

Targeting eosinophils in allergy, inflammation and beyond

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Abstract | Eosinophils can regulate local immune and inflammatory responses, and their accumulation in the blood and tissue is associated with several inflammatory and infectious diseases. Thus, therapies that target eosinophils may help control diverse diseases, including atopic disorders such as asthma and allergy, as well as diseases that are not primarily associated with eosinophils, such as autoimmunity and malignancy. Eosinophil-targeted therapeutic agents that are aimed at blocking specific steps involved in eosinophil development, migration and activation have recently entered clinical testing and have produced encouraging results and insights into the role of eosinophils. In this Review, we describe recent advances in the development of first-generation eosinophil-targeted therapies and highlight strategies for using personalized medicine to treat eosinophilic disorders.

Helminth infections

Infections with parasitic worms; helminth infections are the most common cause of eosinophilia worldwide, particularly in low-income countries. Infections with helminths can be transmitted via mosquito bites, by eating infected food, by drinking contaminated water and by walking on contaminated soil.

Churg–Strauss syndrome

A rare systemic necrotizing vasculitis that affects small to medium-sized vessels and is characterized by asthma, blood eosinophilia and eosinophil-rich granulomatous inflammation in affected tissues. First-line treatment involves the use of corticosteroids.

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Eosinophils are bone marrow-derived leukocytes that normally represent less than 5% of leukocytes in the blood, but they can be found in higher numbers in tissues such as the bone marrow and gastrointestinal tract. Eosinophilia (BOX 1), defined as a peripheral blood eosinophil count greater than 450 cells per microlitre, is associated with numerous disorders, including allergies, drug reactions, helminth infections, Churg–Strauss syndrome, some malignancies, metabolic disorders, eosinophilic gastrointestinal disorders and hypereosinophilic syndrome. The recruitment of activated eosinophils from the bloodstream into tissues can occur under numerous conditions and lead to the release of pre-formed and newly synthesized products — including cytokines, chemokines, lipid mediators and cytotoxic granule proteins — that can initiate, quickly escalate and sustain local inflammatory and remodelling responses (FIG. 1).

Eosinophil-rich inflammation has long been associated with parasitic infestation and allergic inflammation. A substantial body of evidence — including clinical studies and animal models of asthma — has demonstrated a causal role for eosinophils in asthma pathogenesis, including airway hyperreactivity, elevated mucus production and airway remodelling. These studies include elegant experiments in eosinophil-lineage-deficient mice that are protected (although not in all cases) against features of asthma^{1,2}. Clinical studies have revealed an important role for eosinophils in asthma

exacerbations^{3,4}. Recent evidence also supports a broader role for eosinophils in health and disease, with an emerging role in malignancy and in regulating antibody production^{5,6}. In addition, eosinophils can frequently be found surrounding solid tumours^{6,7} and can participate in tumour immune surveillance, thereby influencing the incidence of tumour formation^{8,9}.

Recent studies have proposed a role for eosinophils in humoral immunity as an important source of pro-survival factors for long-lived plasma cells in the bone marrow^{10,11}. In autoimmune disease, in which plasma cells have a pathological role via autoantibody production, eosinophils may prove to be an attractive therapeutic target. Thus, treatments that specifically target eosinophils are likely to be effective in controlling allergy, infectious disease, autoimmunity and malignancy. The increasing incidence of eosinophil-associated disorders, including eosinophilic gastrointestinal disorders and asthma, in both high-income and low-income countries highlights the important and expanding need for eosinophil-targeted therapies^{12,13}.

There have traditionally been two major approaches to drug development for eosinophilic diseases: blocking the recruitment of eosinophils into organs and impairing the survival of mature eosinophils. Below, we discuss innovative new strategies for future drug development, including blockade of eosinophil production in the bone marrow and the inhibition of eosinophil activation.

Box 1 | Blood and tissue eosinophilia

Eosinophilia (that is, an increased number of eosinophils) can be found both in the peripheral blood and/or in tissues in several disorders, including helminthic parasite infections, atopic and allergic diseases as well as adverse drug reactions^{163,168–170}. In the peripheral blood, eosinophilia can be characterized as mild (450–1,500 eosinophils per microlitre), moderate (1,500–5,000 eosinophils per microlitre) or severe (greater than 5,000 eosinophils per microlitre). Hypereosinophilia refers to blood eosinophil levels greater than 1,500 eosinophils per microlitre. In general, blood eosinophilia results from enhanced eosinophilopoiesis (increased production of eosinophils in the bone marrow). Peripheral blood eosinophilia can be further categorized as primary, secondary and idiopathic. Primary eosinophilia usually occurs in the context of haematological malignancies and proliferative disorders that result in increased numbers of progenitors, leading to increased numbers of eosinophils in the bone marrow and blood. Secondary eosinophilia is the most common form of eosinophilia and often occurs in response to other primary disease processes such as overproduction of the cytokine interleukin-5 (IL-5), often by T cell lymphocytes, leading to elevated production of eosinophils. Idiopathic eosinophilia is often moderate to severe eosinophilia and has no identifiable cause.

In healthy individuals, tissue eosinophils can normally be found in the bone marrow and gastrointestinal tract, but they can also be found in lower numbers in the thymus and fat pads. The number of eosinophils that normally reside in tissues can vary dramatically, even within the same organ system. For example, in a healthy gastrointestinal tract, eosinophils can be found in increasing numbers from the stomach to the colon, but no eosinophils are normally present in the oesophagus.

Tissue eosinophilia usually results from increased recruitment of activated eosinophils from the bloodstream. Once recruited into tissues, activated eosinophils have the capability to cause tissue damage and dysfunction. Tissue eosinophilia does not always correlate with blood eosinophilia. Blood eosinophil numbers can be normal, but increased migration of activated eosinophils from the blood increases eosinophil numbers in organs, as observed in patients with eosinophilic oesophagitis and eosinophilic pneumonias. By contrast, blood eosinophilia can occur without any evidence of increased tissue eosinophilia; this is likely to be due to the absence of activating signals in the bloodstream.

Pathogenic actions of eosinophils

Eosinophils develop in the bone marrow from haematopoietic stem cells^{14,15}. During human haematopoiesis, common myeloid progenitors give rise to CD34⁺IL-5Rα⁺ eosinophil progenitors¹⁴, the numbers of which are increased in several diseases including allergies, helminth infections and hypereosinophilic syndrome. This suggests that the increased production of eosinophil progenitors is an important checkpoint in disease-associated eosinophilia^{14,16}. Mature human eosinophils contain crystalloid secondary granules, which are primarily composed of highly charged basic proteins including two eosinophil granule major basic proteins (MBPs), eosinophil cationic protein, eosinophil-derived neurotoxin and eosinophil peroxidase¹⁷. A common finding in eosinophil-associated diseases is the deposition of granules (released from eosinophils) in tissues, which contributes to disease pathogenesis^{18–22}. MBP and eosinophil peroxidase are toxic to several cell types, including airway epithelial cells^{23,24} and cardiac muscle cells²⁵, and may contribute to tissue damage and organ dysfunction in patients with asthma or hypereosinophilic syndrome. Eosinophil-derived neurotoxin and eosinophil cationic protein (which belong to the RNase A family of granule proteins that have ribonuclease activity)²⁶ are associated with host defence against viruses²⁷ and may have a role in tissue remodelling²⁸.

