical trials performed in heterogeneous populations of patients with mild or moderate chronic persistent asthma have shown that the humanized monoclonal antibody mepolizumab is safe and can effectively reduce eosinophil numbers in airways and blood^{78,79}. However, these effects were not paralleled by significant improvements in asthma symptoms, lung function and bronchial hyperresponsiveness. In particular, mepolizumab (given at a single intravenously administered dose of 10 mg per kg) markedly lowered sputum eosinophil counts but did not affect the late asthmatic

reaction to allergen challenge and the airway response to inhaled histamine in patients with mild asthma⁷⁸. Another placebo-controlled study performed in a large number of patients with moderate persistent asthma, who received two different doses of mepolizumab (250 mg or 750 mg intravenously, once every month for 3 months), confirmed that the drug reduced blood and sputum eosinophil counts⁷⁹. Again, these effects were not associated with significant improvements in clinical and functional end points such as asthma symptoms, exacerbation rates, quality of life, forced expiratory volume in 1 second (FEV1) and peak expiratory flow (PEF). Such observations have raised doubts about the relevance of the pathogenic roles of IL-5 and eosinophils in asthma.

More recently, mepolizumab has been tested in patients with selected subtypes of chronic severe asthma, characterized by frequent exacerbations and airway eosinophilia that is refractory to inhaled and systemic corticosteroid therapies^{80,81}. In particular, when it was intravenously administered to such patients at a monthly dose of 750 mg for 4 months, mepolizumab caused a dramatic decrease in levels of blood and sputum eosinophils⁸⁰. These changes were associated with clinically relevant reductions in asthma exacerbations and prednisone consumption, as well as with significant improvements in respiratory function (FEV1) and the Asthma Control Questionnaire (ACQ) score.

In another similar trial, patients receiving intravenously administered mepolizumab at a monthly dose of 750 mg for 1 year experienced a marked reduction in blood and sputum eosinophilia as well as a significant decrease in the frequency of asthma exacerbations⁸¹. A larger, multicentre, double-blind and placebo-controlled trial (named the DREAM study) has recently been carried out in 621 patients with severe, exacerbation-prone, eosinophilic asthma who were randomly assigned to four groups receiving (at 4-week intervals) 13 intravenous infusions of placebo or one of three doses of mepolizumab (75 mg, 250 mg or 750 mg)⁸². At all dosages used, mepolizumab was well tolerated and effectively decreased the frequency of asthma exacerbations (compared to placebo) as well as blood and sputum eosinophil counts⁸². IgE levels and atopic status at baseline were not associated with therapeutic responses to mepolizumab, thus potentially differentiating this treatment from omalizumab⁸².

Taken together, the results of these three placebo-controlled studies demonstrate that mepolizumab is efficacious in patients with specific phenotypes of severe asthma characterized by persistent, corticosteroid-resistant eosinophilia. Such findings thus revalorize the importance of the pathogenic role of the IL-5–eosinophil axis in selected subgroups of patients with severe asthma, with special regard to the recurrence of asthma exacerbations. However, patients with difficult-to-treat, exacerbation-prone eosinophilic asthma should also be tested with respect to their eventual responsiveness to omalizumab, as this drug has proven effectiveness in inducing eosinophil apoptosis and reducing asthma exacerbations. The variable patient responses to mepolizumab are due to the inclusion criteria that were adopted to selectively enrol individuals with asthma in clinical trials, and are

probably due to the pathobiological differences between distinct asthma phenotypes.

Another interesting IL-5-specific biologic is reslizumab, an IgG4κ humanized monoclonal antibody. When administered at a monthly dosage of 3.0 mg per kg for 3 months to patients with poorly controlled eosinophilic asthma, reslizumab significantly decreased sputum eosinophil counts and improved lung function (compared to placebo), as well as inducing a positive trend towards better control of asthma⁸³. The anti-asthma effects of reslizumab were most pronounced in a subgroup of patients characterized by the highest levels of blood and sputum eosinophils, which were associated with the presence of nasal polyposis⁸³.

Therefore, such findings further emphasize the importance of accurately selecting patients based on phenotype in order to tailor anti-asthmatic treatments to specific biological and clinical features of the individual disease. These concepts will eventually also apply to the use of benralizumab, an IgG1 monoclonal antibody directed against the α-chain of IL-5R (IL-5Rα); benralizumab was shown to be safe and effectively lowered peripheral blood eosinophil counts in preliminary investigations⁸⁴. There was a relatively long-lasting depletion of peripheral blood eosinophils after either intravenous or subcutaneous administration of benralizumab; in particular, this effect persisted for at least 2 to 3 months in individuals receiving doses ranging from 0.03 to 3 mg per kg. With regard to these dosages, pharmacokinetic parameters such as mean maximum concentration (1–82 µg per ml) and mean area under the curve (5–775 µg of drug per ml) were approximately proportional to the dose⁸⁵. Moreover, the mean volume of distribution of benralizumab (52–93 ml per kg) was greater than the plasma volume. This suggests that benralizumab binds to IL-5Rα-expressing blood cells (eosinophils and basophils) and can also penetrate into extravascular tissues; the mean predicted elimination half-life was about 18 days⁸⁵.

Several Phase II clinical trials of benralizumab are currently being carried out in adult patients with asthma (ClinicalTrials.gov identifiers: NCT00659659 and NCT00768079). Benralizumab could have a more beneficial effect than mepolizumab and reslizumab (both of which appear to exhibit similar pharmacological features). Indeed, by binding with a high affinity to IL-5Rα, benralizumab effectively blocks IL-5-dependent functions of eosinophils, thereby focusing its action more specifically on cellular targets (that is, eosinophils) than IL-5-targeted monoclonal antibodies. Overall, IL-5-specific drugs are well tolerated and display a good safety profile.

IL-4-specific therapies

IL-4 contributes to asthma pathophysiology by inducing T_H2 cell differentiation and expansion, isotype switching of B cells to IgE synthesis, as well as eosinophil recruitment, development of mast cells and mucous metaplasia¹². Moreover, IL-4 is involved in airway remodelling through upregulation of collagen and fibronectin production¹². Several studies aiming to evaluate the effects

Peak expiratory flow

(PEF). An individual's maximum speed of expiration that acts as an indicator of changes in the functioning of the airway.

Asthma Control Questionnaire

(ACQ). A list of questions that are used to assess how well a patient's asthma is controlled; includes questions about symptoms during the day and at night, limitations in daily activity, airway functioning and the use of rescue bronchodilators.

Atopic status

The propensity to generate allergic responses to antigens, mediated by an exaggerated production of immunoglobulin E.

Rescue bronchodilators

Rapidly acting inhaled drugs, which provide immediate relief of bronchoconstriction.

Forced vital capacity

(FVC). A spirometric indicator of lung function.

FEV1/FVC ratio

A ratio of forced expiratory volume in 1 second (FEV1) to forced vital capacity (FVC). A decrease in this ratio from normal values indicates that a patient has limitations in airflow through the bronchi.

Box 1 | Omalizumab: the first biological drug approved for asthma treatment

Currently, the only biological drug approved for asthma treatment is omalizumab (Xolair; Genentech), a humanized monoclonal immunoglobulin E (IgE)-specific antibody. In Phase III trials, patients receiving omalizumab had fewer asthma exacerbations, experienced improvements in asthma symptoms and quality of life, and had decreased requirements for both inhaled corticosteroids and rescue bronchodilators^{140–145}. Moreover, compared to placebo-treated patients, omalizumab-treated patients with asthma had fewer hospitalizations, unscheduled outpatient visits and emergency hospital visits. In our own experience, the best results can be obtained using omalizumab as an add-on therapy in individuals with severe, uncontrolled and oral steroid-dependent allergic asthma that is characterized by the exacerbation-prone phenotype. During an uncontrolled trial in these patients, we observed dramatic reductions in exacerbation rate and oral corticosteroid intake, which were associated with a significant improvement in lung function (measured by forced expiratory volume in 1 second (FEV1) and the ratio of FEV1 to forced vital capacity (FVC); the FEV1/FVC ratio) and a decrease in the number of peripheral blood eosinophils¹⁴⁶.

Overall, omalizumab is well tolerated. Pivotal Phase III clinical trials have shown that the frequencies of adverse events — which included local reactions at the injection sites, headaches, fatigue and nausea — were similar between omalizumab-treated and control groups. Such a side-effect pattern has also been confirmed in long-term follow-up studies^{147,148}. A major concern is the small increase in the number of malignancies that were detected in initial clinical trials in omalizumab-treated patients¹⁴⁹. However, there was no difference in cancer incidence between individuals undergoing omalizumab therapy and the general population¹⁵⁰.