Hypereosinophilic syndrome

A heterogeneous group of disorders characterized by a persistently elevated peripheral blood eosinophil count (> 1,500 eosinophils per mm³), typically without any recognizable cause.

Eosinophilic oesophagitis

A chronic antigen-induced disease characterized by symptoms of oesophageal dysfunction, evidence of eosinophil infiltration of at least 15 eosinophils per high-power microscopy field on oesophageal biopsy and the exclusion of other possible causes of oesophageal eosinophilia, especially oesophageal eosinophilia induced by gastroesophageal reflux disease.

In addition to these cationic proteins, eosinophil granules contain a plethora of preformed cytokines, chemokines, enzymes and growth factors, resulting in a diverse biological activity of eosinophils in infection and inflammation²⁹ (FIG. 1). In addition to the preformed mediators stored in the granules, eosinophils can release *de novo*-synthesized mediators upon activation, including interleukin-4 (IL-4), which has the capacity to stimulate adaptive immunity.

Current therapies and moving forward

Glucocorticoids are currently the most effective form of therapy used to reduce eosinophil numbers in the blood and tissue (TABLE 1), but the pleiotropic effects of corticosteroids can result in potentially harmful side effects and limit their therapeutic use. Glucocorticoids promote eosinophil clearance by directly inducing apoptosis and by inhibiting pro-survival signals induced by cytokines such as IL-3, IL-5 and granulocyte-macrophage colony-stimulating factor (GM-CSF)^{30–32} (FIG. 2). The tyrosine kinase inhibitor imatinib (Gleevec; Novartis), the first and only drug approved for the treatment of hypereosinophilic syndrome, can reduce blood eosinophil levels but only in patients with genetic alterations involving fusion genes that result in hypereosinophilic syndrome, such as the fusion of the gene encoding FIP1-like 1 protein (FIP1L1) with the gene encoding platelet-derived growth factor receptor α (PDGFRA)^{33,34}.

Therapy directed against the eosinophil growth factor IL-5 is effective in animal studies³⁵ and has recently been tested in clinical trials (as discussed below); although targeting IL-5 substantially decreases blood eosinophil counts in several different diseases, such as hypereosinophilic syndrome, eosinophilic asthma and eosinophilic oesophagitis, the reduction of tissue eosinophilia and improvements in symptoms have been variable depending on the disease and patient subgroup^{4,36–38}. Along with these drugs, other new drugs that are based on an improved understanding of the mechanism of eosinophilia and the effector functions of eosinophils are thus urgently needed.

Blockade of eosinophil recruitment

A network of cytokines, adhesion molecules, chemoattractants and receptors regulate eosinophil trafficking¹⁷. Several of these molecules have been investigated as candidates for blocking eosinophil accumulation in tissues.

CCR3 and CCL11. CC chemokine receptor 3 (CCR3), a seven-transmembrane cell surface G protein-coupled receptor, is a promiscuous chemokine receptor that is selectively and abundantly expressed on eosinophils and promotes their accumulation; CCR3 has up to 11 different ligands^{39,40}. These ligands include the eotaxins (CC chemokine ligand 11 (CCL11; also known as eotaxin 1), CCL24 (also known as eotaxin 2) and CCL26 (also known as eotaxin 3)), which are high-affinity agonists for CCR3. In addition to eosinophil chemoattraction, eotaxin-mediated activation of CCR3 triggers the respiratory burst apparatus, induces eosinophil degranulation and upregulates the expression of adhesion molecules^{41,42}.

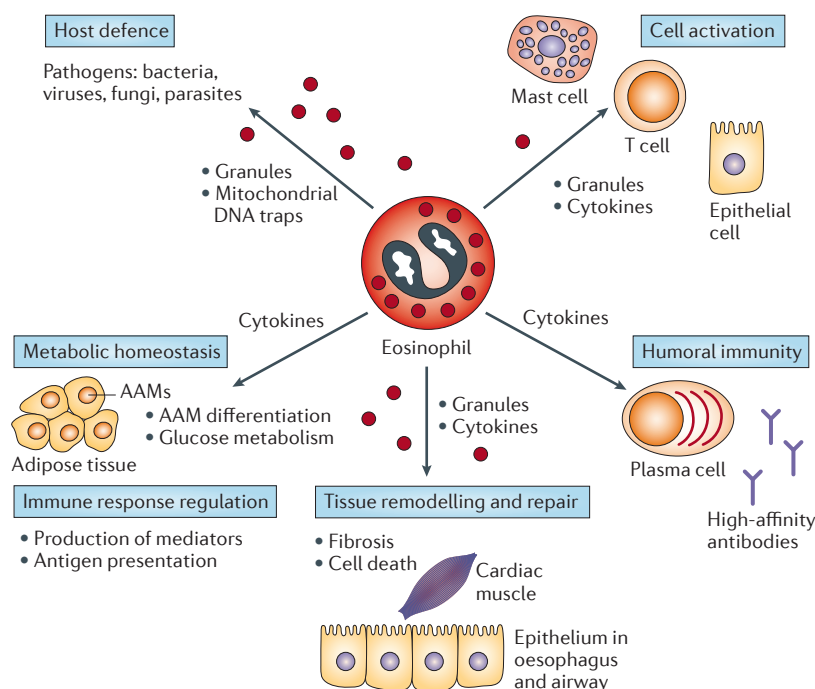


Figure 1 | Effector functions of eosinophils. Eosinophils are pleiotropic cells that have the capability to release both preformed and newly synthesized granule proteins, cytokines, lipid mediators and growth factors. Eosinophils have roles in host defence against several pathogens, in the activation of neighbouring cells including mast cells, T cells and epithelial cells, in promoting the survival of long-lived plasma cells in the bone marrow, in metabolic homeostasis through the maintenance of alternatively activated macrophages (AAMs) in adipose tissue, in regulating immune responses as well as in tissue remodelling and repair.

Although *in vitro* and *in vivo* preclinical studies have supported a crucial role for CCR3 and its ligands in recruiting eosinophils into inflamed tissues, the lack of statistically significant efficacy in clinical trials to date may be related to the activity of redundant pathways in the diseases being studied. The redundancy of the chemokine and chemokine receptor system as well as the exquisite sensitivity of eosinophils to other chemoattractant molecules probably contributes to the decreased therapeutic potential of CCR3 blockade. However, some diseases are likely to be more selectively driven by an eotaxin-dependent pathway, such as eosinophilic oesophagitis, in which *CCL26* is the most highly induced gene in the oesophagus and there is limited induction of other major eosinophil chemoattractants⁵¹. Indeed, mice that are genetically engineered to be deficient in *CCL11* or *CCR3* are protected from the development of oesophageal eosinophilia^{51,52}. In addition, as eotaxin elicits eosinophilia in concert with other eosinophil-directed cytokines such as IL-5, combination therapy with IL-5-targeted and eotaxin- or CCR3-targeted treatment may be more effective. Indeed, mice in which both IL-5 and eotaxin 1 are ablated have increased protection against experimental asthma than mice in which there is targeted ablation of either gene alone⁵³.