Although omalizumab is considered to be a non-anaphylactogenic antibody, a warning has been issued by the US Food and Drug Administration (FDA) about the potential occurrence of anaphylactic and anaphylactoid reactions. In a publication that reviewed data referring to anaphylaxis and anaphylactoid reactions reported in Phase III clinical trials and post-marketing surveillance studies, it was noted that among 39,510 patients receiving omalizumab, 35 individuals manifested 41 episodes of anaphylaxis associated with omalizumab administration, corresponding to an anaphylaxis-reporting rate of 0.09% of patients¹⁵¹. All patients responded to anti-anaphylactic treatments, and there were no fatalities or respiratory failures requiring intubation.

Although other concerns have been raised by the FDA about the potential occurrence of cardiovascular and cerebrovascular adverse effects in patients treated with omalizumab, the agency has not recommended any changes to the prescribing information for this drug¹⁵². Furthermore, a recent systematic analysis of eight selected placebo-controlled trials involving a total of 3,429 individuals did not detect an increased cardiovascular risk associated with the use of omalizumab¹⁶.

of anti-IL-4 therapies in asthma treatment have yielded conflicting results, and some findings obtained in mouse models of allergen-induced asthma have not been reproduced in humans.

IL-4-targeted monoclonal antibodies decreased IgE synthesis and airway hyperresponsiveness in mouse models of atopic asthma and acute bronchial hyper-reactivity^{86,87}. The humanized IL-4-targeted monoclonal antibody pascolizumab neutralized the bioactivity of human IL-4 *in vitro*, and was well tolerated by cynomolgus monkeys receiving monthly intravenous doses (up to 100 mg per kg) for 9 months⁸⁸. In a randomized, placebo-controlled, dose-escalation Phase I trial carried out in adult patients with mild-to-moderate asthma who were followed for 55 days, pascolizumab was well tolerated at single intravenous doses of 0.5–10 mg per kg and had an elimination half-life of more than 2 weeks⁸⁹. However, a subsequent large-scale, multidose Phase II trial was discontinued because pascolizumab did not provide clinical efficacy in patients with symptomatic, steroid-naïve asthma (ClinicalTrials.gov identifier: NCT00024544).

The soluble recombinant human IL-4R altrakinecept contains the extracellular portion of the IL-4Rα chain but lacks the transmembrane and cytoplasmic domains, and so is unable to activate receptor-mediated intracellular signalling pathways. When administered during allergen challenge in mouse models of asthma, this soluble IL-4R inhibited airway eosinophil infiltration, late-phase lung inflammation and mucus hypersecretion⁹⁰. These positive

results prompted the use of altrakinecept in clinical trials involving patients with moderate persistent asthma^{91,92}. Given that a nebulized form of altrakinecept has a half-life of approximately 5 days, a once-weekly administration was considered to be practical. In a Phase I/II trial, a single inhalation of the drug was safe and effective in patients with moderate asthma; it improved lung function and reduced airway inflammation (as shown by decreased levels of exhaled nitric oxide)⁹¹. These positive preliminary results were later confirmed using repeated doses for 12 weeks⁹². However, asthma symptoms and respiratory function (FEV1) were not improved in later clinical trials⁹³ (ClinicalTrials.gov identifier: NCT00001909).

Altogether, the reported discrepancies between studies in animal models and clinical studies could be due to several reasons. Experimental animal models have provided important information about asthma pathophysiology. In particular, mice can be extensively used and manipulated to evaluate the effects of gene deletion⁹⁴, especially the biological consequences of cytokine suppression. Furthermore, new drugs can rapidly be tested in mouse models during early development. However, mouse models also have many limitations, and thus cannot faithfully reproduce the complexity of human immune responses. Mice do not spontaneously develop asthma, and mouse models do not reflect the phenotypic heterogeneity of human asthma. In particular, mouse models are mainly based on the induction of antigen-dependent,

IgG4κ

A subclass of immunoglobulin G4 that has a structure characterized by the presence of κ light chains.

Nasal polyposis

Mucosal protrusions that contain oedema fluid and variable levels of eosinophils.

Area under the curve

A pharmacokinetic parameter that estimates drug bioavailability. It is extrapolated from the area under the graph of drug plasma concentration plotted against time after administration.

Volume of distribution

The amount of drug in the body divided by the concentration of the drug in blood or plasma; the theoretical volume in which the total amount of drug would need to be uniformly distributed to produce its desired blood concentration.

T_H2 -mediated inflammation⁹⁵. In humans, in addition to being triggered by aeroallergens, asthma can be triggered by multiple environmental agents such as viral and bacterial infections, drugs such as aspirin, cigarette smoke and other airborne pollutants, dietetic factors and occupational exposures as well as other risk factors¹. Therefore, in humans asthma can be driven and sustained not only by a T_H2 -polarized cellular response but also by mixed patterns of eosinophilic–neutrophilic airway infiltrates resulting from a possible concomitant involvement of T_H2 , T_H1 and/or T_H17 cells.

Overall, the relative therapeutic failure of strategies targeting IL-4 might be explained at least in part by the biological redundancy between IL-4 and IL-13. Therefore, a combined approach targeted to both of these cytokines may be eventually more successful. In this regard, an IL-4-mutant mouse protein prevented antigen-induced airway eosinophilia and bronchial hyperresponsiveness in mouse models of asthma⁹⁶. These findings were later confirmed in cynomolgus monkeys using pitrakinra⁹⁷, a bioengineered variant of IL-4 containing two mutations in the IL-4 amino acid sequence at position 121 (an arginine to aspartic acid mutation) and position 124 (a tyrosine to aspartic acid mutation). Pitrakinra acts as an antagonist of the heterodimeric receptor complex (IL-4R α –IL-13R α 1) that is shared by IL-4 and IL-13 (REF. 98).

When administered by either subcutaneous or inhaled routes, pitrakinra is safe and inhibits allergen-induced early and late asthmatic responses as well as disease exacerbations in patients with selected phenotypes of eosinophilic asthma^{99,100}. Moreover, in the first large pharmacogenetic, placebo-controlled investigation of the IL-4–IL-13 pathway, three doses (1 mg, 3 mg or 10 mg twice daily for 12 weeks) of inhaled pitrakinra were tested in patients with moderate-to-severe asthma¹⁰¹. Although this trial failed to demonstrate clinical efficacy in the whole study population, pitrakinra (at the 10 mg dose) significantly lowered the frequency of asthma exacerbations in individuals with specific single nucleotide polymorphisms in the gene encoding IL-4R α , located within the 3' untranslated region (rs8832GG and rs1029489GG genotypes)¹⁰¹.

Monoclonal antibodies against the α -chain of IL-4R represent another approach for disrupting the IL-4–IL-13 pathway. In mouse models of asthma, IL-4R α -targeted antibodies reduced lung inflammation, airway hyperresponsiveness and goblet cell hyperplasia¹⁰². The fully human IL-4R α -targeted monoclonal antibody AMG 317 not only blocks the binding of IL-4 to its receptor but also impedes signal transduction activated by IL-13 (REF. 103). In Phase I and Phase II clinical trials, single and multiple intravenous (10–1,000 mg) or subcutaneous (75–600 mg) doses of AMG 317 induced a significant decrease in total serum IgE levels¹⁰⁴; pharmacokinetic profiles were nonlinear over the dose ranges studied. In patients with moderate-to-severe asthma, AMG 317 was safe and well tolerated¹⁰⁵. Although no efficacy was detected across the entire study population after once-weekly administration of subcutaneous injections of AMG 317 (75 mg, 150 mg or 300 mg), the antibody induced significant clinical improvements in patients with higher baseline ACQ

scores¹⁰⁵ (ClinicalTrials.gov identifier: NCT00436670). Another monoclonal antibody targeted against IL-4R (REGN668) is currently being investigated to evaluate its potential effects on asthma exacerbations in patients with a moderate-to-severe, persistent eosinophilic form of the disease (ClinicalTrials.gov identifier: NCT01312961).

A further potential strategy to interfere with the IL-4–IL-13 pathway is blockade of signal transducer and activator of transcription 6 (STAT6), which is an essential component of the intracellular signalling network that mediates the biological actions of IL-4 and IL-13 (REF. 12). In this regard, intranasal delivery of a STAT6-inhibitory peptide decreased allergen-induced mucus production and lung eosinophilia in mouse models of asthma¹⁰⁶. Furthermore, when administered intraperitoneally in mice, a small-molecule inhibitor (AS1517499) of STAT6 reduced antigen-induced bronchial hyperresponsiveness and antigen-induced IL-13 upregulation¹⁰⁷. However, these compounds have not yet been tested in clinical trials.

IL-13-specific therapies

IL-13 is a key target for new anti-asthma therapeutic strategies because of its involvement — together with IL-4 — in several aspects of airway inflammation and remodelling, including mucus production, IgE synthesis, recruitment of eosinophils and basophils, as well as proliferation of bronchial fibroblasts and airway smooth muscle cells¹⁰⁸. Preclinical studies in mouse models of asthma have shown that the intravenous administration of IL-13-targeted monoclonal antibodies can inhibit allergen-induced inflammation, goblet cell hyperplasia and airway remodelling¹⁰⁹. These findings have been confirmed in mice using the fully human IL-13-targeted antibody tralokinumab, which has been shown to markedly attenuate airway eosinophilia and bronchial hyperresponsiveness¹¹⁰.