Adhesion molecules. The migration of eosinophils from the bloodstream into various tissues results from the specific interactions between integrins on the surface of eosinophils and adhesion receptors on the surface of the vascular endothelium¹⁷. In experimental models, the recruitment of eosinophils into the lung in response to allergen challenge is dependent on very late antigen 4 (VLA4; also known as integrin $\alpha 4 \beta 1$), an integrin dimer composed of integrin $\alpha 4$ (also known as CD49D) and integrin $\beta 1$ (also known as CD29)⁵⁴. This finding prompted investigations targeting VLA4 in order to inhibit eosinophil accumulation in inflamed lungs. Preclinical studies demonstrated that blockade of VLA4 using a CD49D-specific antibody inhibited airway eosinophilia in an experimental asthma model⁵⁵. Small-molecule VLA4 antagonists have also been evaluated for potential clinical utility, with studies demonstrating inhibition of eosinophil adhesion to VLA4 and a significant reduction (of up to 80%) of skin eosinophilia induced by the intradermal injection of CCL11 into mice⁵⁶.

Of note, marked peripheral blood eosinophilia (more than 2,000 eosinophils per mm³) has been observed in three patients with multiple sclerosis following treatment with natalizumab (Tysabri; Elan/Biogen Idec), a humanized monoclonal antibody against CD49D⁵⁷. It will be interesting to determine whether this was mediated by a direct inhibitory effect of natalizumab on eosinophil adhesion that resulted in the retention of eosinophils in the bloodstream.

Taken together, these data suggest that although blockade of the interaction between integrins and adhesion receptors could result in decreased eosinophil accumulation in tissues, there is the potential to induce secondary blood eosinophilia (with its associated risks), which could limit the therapeutic benefits of this strategy.

The CCR3–CCL11 axis is crucial for the recruitment and accumulation of eosinophils in numerous disease states, including experimental models of asthma and eosinophilic gastrointestinal disorders^{43–46}. In addition, increased expression of CCR3 and its ligands correlates with disease severity in patients with asthma⁴⁷. Thus, antagonism of CCR3 has been a promising target for therapeutic intervention in eosinophil-associated inflammation. In preclinical studies, low-molecular-mass CCR3 antagonists reduced airway eosinophilia in models of chronic experimental asthma^{48,49}.

A selective and competitive CCR3 antagonist was evaluated in a Phase II trial (ClinicalTrials.gov identifier: NCT01160224) for its effectiveness in reducing sputum eosinophilia in individuals with mild to moderate asthma, but no results have yet been reported. Bertilimumab, a neutralizing antibody directed against CCL11, has been evaluated for reducing nasal congestion in a Phase II trial; allergen-induced nasal obstruction was significantly reduced and there was a trend towards reduced numbers of eosinophils in nasal mucosa biopsy samples. However, this trend did not reach statistical significance, perhaps owing to the small sample size of patients⁵⁰. The application of this drug for other eosinophil-associated disorders, such as subgroups of patients with ulcerative colitis, is currently being pursued (ClinicalTrials.gov identifier: NCT01671956).

Eotaxins

A subset of structurally related chemokines that bind to the eotaxin receptor (CC chemokine receptor 3) and are involved in selectively activating and chemoattracting eosinophils.

Respiratory burst

The rapid release of destructive reactive oxygen species from eosinophils and other immune cells that have an important role in the immune response to infection; this contributes to oxidative damage during inflammatory responses.

Table 1 | **Current therapies used to treat severe eosinophilia**

Therapy	Mechanism of action
Corticosteroids (systemic, topical and inhaled)	Inhibit eosinophil survival and promote eosinophil clearance from tissues
Imatinib	Inhibits receptor tyrosine kinase activity
Hydroxyurea	Cytotoxic agent; precise mechanism of action is unclear
Interferon- α	Inhibits eosinophil development and functional responses

CRTH2 and PGD₂. Prostaglandin D₂ (PGD₂) is a product of arachidonic acid metabolism that is generated and released by activated mast cells (and other inflammatory cells including eosinophils themselves) during an allergic response⁵⁸. PGD₂ induces eosinophil chemotaxis and the mobilization of mature eosinophils from the bone marrow^{59,60}. The effects of PGD₂ are mediated through two G protein-coupled receptors: the PGD₂ receptor DP1 and chemoattractant receptor of T_H2 cells (CRTH2; also known as PTGDR2). CRTH2 is expressed on the surface of T helper 2 (T_H2) cells, eosinophils and basophils⁶¹.

In experimental models of asthma, CRTH2 mediates the recruitment of eosinophils into the lung^{61,62}. Furthermore, PGD₂ activates eosinophils via CRTH2, resulting in the release of granule proteins and respiratory burst activity⁶³. As PGD₂ signalling results not only in eosinophil recruitment but also in eosinophil activation and the mobilization of mature eosinophils from the bone marrow, intervening in this pathway has the potential to result in substantial clinical benefit. Thus, antagonizing CRTH2 has been pursued as a potentially useful strategy for the treatment of eosinophil-associated disorders.

Low-molecular-mass CRTH2 antagonists partially attenuate pulmonary eosinophilia in various models^{64,65}. A published Phase II study of the effectiveness of a CRTH2 antagonist in patients with moderate persistent asthma showed a significant reduction in the geometric mean sputum eosinophil count from 2.1% to 0.7% after treatment⁶⁶. Although this may appear to be a small difference, reductions in sputum eosinophil counts (even at this low level) correlate very well with improved asthma control⁶⁷. Another Phase II study is in progress to evaluate the effects of CRTH2 on reducing sputum eosinophilia in patients with persistent asthma (ClinicalTrials.gov identifier: NCT01545726). Preliminary data from a clinical trial of patients with active eosinophilic oesophagitis show that treatment with a CRTH2 antagonist results in a moderate reduction in tissue eosinophilia⁶⁸. More clinical studies are needed to evaluate the effectiveness of CRTH2 blockade, and possibly DP1 blockade, on blood and tissue eosinophilia in human disease.

Histamine H₄ receptor. The histamine H₄ receptor is a G protein-coupled receptor that is expressed on cells of the immune system, including eosinophils. Activation of the H₄ receptor by its ligand histamine results in eosinophil chemotaxis to sites of allergic inflammation and increased expression of adhesion molecules on the surface of eosinophils^{69,70}. Small-molecule antagonists of the

H₄ receptor inhibit eosinophil migration *in vitro*^{71,72}. In addition, antagonism of the H₄ receptor inhibits eosinophil infiltration into the oesophageal epithelium in an allergen-induced model of eosinophilic oesophagitis in guinea pigs⁷³. To date, only preclinical assessments of candidate drugs targeting H₄ receptors have been completed, but the results from these studies advocate the H₄ receptor as a potential drug target for the treatment of eosinophil-associated inflammatory disorders^{72,74}.

IL-13 and IL-4. Several studies have shown that IL-13 has an important role in eosinophil-associated disorders^{75,76}. In animal models, IL-13 is a key cytokine that regulates the recruitment of eosinophils at inflammatory sites, primarily through the induction of chemokine expression^{77,78}. For example, a whole-genome expression analysis of primary human oesophageal epithelial cells showed that *CCL26* exhibited the greatest increase in expression levels following induction with IL-13 (REF. 79).

In patients with asthma, IL-13 expression has been associated with eosinophil recruitment into the airway in response to allergen challenge^{80,81}. Thus, it has been hypothesized that IL-13 neutralization could inhibit allergen-induced eosinophil inflammation. However, when the effectiveness of two fully humanized IL-13-specific antibodies on allergen-induced responses in patients with mild asthma was recently assessed, there were no changes in the numbers of peripheral blood eosinophils or sputum eosinophils⁸². The treatment of patients with uncontrolled asthma using lebrikizumab, a humanized monoclonal antibody that binds to IL-13, resulted in modestly increased peripheral blood eosinophil counts, suggesting that IL-13 blockade decreased eosinophil recruitment from the bloodstream into the lungs; however, in patients with persistently increased eosinophil production this may lead to blood eosinophilia⁸³. In addition, an IL-13-blocking antibody had no effect on nasal lavage eosinophil numbers in patients with allergic rhinitis in a nasal allergen challenge model⁸⁴.

These studies suggest that IL-13 blockade alone may be insufficient to inhibit tissue eosinophilia and blood eosinophilia, perhaps owing to the overlapping roles of IL-13 and IL-4 in promoting eosinophil-rich inflammation^{85,86}. Accordingly, the therapeutic effects of pitakinra, an IL-4 variant and fully human monoclonal antibody against IL-4 receptor subunit α (IL-4Ra) that blocks both IL-4 and IL-13 activity, have been investigated in Phase II clinical trials^{87,88}. However, inhibition of the biological activity of both IL-4 and IL-13 resulted in no significant change in the number of sputum eosinophils^{87,88} or peripheral blood eosinophils⁸⁸ in patients with atopic asthma.

Summary of blockade of eosinophil recruitment

Preclinical studies have suggested that blockade of eosinophil migration from the bloodstream into tissues has the potential to be therapeutically beneficial, but there has been limited clinical success to date with current targets and approaches. Studies have emphasized the importance of selecting patients for clinical trials on the basis of the mechanism of action of the drug^{4,83,89},

Atopic asthma

Also known as allergic asthma; a form of asthma that is characterized by recurrent attacks of difficulty in breathing with wheezing and inflammation and is caused by exposure to inhaled airborne allergens.

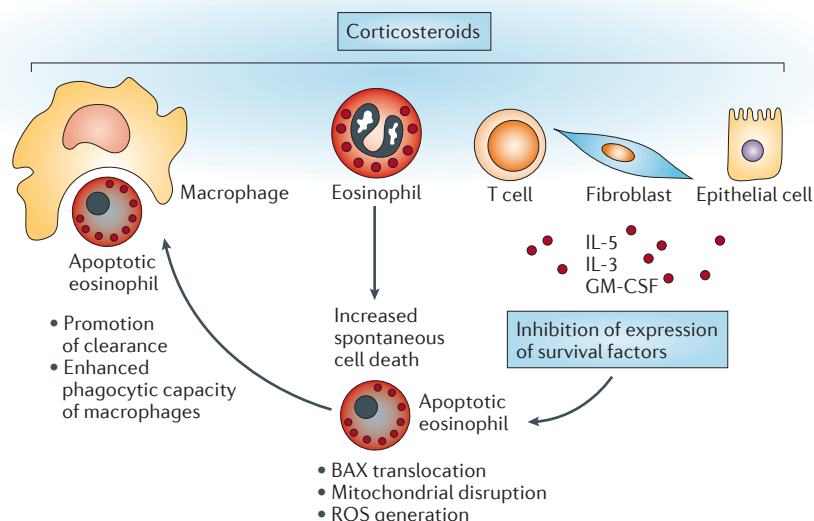


Figure 2 | The effects of corticosteroids on eosinophils. Corticosteroids reduce the number of eosinophils in the blood and tissues through mechanisms that promote cell death and clearance of eosinophils. Corticosteroids impair eosinophil survival and differentiation by inhibiting the expression of pro-eosinophil cytokines (that is, interleukin-5 (IL-5), IL-3 and granulocyte-macrophage colony-stimulating factor (GM-CSF)) by T cells and structural cells such as fibroblasts and epithelial cells. Corticosteroids amplify the rate of spontaneous cell death of eosinophils by having direct effects on pro-apoptotic signalling pathways and through the increased generation of cell-damaging molecules such as reactive oxygen species (ROS) by eosinophils. Corticosteroids also promote the clearance of apoptotic eosinophils from tissues by enhancing the phagocytic capacity of macrophages. BAX, BCL-2-associated X protein.

and it has been suggested that the lack of efficacy of current approaches may be due to the inclusion of patients who are not likely to respond to the drug owing to varying patient phenotypes⁹⁰. For example, lebrikizumab treatment was more effective in patients with high periostin and blood eosinophil levels, suggesting that these biomarkers could be used to identify patients with an asthma phenotype that is driven by IL-13 (REF. 83). Notably, this particular group of patients may be more likely to respond to specific and effective anti-eosinophil therapeutics, either alone or in combination with agents that block IL-13 and IL-4.

More work is needed to identify those patients who are most likely to respond to migration-targeted therapy. The lack of effectiveness of current targets may also be due to the complex regulation of eosinophil recruitment into inflammatory tissue, especially within the lung, which is often the first organ for which eosinophil-targeted therapy is tested (because of the large potential market for asthma); a single target may be insufficient to block tissue eosinophilia and prevent eosinophil-mediated pathology.

Inhibition of eosinophil survival

Eosinophils differentiate from multipotent stem cells in the bone marrow under the influence of IL-3, GM-CSF and IL-5 (REF. 91). The most eosinophil-specific of these cytokines is IL-5, which has key roles in the proliferation

and maturation of the committed progenitors as well as in the migration and survival of mature eosinophils^{92,93}. As differentiation and maturation of the developing eosinophil progresses, the cell loses the capacity to proliferate. The life cycle of the terminally differentiated mature eosinophil usually ends physiologically by apoptosis, although necrosis (involving the release of functionally active granules, especially following cellular activation) can also occur. It is notable that eosinophilic inflammatory tissue often contains abundant and intact granules that are extracellular to the eosinophil, suggesting that these may be functionally relevant in the pathogenesis of disease⁹⁴. Indeed, these granules are functionally active in a number of assays⁹⁴. Eosinophils have high rates of spontaneous apoptosis⁹⁵, and several mediators — including IL-5 — promote eosinophil survival^{32,96,97}. Agents that interfere with the survival signals for eosinophils are being actively developed and are now being tested for therapeutic efficacy.

IL-5 and IL-5Rα. As IL-5-mediated signal transduction has been shown to participate in the development, survival and effector functions of eosinophils (FIG. 3), IL-5 and its specific receptor subunit IL-5Rα are attractive therapeutic targets for eosinophil-associated disorders⁹³. Two different humanized IL-5-specific antibodies — mepolizumab and reslizumab — have been developed and tested in clinical trials for asthma^{3,4,89}, hypereosinophilic syndrome³⁶, eosinophilic oesophagitis^{37,98,99} and nasal polypsis¹⁰⁰. Treatment with an IL-5-targeted therapy results in a dramatic decline in blood eosinophilia but has variable efficacy in reducing tissue eosinophilia and no effect on the numbers of eosinophil progenitors in the bone marrow. IL-5-targeted therapy reduced oesophageal eosinophil infiltration⁹⁹ and intraepithelial eosinophil counts^{37,101} in patients with eosinophilic oesophagitis but had variable effects on the symptoms, which was in part likely to be related to the use of non-disease-specific patient-reported outcome measures^{37,101}.