In a multiple-dose, randomized, double-blind and placebo-controlled Phase I clinical trial, tralokinumab had linear pharmacokinetics and an acceptable safety profile after it was intravenously administered at doses of 1.5 mg per kg or 10 mg per kg (injected at intervals of 28 days)¹¹¹. However, similarly to other biologics currently in clinical development for asthma, larger Phase III studies are required to obtain more reliable information about potential adverse events. In a Phase II clinical trial carried out in patients with mild atopic asthma, anrukinzumab — a humanized IL-13-specific monoclonal antibody — significantly inhibited allergen-induced late asthmatic responses within 14 days (but not at 35 days) after subcutaneous administration (two doses of 2 mg per kg, given 1 week apart)¹¹².

Furthermore, the IL-13-specific monoclonal antibody lebrikizumab exerts an effective anti-asthmatic effect in the so-called ' T_H2 -high' asthmatic phenotype, which is characterized by an overexpression of IL-13-inducible genes such as the gene encoding periostin, an extracellular matrix protein produced by bronchial epithelial cells²⁴. In a randomized multicentre study²⁵, the overall frequency of adverse events was similar regardless of whether asthmatic individuals had received lebrikizumab or placebo

(in addition to standard inhaled therapy). In this study, 219 adults with asthma were enrolled, whose disease was inadequately controlled by inhaled corticosteroid therapy. Lebrikizumab was administered at monthly subcutaneous doses of 250 mg for 6 months. It elicited a better improvement in lung function in patients with moderate-to-severe asthma who had high serum levels of periostin. Indeed, at week 12 the reported FEV1 increase, with respect to baseline values, was 5.5% in the whole lebrikizumab-treated group, 8.2% in the high-periostin subgroup and 1.6% (not significant) in the low-periostin subgroup.

This implies that easily detectable biomarkers such as periostin could be routinely used in clinical practice to identify specific asthmatic phenotypes in which IL-13 has a key pathogenic role; such asthmatic phenotypes will thus be potentially responsive to therapeutic strategies targeted against this pleiotropic cytokine. However, in the same trial there were similar increases (of 8.6%) in the post-lebrikizumab FEV1 in individuals who had high pre-treatment levels of the fraction of exhaled nitric oxide²⁵ (a less specific asthma biomarker) and in patients with high pre-treatment concentrations of serum periostin.

Moreover, ongoing early-stage clinical studies are evaluating the efficacy of two other IL-13-targeted antibodies — ABT-308 (ClinicalTrials.gov identifier: NCT00986037) and QAX576 (ClinicalTrials.gov identifier: NCT01130064) — in patients with moderate-to-severe persistent asthma.

IL-9-targeted therapies

IL-9 is overexpressed in airways affected by asthma, where it stimulates mast cell proliferation and mucus hyperplasia¹². In mice, IL-9 blockade reduces airway inflammation and hyperresponsiveness¹¹³. In healthy individuals, the humanized IL-9-targeted monoclonal antibody MEDI-528 was safe and well tolerated, displaying linear pharmacokinetics when delivered intravenously or subcutaneously¹¹⁴. In one of two randomized Phase IIa trials carried out in individuals with mild-to-moderate asthma, MEDI-528 induced a trend towards an improvement in AQLQ (Asthma Quality of Life Questionnaire) scores and an improvement in disease exacerbation rates¹¹⁵. The second of these two clinical studies showed that 50 mg of MEDI-528, administered subcutaneously twice weekly, can exert a protective effect against bronchoconstriction triggered by exercise¹¹⁵.

GM-CSF

GM-CSF is a growth factor that is upregulated in airways affected by asthma and has a key role in eosinophil differentiation and survival¹². In a mouse model of allergic asthma, intranasal administration of a GM-CSF-specific polyclonal antibody significantly inhibited airway inflammation, mucus production and bronchial hyperresponsiveness¹¹⁶. A human anti-GM-CSF monoclonal IgG1 antibody (MT203) has been developed that decreased the survival and activation of peripheral human eosinophils¹¹⁷. A Phase II, double-blind, placebo-controlled and randomized clinical trial is planning to evaluate 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 that is inadequately controlled by corticosteroids (ClinicalTrials.gov identifier: NCT01603277).

TNF α

In mouse models of allergen-dependent asthma, the pro-inflammatory cytokine TNF α — produced by T_H1 lymphocytes, macrophages and mast cells — induced the recruitment of neutrophils and eosinophils into airways via the upregulation of epithelial and endothelial adhesion molecules¹¹⁸. Moreover, TNF α is overexpressed in the airways of patients with severe asthma and also directly stimulates airway smooth muscle contraction by causing changes in intracellular calcium fluxes. Therefore, several drugs targeting TNF α have been evaluated for asthma treatment, including TNF α -blocking antibodies such as infliximab (Remicade; Centocor Ortho Biotech) and golimumab (Simponi; Centocor Ortho Biotech), as well as the soluble TNF α receptor fusion protein etanercept (Enbrel; Amgen/Pfizer)¹⁷. Overall, conflicting results have been obtained and serious concerns have been raised with regard to the safety of TNF α blockade, which may enhance the susceptibility of developing respiratory infections and cancers.

When etanercept was given to patients with severe asthma (subcutaneously, twice weekly for 12 weeks at a dose of 25 mg), there was a significant improvement in ACQ score, FEV1, PEF and bronchial hyperresponsiveness to methacholine¹¹⁹. Similar effects on asthma symptoms and lung function were observed during another study carried out in patients with severe refractory asthma who expressed high monocyte levels of TNF α and TNF α receptor, and received 25 mg of etanercept¹²⁰ (given subcutaneously twice weekly for 10 weeks). However, in a more recent and larger randomized trial in patients with moderate-to-severe persistent asthma, no significant differences in respiratory function, airway hyperresponsiveness, quality of life and exacerbation rate were observed in patients treated with etanercept (25 mg subcutaneously administered twice weekly for 12 weeks) versus those treated with placebo¹²¹.

In individuals with moderate asthma, the recombinant human–murine chimeric anti-TNF α monoclonal antibody infliximab (administered at a dose of 5 mg per kg on weeks 0, 2 and 6) reduced circadian PEF oscillations and the related disease exacerbations¹²².

The effects of golimumab, a fully human TNF α -blocking antibody, were assessed in a large multicentre, placebo-controlled, dose-ranging study (at doses of 50 mg, 100 mg or 200 mg every 4 weeks for 52 weeks) in 309 patients with uncontrolled severe asthma¹²³. No significant improvements in lung function and disease exacerbations were detected. Moreover, 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). Therefore, the trial was interrupted and at present it appears to be very unlikely that anti-TNF α antibodies will be further evaluated for the treatment of severe asthma.

The discordances between these two studies (investigating infliximab and golimumab) might be due to phenotypic differences between the enrolled patients, because it is known that patients with more severe disease have a greater susceptibility to infections and other comorbidities — which could be exacerbated by TNF α blockade — than individuals with moderate persistent asthma. A subgroup analysis of the patients enrolled in the golimumab trial demonstrated that the drug was beneficial in 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 >12%¹²³. These observations suggest that patient stratification, based on both clinical and functional parameters, is important in order to identify those patients who predominantly have the most severe disease and could potentially be more responsive to a TNF α -based therapeutic strategy.

Targeting IL-17 and IL-23

IL-17A and IL-17F — pro-inflammatory cytokines that are released by T_H17 cells and crucially involved in neutrophilic inflammation as well as in airway remodelling — are upregulated in bronchial biopsy samples obtained from patients with severe asthma¹³. In this regard, it is noteworthy that neutralizing monoclonal antibodies against IL-17 lowered the numbers of neutrophils, eosinophils and lymphocytes in bronchoalveolar lavage fluid in mouse models of allergic asthma¹²⁴.

Ongoing Phase II clinical trials are currently evaluating the efficacy and safety of a fully human IL-17A-specific monoclonal antibody (secukinumab (also known as AIN457); ClinicalTrials.gov identifier: NCT01478360), as well as of a human IL-17R-specific monoclonal antibody (brodalumab (also known as AMG827); ClinicalTrials.gov identifier: NCT01199289), in patients with severe asthma that is not adequately controlled by inhaled corticosteroids and long-acting β_2 -adrenergic receptor agonists. Another potential therapeutic approach is the use of antibodies directed against the IL-17-regulating cytokine IL-23, blockade of which results in inhibition of antigen-dependent recruitment of neutrophils, eosinophils and lymphocytes into the airways of sensitized mice¹²⁵. In these animal models of asthma, a dramatic reduction in lung inflammation was also obtained through RNA interference-mediated knockdown of IL-23 (REF. 126). However, these experimental strategies should be considered with extreme caution, because IL-17 is also involved in immune protection against infectious and carcinogenic agents¹²⁷, and hence inactivation of this cytokine could result in an increased risk of opportunistic infections and cancer development.