In asthma, initial studies showed no improvement in lung function in patients with asthma who were treated with reslizumab, but a post-hoc analysis of patient data to identify patients with sputum eosinophilia at the baseline showed a significant increase in pulmonary function in patients receiving reslizumab compared with those receiving placebo^{3,102}. In other studies that included patients with persistent asthma and with baseline sputum eosinophilia, reductions in severe exacerbations⁴ and in daily corticosteroid dose⁸⁹ as well as improvements in lung function^{3,38} were observed in those patients receiving the IL-5-targeted therapy mepolizumab. These findings emphasize the importance of selecting patients with eosinophilia in disease-related tissues — for example, the airway in patients with asthma — in order to achieve maximum therapeutic benefit.

Another approach for blocking eosinophils is to therapeutically target the IL-5R with a neutralizing and cytotoxic antibody. Benralizumab is a humanized monoclonal antibody that binds to IL-5Rα and blocks the biological activity of IL-5. Importantly, benralizumab also induces death of eosinophils through

Periostin

An extracellular matrix protein that interacts with integrin molecules on epithelial and leukocyte cell surfaces.

Periostin expression is induced by T helper 2 (T_H2) cytokines such as interleukin-4 (IL-4) and IL-13. Periostin promotes allergic inflammation, including the accumulation of eosinophils in the skin.

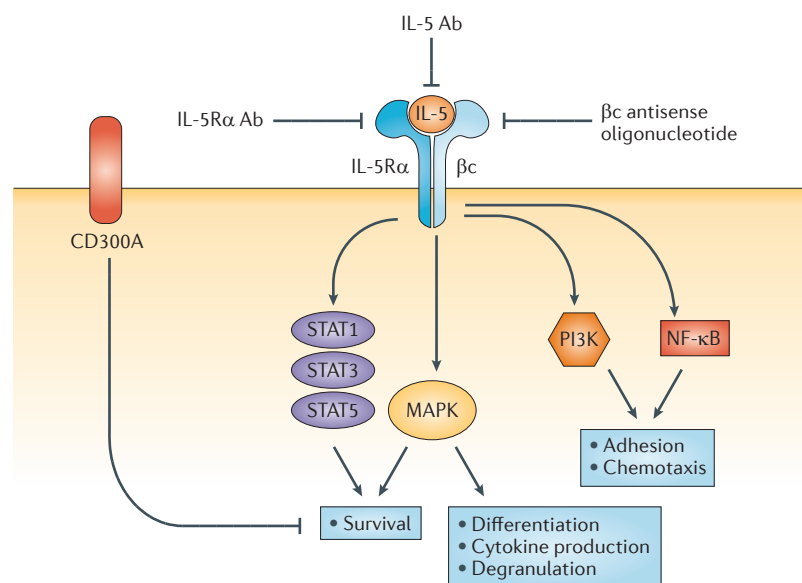


Figure 3 | IL-5-mediated signalling. Interleukin-5 (IL-5)-mediated signal transduction regulates eosinophil differentiation, proliferation and survival. IL-5-mediated stimulation of eosinophils begins with the high-affinity binding of IL-5 to IL-5 receptor subunit α (IL-5Rα), followed by the binding of this complex to the common β-chain (βc) to initiate signal transduction. IL-5 stimulates signal transducer and activator of transcription 1 (STAT1), STAT3 and STAT5 pathways in eosinophils, resulting in the expression of genes that are important in promoting eosinophil survival. The activation of mitogen-activated protein kinase (MAPK) and related pathways results in eosinophil differentiation and degranulation as well as cytokine production. These pathways, along with the phosphoinositide 3-kinase (PI3K) and nuclear factor-κB (NF-κB) pathways, have been shown to promote adhesion and chemotaxis of eosinophils. Therapeutics that bind to IL-5, antagonize IL-5Rα or decrease the expression of the βc subunit inhibit IL-5-mediated effects on eosinophils. The activation of inhibitory receptors, such as CD300A, has also been shown to inhibit IL-5-mediated survival. Ab, antibody.

Eosinopenia

A lower than expected number of eosinophils in the blood or tissue. Eosinopenia can be caused by stress reactions, bacterial infections and the use of corticosteroids.

Antisense oligonucleotide

A synthesized strand of nucleic acid (DNA or RNA) that is complementary to a specific mRNA. Antisense oligonucleotides bind to their target mRNA to promote degradation of the mRNA and prevent its translation, which can ultimately lead to decreased expression of a particular protein.

Splice-switching oligonucleotides

Oligonucleotides that target and bind to a particular mRNA and can be designed to promote favourable splice variants (rather than promoting degradation of the mRNA of targeted genes).

antibody-dependent cell-mediated cytotoxicity¹⁰³. As benralizumab reduced the numbers of peripheral blood eosinophils and eosinophil precursors in the bone marrow of non-human primates¹⁰³, there is considerable potential in targeting IL-5Rα for clinical efficacy in eosinophil-associated diseases. In early Phase I/II studies, benralizumab treatment resulted in significant and prolonged peripheral blood eosinopenia in patients with mild atopic asthma¹⁰⁴.

Another strategy that is being used to inhibit the IL-5-mediated effects on eosinophils involves downregulation of the other subunit of the IL-5R — the common β-chain (βc), which is shared with IL-3, IL-5 and GM-CSF receptors. The inhaled antisense oligonucleotide drug candidate TPI ASM8 contains antisense oligonucleotides directed against the mRNA encoding human CCR3 and βc. In an open-label study, TPI ASM8 reduced the recruitment of allergen-induced sputum eosinophils and eosinophil progenitors into the airway¹⁰⁵. Further studies are underway to test the clinical usefulness of this strategy — downregulating the expression of receptors that are important for the growth and recruitment of eosinophils via degradation of their respective mRNAs. Improvements in the delivery and longevity of antisense oligonucleotides will help enhance the clinical potential

of this technology^{106,107}. Another possibility, although not yet tested for eosinophil-associated diseases, is to promote the expression of the soluble form of IL-5Rα using splice-switching oligonucleotides. This strategy was shown to be effective in mouse models of arthritis in which a splice-switching oligonucleotide changed the expression of the tumour necrosis factor (TNF) receptor from a membrane-bound form to a secreted, soluble form that bound to TNF in the bloodstream and antagonized its activity¹⁰⁸.

SIGLEC-8. Sialic acid-binding immunoglobulin-like lectins (SIGLECs) are surface proteins that are expressed predominantly by leukocytes. SIGLEC-8 is selectively expressed on human eosinophils and mast cells, whereas its murine functional paralogue SIGLEC-F is expressed by eosinophils but not by mast cells¹⁰⁹. The engagement of SIGLEC-8 on the surface of eosinophils by a crosslinking antibody resulted in pronounced cell death via apoptosis¹¹⁰. Preclinical studies with SIGLEC-F-deficient mice demonstrated that SIGLEC-F is important in regulating eosinophil survival in experimental models of asthma¹¹¹. Furthermore, administration of SIGLEC-F-targeted antibodies to mice overexpressing IL-5 (which leads to hypereosinophilia) resulted in the induction of eosinophil apoptosis and a rapid reduction in blood and tissue eosinophilia¹¹². The treatment of mice with a SIGLEC-F-targeted antibody also resulted in decreased eosinophil accumulation in the intestinal mucosa and decreased blood and bone marrow eosinophilia in a model of allergen-induced gastrointestinal inflammation¹¹³.