Targeting IL-25, IL-33 and TSLP

IL-25, IL-33 and TSLP, which are mainly released from the airway epithelium, are overexpressed in airways of patients with asthma and have a crucial role in driving and stimulating T_H2-mediated immune-inflammatory responses²³. Therefore, these cytokines are potential targets for novel anti-asthma therapies.

In mice, an IL-25-specific monoclonal antibody suppressed T_H2-dependent allergic airway inflammation¹²⁸. Moreover, allergic inflammation and airway hyperresponsiveness in mice can be attenuated by antibodies directed against IL-33R¹²⁹. These antibodies can also markedly inhibit IL-17F expression in human bronchial epithelial cells¹³⁰. An attenuation of allergic airway inflammation in mice has also been observed as a consequence of antibody-induced neutralization of the TSLP receptor¹³¹.

Antibodies targeting TSLP are currently under clinical development, and one of these (AMG157) is being investigated in Phase Ib studies for the potential intravenous treatment of individuals with mild atopic asthma (ClinicalTrials.gov identifier: NCT01405963).

IL-27

IL-27 is a monocyte- and macrophage-derived innate cytokine that is probably involved in the pathogenesis of severe, corticosteroid-resistant asthma. Indeed, IL-27 levels are increased in the airways of patients with severe neutrophilic asthma¹³². Moreover, in mouse lung macrophages, IL-27 itself inhibited nuclear translocation of glucocorticoid receptors¹³², which is an essential cellular event for the biological and pharmacological effects of corticosteroids. Therefore, IL-27 may represent a potential target for new therapeutic strategies aimed to provide better control of severe, steroid-refractory asthma.

IFN β

Based on the findings regarding the deficient production of antiviral IFN β in airway epithelial cells, which is typically observed in patients with asthma, a randomized, multicentre, placebo-controlled study has been recently completed in 134 adult patients with mild-to-moderate to severe asthma in order to evaluate the potential therapeutic effects of inhaled IFN β (SNG001; ClinicalTrials.gov identifier: NCT01126177). In particular, after the onset of an airway viral infection, SNG001 attenuated the symptoms caused by respiratory viruses, thus preventing asthma from worsening in the first week of infection, as shown by a 65% reduction in the number of patients experiencing moderate exacerbations during the treatment period (see the 19 April 2012 press release on the University of Southampton website).

Mast cell-related kinases

Spleen tyrosine kinase (SYK), which is involved in IgE receptor-dependent intracellular signalling, has a key role in mast cell activation¹³³. In this regard, it is noteworthy that an aerosolized SYK antisense oligonucleotide reduced allergic airway inflammation in the Brown Norway rat model of ovalbumin-induced asthma¹³⁴.

Translating this therapeutic approach from animal models to clinical studies, intranasal delivery of the small-molecule SYK inhibitor R112 significantly improved the symptoms of patients with seasonal allergic rhinitis¹³⁵. Furthermore, a more potent SYK inhibitor — R343 — is currently in development for use as an inhalant in patients with asthma; Phase I clinical trials have been completed, and R343 is scheduled to enter Phase II development very soon. In this regard, approximately 270 adult patients

with allergic asthma will be randomized to undergo the so-called SITAR ('SYK Inhibition for Treatment of Asthma with R343') study (see [the Rigel website](#) for further details).

Phenotype-selective biological therapies

An accurate characterization of the distinct asthma phenotypes is essential for the development and implementation of biological treatments. Whereas corticosteroids interfere with several pro-inflammatory pathways that are widely involved in asthma pathophysiology, a cytokine-based therapy usually targets a restricted pathogenic cascade that is relevant for a specific phenotype. For example, atopic patients with T_H2 -driven eosinophilic asthma, which is not adequately controlled by corticosteroids, could greatly benefit from inhibition of IL-5, IL-4 and IL-13. Patients with severe neutrophilic asthma might instead be more susceptible to a blockade of IL-17, which also contributes to steroid resistance. Patients with mixed neutrophilic-eosinophilic phenotypes, which are quite frequent in the exacerbation-prone variant of severe asthma, could thus be treated with combinations of biological drugs targeting several different cytokines.

Moreover, the prospective approaches that are aimed at neutralizing the effects of the innate cytokines IL-25, IL-33 and TSLP, which are very important in the initial priming of T_H2 -mediated airway inflammation, appear to be very promising. Such cytokine-based biological strategies could thus make it possible to disrupt the tight link between adaptive and innate immune responses, which probably lead to the development of severe and difficult-to-treat subtypes of asthma in susceptible individuals.