Together, these preclinical studies strongly support continued investigations into SIGLEC-8-targeted therapies for eosinophil-associated disorders, as the engagement of SIGLEC-8 results in robust and selective apoptosis of eosinophils. Active research is currently underway to fully characterize the endogenous ligands for SIGLEC-8 as well as the enzymes required for its synthesis¹¹⁴.

Immunoglobulin E. Omalizumab (Xolair; Genentech/Novartis) is a recombinant monoclonal antibody that binds to immunoglobulin E (IgE) and prevents it from binding to its receptor Fcε receptor 1 (FcεRI) on the surface of mast cells and basophils. Several clinical trials have demonstrated that omalizumab is effective in reducing exacerbations, corticosteroid use and other clinical outcomes in patients with moderate to severe asthma^{115–118}. In studies in which eosinophilia was measured, there was evidence that IgE-targeted treatment reduced blood eosinophilia. For example, individuals with allergic asthma receiving omalizumab therapy had reduced mean percentages of sputum eosinophils (from 6.6% to 1.7%) and bronchial eosinophil counts (from 8 to 1.5 cells per mm³) compared to patients receiving placebo¹¹⁹.

Pooled analysis from five randomized, double-blinded, placebo-controlled trials in patients with moderate to severe persistent asthma revealed that treatment with omalizumab decreased baseline mean blood eosinophil counts by a median of 18.8% post-treatment, compared

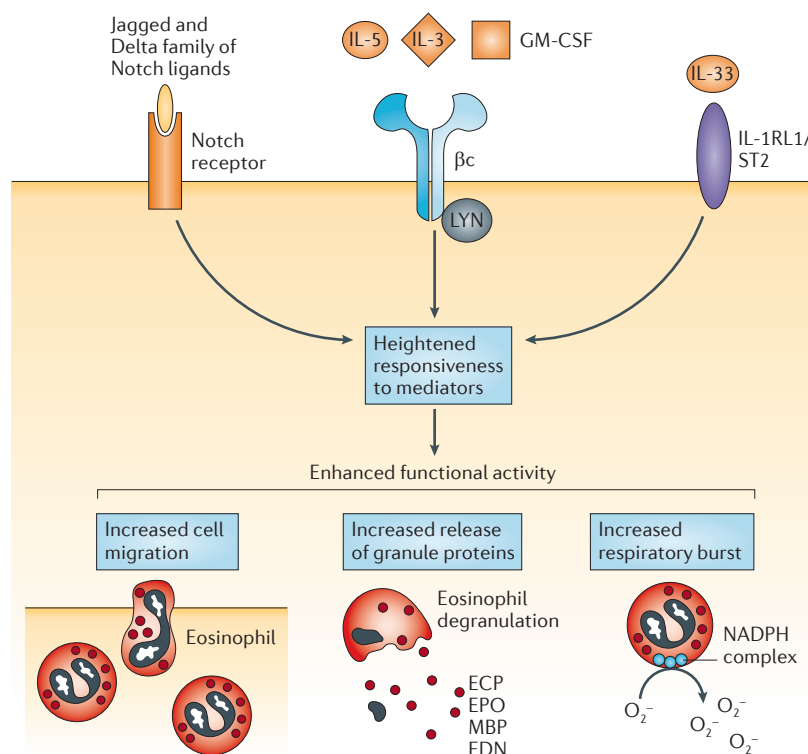


Figure 4 | Eosinophil priming results in amplified responses. The activation of eosinophils by a priming agent, such as interleukin-5 (IL-5), IL-3, IL-33, granulocyte–macrophage colony-stimulating factor (GM-CSF) or Notch ligands, results in enhanced functional responses to stimuli, including increased cell migration, release of granule proteins and respiratory burst activity. The SRC family member LYN is involved in upregulating the activity of downstream pathways that are important for effector functions and results in enhanced eosinophil responsiveness. ECP, eosinophil cationic protein; EDN, eosinophil-derived neurotoxin; EPO, eosinophil peroxidase; IL-1RL1, IL-1 receptor-like 1 (ST2 protein); MBP, eosinophil granule major basic protein.

to a 2.1% decrease with placebo¹²⁰. The mechanism by which the reduction in circulating IgE levels with omalizumab therapy also leads to a reduction in blood and tissue eosinophil numbers remains unclear. There is an increased level of eosinophil apoptosis in omalizumab-treated patients compared to placebo-treated patients, but the reason for this is unknown¹²¹.

In a small open-label trial evaluating omalizumab in patients with eosinophilic gastrointestinal disorders, mean blood eosinophil counts were significantly decreased (by 47%) following omalizumab treatment¹²². Numbers of eosinophils in the duodenum and stomach declined (but not significantly), whereas oesophageal eosinophilia remained unchanged. Studies that are focused on understanding how IgE modulates blood and tissue eosinophilia are needed, and results may yield potential new therapeutic targets.

Inhibitory receptors. Immune responses are regulated by an intricate network of positive and negative signals¹²³. Although most studies have focused on activation receptors on cells of the immune system, recent evidence supports an important role for inhibitory receptors in maintaining immune homeostasis¹²⁴. Eosinophils are

known to express various different inhibitory receptors in addition to SIGLEC-8 (described above) such as paired immunoglobulin-like receptor B^{125,126}. Preclinical studies have emphasized a key role for inhibitory receptors in the regulation of eosinophil survival and activation. The inhibitory receptor CD300A has been shown to override eosinophil survival signals mediated by IL-5, IL-3 and GM-CSF¹²⁷. In addition, a recent study provides evidence that the inhibitory receptor CD172A is a negative regulator of eosinophil survival, as well as eosinophil degranulation, in the intestine¹²⁸. Eosinophil chemotaxis is also counter-regulated by inhibitory receptors¹²⁹, emphasizing that inhibitory receptors are key regulators not only of cell survival but also of effector functions. The potential for targeting these inhibitory receptors in eosinophil-rich inflammatory states was shown in a study in which treatment with a bispecific antibody fragment that recognized both CD300A and CCR3 reversed airway eosinophilia in an experimental model of chronic asthma¹³⁰.

Thymic stromal lymphopoietin. Thymic stromal lymphopoietin (TSLP) is an IL-7-like cytokine expressed primarily by activated epithelial cells that signals through a heterodimeric receptor (composed of a TSLP-specific binding chain and the IL-7Rα chain)¹³¹. TSLP has diverse effects but its primary target appears to be dendritic cells, through which it promotes the differentiation of T_H2 cells that produce IL-4, IL-13 and IL-5 (REFS 132–134). Transgenic mice that overexpress TSLP have elevated serum levels of IL-5, suggesting that TSLP has a role in the promotion of eosinophilia via the induction of T_H2 cytokine expression¹³⁵.