It is therefore crucial to select reliable biomarkers that can be assessed in clinical studies in order to characterize patients with specific asthma phenotypes and their responses to biological treatments¹³⁶. For example, peripheral blood eosinophil counts can be useful to monitor the anti-inflammatory effects of omalizumab. A recent pooled analysis of data from several trials, involving patients with moderate-to-severe persistent allergic asthma who were treated with this drug, has found some degree of correlation between the omalizumab-induced decrease in peripheral blood eosinophil counts and a positive global evaluation of treatment effectiveness, which was associated with an increased FEV1 and a reduced requirement for the management of exacerbations with oral steroid bursts¹³⁷. The persistence of induced sputum eosinophilia in individuals with exacerbation-prone severe asthma (despite receiving inhaled and oral corticosteroid therapy) has been shown to be a very useful criterion for detecting patients who have a marked susceptibility to anti-IL-5 treatment with mepolizumab^{80–82}. Furthermore, the blood biomarker periostin is valuable in detecting individuals with a ' T_H2 -high' phenotypic profile who are likely to benefit from the IL-13-specific antibody lebrikizumab^{24,25}.

However, a better stratification of patients with asthma — based on phenotypic differences — probably requires more sophisticated approaches related to 'omics'-based system-wide tools such as genomics, proteomics¹³⁸

and metabolomics. In this regard, it is particularly interesting that a recent study based on genotypic identification of specific single nucleotide polymorphisms in the *IL4RA* gene detected patients with asthma who were more susceptible to the exacerbation-inhibitory effect of the dual IL-4- and IL-13 antagonist pitrakinra¹⁰¹. Therefore, in order to optimize personalized therapies with biological drugs, current and future investigations aimed at developing a better understanding of asthma heterogeneity should focus on molecular characterization of the different phenotypes, with the aim of linking clinical presentation to the underlying biology within a 'bench to bedside' translational framework.

Conclusions

As von Mutius and Drazen¹³⁹ elegantly pointed out in a recent editorial: "Asthma is both easy and hard to treat. It is easy to treat because the vast majority of patients require little medication for a lot of benefit." However, "asthma becomes hard to treat when asthma control is not obtained with the health care provider's first choice of a controller; this usually means that treatments need to be stepped up and leads to the question, [that is] my patient needs more treatment, but what will offer the greatest likelihood of improvement?"^{91,39}. In this regard, on the basis of our experience, we think that substantial efforts must be made to characterize the phenotypic pattern of each patient with difficult-to-treat asthma. Indeed, only by following this approach will it be eventually possible to identify, across the heterogeneity and redundancy of asthma pathophysiology, the clinical and biological profiles that make up the individual expressions of this disease.

Therefore, such a methodological approach could allow the delineation of personalized therapies, which will hopefully be capable of satisfying the unmet medical needs of patients with difficult-to-control asthma. Within this context, we believe that biological drugs could provide a diversified choice of tailored anti-asthma medications. During the past few years, basic and clinical research strategies have identified many attractive molecular targets for asthma treatment. In particular, IgE- and cytokine-targeted therapies (used in addition to conventional treatments and eventually used in various combinations, according to the patient's individual requirements) could lead to considerable improvements in the control of severe asthma.

The relative variability observed in the individual responses to these novel biological therapies further emphasizes the necessity of accurate asthma phenotyping in order to achieve the best possible patient-focused strategy for disease management. Because it is frequently reported that the blockade of a single mediator or cytokine results in only partial efficacy, the next research challenge might be to explore, in carefully selected individuals with asthma, the effects of different cocktails of biologics targeting the key pathogenic pathways that underlie the various phenotypic subgroups of asthma. Ongoing advances in our understanding of asthma pathobiology could make it possible, in the near future, to further extend the already promising scenario of biological therapies for this — sometimes hard to manage — disease.

Innate immune responses
Rapid and non-specific cellular responses to pathogens and/or tissue injury, which stimulate and influence the relatively slow development of specific adaptive immune responses.

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Competing interests statement

The authors declare no competing financial interests.

FURTHER INFORMATION

ClinicalTrials.gov website: <http://www.clinicaltrials.gov>
Rigel website (R343 — Asthma): <http://www.rigel.com/rigel/aa>
University of Southampton website — “Positive results in Southampton-led patient trial for new asthma treatment” (19 April 2012 press release): https://www.soton.ac.uk/mediacentre/news/2012/apr/12_64.shtml

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