TSLP has also been shown to have direct effects on human eosinophils — providing pro-survival signals, modulating the expression of cell surface adhesion molecules and stimulating eosinophil-mediated bacterial killing^{136,137}. A human gene sequence variant associated with elevated blood eosinophil counts was identified in the 5q22 chromosomal region, which contains *TSLP*, supporting an association between eosinophilia and *TSLP*¹³⁸. In a genome-wide association study that aimed to identify susceptibility loci for eosinophilic oesophagitis, *TSLP* at chromosome 5q22 was identified as an important candidate gene in disease pathogenesis; *TSLP* was overexpressed in oesophageal biopsy samples from patients with eosinophilic oesophagitis compared to controls¹³⁹. These and other studies have prompted the development of therapeutic agents targeting TSLP, such as a fully monoclonal antibody (AMG 157) that blocks the interaction of TSLP with its receptor. A Phase I trial (ClinicalTrials.gov identifier: NCT01405963) is underway to evaluate the efficacy, safety and pharmacokinetics of AMG 157 in patients with mild atopic asthma.

Strategies for the future

Eosinophil activation. The clinical severity of many eosinophil-associated disorders is believed to be correlated with the extent of eosinophil activation in the tissues. Indeed, there is a subgroup of patients with marked blood eosinophilia but no evidence of clinical pathology

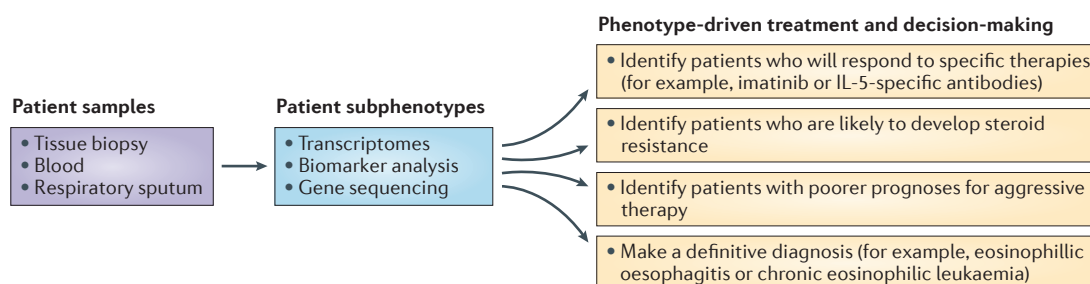


Figure 5 | Subphenotyping patients with eosinophilia for personalized treatment plans. In the future, samples from patients (for example, tissue biopsy samples from the oesophagus, blood cells or respiratory sputum) will be analysed for the expression of a subset of genes, proteins and/or biomarkers for the purpose of phenotyping patients to tailor their treatment plans. For example, such analyses will identify those patients who have a specific disease (such as eosinophilic oesophagitis), those who are more likely to respond to specific treatment plans (such as imatinib or interleukin-5 (IL-5)-targeted antibodies), those who are likely to develop steroid resistance and those with poorer prognoses who can be targeted for more aggressive treatment plans.

or organ dysfunction¹⁴⁰. The eosinophils from these patients have reduced markers of activation compared to those from patients with eosinophil-mediated organ damage, and their eosinophils show little to no evidence of degranulation when examined by electron microscopy. By contrast, eosinophils isolated from the airways of patients with asthma after allergen challenge have enhanced responsiveness to numerous mediators and greater superoxide generation and degranulation compared to blood eosinophils^{141,142}. The increased capacity of tissue-derived eosinophils to respond to mediators results from a process referred to as priming (FIG. 4), and this has been recapitulated *in vitro* via exposure to IL-5, GM-CSF and other cytokines^{143,144}.

Collectively, these studies suggest that inhibiting eosinophil priming and activation in patients may result in decreased tissue eosinophilia and organ damage. It is notable that IL-5-specific antibody therapy reduces the levels of circulating eosinophil-derived neurotoxin³⁶ and the responsiveness of eosinophils to CCL11, providing evidence that an IL-5-targeted antibody may be the first agent to selectively inhibit the effector function of eosinophils¹⁴⁵.

In addition to mediators such as PGD₂ and IL-5 (discussed above), several other mediators can induce eosinophil activation and thus might be beneficial therapeutic targets. The cytokine IL-33, which is a member of the IL-1 family of cytokines and a ligand for IL-1RL1 (also known as ST2), is a potent eosinophil activator^{146–148}. In preclinical studies, treatment with an IL-33-blocking antibody and soluble ST2 markedly decreased tissue eosinophilia in experimental models of asthma and allergic rhinitis^{149–151}, thus supporting further investigations into the potential therapeutic effects of blocking this pathway.

Similarly to the priming of eosinophils by cytokines, Notch signalling has a role in regulating the activation state and effector functions of eosinophils^{152,153} (FIG. 4). The Notch pathway is an evolutionarily conserved signalling pathway that has a key role in determining the fate of progenitor cells¹⁵⁴ as well as regulating immune

cell activation¹⁵⁵. In addition to eosinophil priming, Notch signalling regulates eosinophil maturation^{152,156}. These findings are relevant, as treatment with an inhibitor of Notch signalling (semagacestat) in a clinical trial for Alzheimer's disease unexpectedly resulted in a statistically significant mean increase (of 47%) in the number of blood eosinophils in patients¹⁵⁷.

Further studies are needed to identify the key regulators and potential checkpoints that result in eosinophil activation and priming and thereby lead to enhanced tissue infiltration, degranulation and synthesis of mediators. Knowledge of these regulators and checkpoints may lead to the design of novel therapeutics.

Eosinophil production. Atopy, helminth infections and allergen challenge have all been shown to be associated with increased numbers of eosinophil progenitor cells (that is, CD34⁺IL-5Rα⁺ cells) in the bone marrow, highlighting the importance of the bone marrow in contributing to eosinophil-associated disorders^{14–16}. Furthermore, increased numbers of eosinophil progenitors have been identified in allergic nasal mucosa, airway mucosa and sputum from patients with asthma, suggesting that eosinophil differentiation *in situ* may contribute to the accumulation of effector eosinophils in tissues^{158–160}.

Although IL-5-targeted therapy is very effective at reducing mature eosinophil counts in the blood and bone marrow, the number of eosinophil progenitors and the capacity to produce eosinophils from the bone marrow is unchanged¹⁶¹, which has important implications for patients when therapy is discontinued. An agent that is directed at specifically blocking the production or survival of eosinophil progenitors has the potential to be a long-lasting and specific therapy. As mentioned above, the number of eosinophil precursors in the bone marrow of non-human primates was reduced following treatment with benralizumab¹⁰³, indicating that targeting progenitors via cell surface markers is a viable approach for inhibiting eosinophilia. Research that is focused on further characterizing the biology of eosinophil

IL-1RL1

Interleukin-1 receptor-like 1; also known as ST2 protein. A component of the receptor for IL-33 that is widely expressed by innate immune cells and a subset of T cell lymphocytes.

Atopy

The predisposition to develop allergic hypersensitivity (that is, immunoglobulin E-mediated) reactions that can result from both hereditary and environmental components.

Table 2 | New therapeutic strategies for treating eosinophilia

Mechanism	Examples	Phase	Refs
Blockade of eosinophil recruitment			
CCR3 and CCL11 inhibition	Small-molecule CCR3 antagonists (for example, piperidine derivative LH31407)	II	48
	Eotaxin 1-specific antibodies (for example, bertilimumab)	II	50
Adhesion molecule inhibition	CD49D-specific antibodies (for example, natalizumab)	NE*	57
	Small-molecule VLA4 antagonists (for example, compound 1)	Preclinical	56
CRTH2 and PGD ₂ inhibition	CRTH2 antagonists (for example, OC000459)	II	66
Histamine H ₄ receptor inhibition	Small-molecule histamine H ₄ receptor antagonists (for example, INCB38579)	Preclinical	71
IL-13 and/or IL-4 blockade	IL-13-specific antibodies (for example, lebrikizumab)	II	83
	IL-4R α antagonists (for example, AMG 317)	II	87
	IL-4 variants (for example, pitrakinra)	II	88
Inhibition of eosinophil survival			
IL-5 and/or IL-5R α blockade	IL-5-specific antibodies (for example, mepolizumab and reslizumab)	III	89
	IL-5R α -specific antibodies (for example, benralizumab)	II	104
	Antisense oligonucleotides directed against the common β -chain (for example, TPI ASM8)	II	105
SIGLEC-8 agonism	SIGLEC-8-specific antibodies	Preclinical	113
IgE blockade	IgE-specific antibodies (for example, omalizumab)	IV; marketed	118
Activation of inhibitory receptors	CD300A-specific antibodies	Preclinical	127
TSLP inhibition	TSLP-specific antibodies (for example, AMG 157)	I	
Inhibition of eosinophil activation			
IL-33 blockade	IL-33-specific antibodies	Preclinical	149
Notch inhibition	Notch signalling inhibitors (for example, semagacestat)	Preclinical; NE*	157
Blockade of eosinophil production			
IL-5R blockade	IL-5R α -specific antibodies (for example, benralizumab)	II	104

CCL11, CC chemokine ligand 11; CCR3, CC chemokine receptor 3; CRTH2, chemoattractant receptor of T_H2 cells; IgE, immunoglobulin E; IL-4, interleukin-4; IL-4R α , IL-4 receptor subunit α ; PGD₂, prostaglandin D₂; SIGLEC-8, sialic acid-binding immunoglobulin-like lectin 8; TSLP, thymic stromal lymphopoietin; VLA4, very late antigen 4 (integrin α 4 β 1). *NE* means that the drug has not been tested for efficacy in an eosinophil-associated disorder, but has been found to have an effect on eosinophilia in clinical trials for other disorders.

progenitors, to identify specific surface markers as well as proliferative and survival signals, could result in new approaches for targeting eosinophils.

Subphenotyping eosinophilic disorders. Post-hoc analyses of data from clinical trials have emphasized the importance of including patients who are likely to receive the most therapeutic benefit from a specific agent. For example, in a clinical study of individuals with severe persistent asthma, a subgroup of patients with baseline sputum eosinophil levels greater than 3% showed an increase in lung function (a mean increase in FEV₁ of 0.29 l) after receiving IL-5-specific antibody therapy¹⁰². In individuals with baseline sputum eosinophil levels that were lower than 3%, there was no difference in lung function between patients receiving IL-5-specific antibody therapy or those receiving placebo, emphasizing the importance of phenotyping patients for baseline eosinophilia to maximize therapeutic benefit.

It is interesting to note that patients with eosinophilic asthma are particularly sensitive to the effects of IL-5- and IL-13-specific antibodies^{38,83}. Elevated levels of

serum periostin correlate with elevated eosinophil levels and responsiveness to IL-13-specific antibodies, raising the possibility that this subgroup of patients should also be treated with anti-eosinophil therapy, either alone or in combination with IL-13-specific antibodies.

Blood and tissue eosinophilia can occur in many disease processes¹⁶², and hypereosinophilic syndrome is a heterogeneous disorder that often has no identifiable cause¹⁶³. Thus, it is necessary to identify subphenotypes of patients with eosinophilic disorders in order to predict treatment responses on the basis of a patient's genetics or the gene expression profiles of affected organs and to tailor therapy for their eosinophil-associated disease accordingly. This approach has proven to be very effective in patients with hypereosinophilic syndrome, with treatment plans being selected on the basis of the mechanism of eosinophilia (that is, fusion-gene expression). We believe this approach should now be implemented in other more prevalent eosinophilic disorders such as eosinophilic asthma and eosinophilic gastrointestinal disorders.

Indeed, whole-genome transcript expression profile analysis has identified a disease-specific tissue

FEV1

Forced expiratory volume in 1 second; the maximal amount of air that an individual can forcefully exhale in 1 second as calculated during pulmonary function testing. A normal FEV₁ is predicted based on height, weight and race. A lower than normal FEV₁ is a marker of an obstructive process such as asthma.

transcriptome in the oesophagus of patients with eosinophilic oesophagitis^{51,79}. The transcriptome analysis has been limited to oesophageal biopsy samples, which typically represent mucosa (epithelium and lamina propria) rather than deeper layers of the oesophagus and so may not fully reflect the inflammatory response in the oesophagus. Notably, the transcript is not enriched for eosinophil-specific genes because eosinophils contain relatively low amounts of mRNA compared with other inflammatory and resident cells. Rather, the eosinophilic oesophagitis transcriptome provides insight into the effector pathways that are involved in eosinophilic inflammation and can distinguish between patients with differing levels of mucosal inflammation as well patients who have been exposed to specific drugs such as topical glucocorticoids¹⁶⁴. Thus, the use of tissue transcript profiling enables an eosinophilic oesophagitis diagnostic panel to be derived to monitor disease diagnosis, patient prognosis and responsiveness to therapy^{51,164–166}. It is anticipated that patient-specific information, derived from tissue transcriptome or cytokine expression analysis, would enable and customize key decisions about the degree and mechanism of tissue inflammation, compliance with medications and responsiveness to therapeutic intervention^{51,165,167} (FIG. 5).

Conclusions

Eosinophil accumulation in blood and tissue is associated with several prevalent infectious and inflammatory disorders. Traditionally, therapy for eosinophilic disorders has primarily focused on glucocorticoids, but these agents are often toxic and variable degrees of drug resistance are common. More recently, drug development has

focused on blocking eosinophil recruitment into organs as well as impairing eosinophil survival and activation (TABLE 2). Clinical trials with first-generation eosinophil-targeted therapeutic agents are now underway and appear to be promising, especially those that target IL-5 and its receptor.

Challenges that need to be addressed as new agents are evaluated include the occurrence of residual tissue eosinophilia (as reported in clinical trials) in order to evaluate the efficacy of IL-5-specific antibodies as well as the refractory response that was seen in some patients who were treated with glucocorticoids. In addition, as therapeutic agents that are more effective at blocking eosinophilia are identified, the potential beneficial role for eosinophils in maintaining good health may come to the forefront and need to be addressed.

Another substantial challenge is identifying the subphenotypes of patients with other complex eosinophilic diseases, such as allergic asthma and eosinophilic gastrointestinal disorders, and determining how particular subphenotypes of patients respond to specific agents on the basis of their disease characteristics and pathophysiology. Indeed, personalized medicine has already proven to be crucial and effective, as demonstrated by the observation that patients with the *PDGFRA*-associated myeloproliferative form of hypereosinophilic syndrome are generally very responsive to the tyrosine kinase inhibitor imatinib. The application of tissue transcriptome analysis offers the opportunity to further advance personalized and predictive medicine for eosinophilic disorders, particularly for eosinophilic oesophagitis, as tissue biopsy samples are the standard of care and such analyses have already proven to be feasible and useful.

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Competing interests statement

The authors declare [competing financial interests](#): see Web version for details.

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