

REVIEW

Molecular and clinical rationale for therapeutic targeting of interleukin-5 and its receptor

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Summary

Interleukin-5 is a Th2 homodimeric cytokine involved in the differentiation, maturation, migration, development, survival, trafficking and effector function of blood and local tissue eosinophils, in addition to basophils and mast cells.

The IL-5 receptor (IL-5R) consists of an IL-5-specific α subunit that interacts in conformationally dynamic ways with the receptor's β c subunit, an aggregate of domains it shares with binding sites of IL-3 and granulocyte-macrophage colony-stimulating factor. IL-5 and IL-5R drive allergic and inflammatory immune responses characterizing numerous diseases, such as asthma, atopic dermatitis, chronic obstructive pulmonary disease, eosinophilic gastrointestinal diseases, hyper-eosinophilic syndrome, Churg-Strauss syndrome and eosinophilic nasal polyposis. Although corticosteroid therapy is the primary treatment for these diseases, a substantial number of patients exhibit incomplete responses and suffer side-effects.

Two monoclonal antibodies have been designed to neutralize IL-5 (mepolizumab and reslizumab). Both antibodies have demonstrated the ability to reduce blood and tissue eosinophil counts. One additional monoclonal antibody, benralizumab (MEDI-563), has been developed to target IL-5R and attenuate eosinophilia through antibody-dependent cellular cytotoxicity. All three monoclonal antibodies are being clinically evaluated. Anti-sense oligonucleotide technology targeting the common β c IL-5R subunit is also being used therapeutically to inhibit IL-5-mediated effects (TPI ASM8). Small interfering RNA technology has also been used therapeutically to inhibit the expression of IL-5 in animal models.

This review summarizes the structural interactions between IL-5 and IL-5R and the functional consequences of such interactions, and describes the pre-clinical and clinical evidence supporting IL-5R as a therapeutic target.

Keywords asthma, benralizumab, eosinophils, interleukin-5, interleukin-5 receptor, mepolizumab, reslizumab

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Introduction

The management of human allergic disease has focused on the study of specific cytokines, chemokines and their receptors in animal models and clinical trials for more than a decade [1]. Commonality of receptor-ligand involvement across a variety of allergic disease states acquires clinical relevance as treatments become more immunomodulatory in nature, most commonly towards the use of human or humanized monoclonal antibodies (mAbs), fusion proteins and cytokines [2]. As the eosinophil has been clinically identified as hav-

ing key roles in the pathology of allergic disease [3–5], interfering with eosinophil function or reducing its numbers has been the most common goal of mAb therapeutic targeting of cytokine-receptor interaction in allergic processes. Such targeting has focused most commonly on asthma [3, 4] as well as on Churg-Strauss syndrome [6] due to strong eosinophilic inflammation in affected tissues, and has also been used to treat eosinophil-associated gastrointestinal disorders [7], hyper-eosinophilic syndrome (HES) [8], atopic dermatitis (AD) [9] and idiopathic pulmonary fibrosis [10].

An extensive PubMed/MEDLINE literature search has identified IL-4, IL-5, IL-9 and IL-13 as the most studied cytokines in the management of allergic diseases. Targeting of the receptor rather than the ligand has now focused emerging interest on the eotaxin chemokine receptor CCR3, and on the multi-domain receptors of the aforementioned ILs, with particular attention to the IL-5 receptor (IL-5R) [11, 12]. Although mAb-targeted inhibition of IL-5 itself (e.g. with mepolizumab and reslizumab) has been effective in reducing eosinophil counts in several disease states, the clinical responses have been consistently suboptimal and are not entirely understood. This article reviews the landscape of eosinophil-related diseases and the anti-IL-5 therapies being used to treat them. It also comparatively summarizes data for emerging trends in anti-IL-5R treatments for asthma.

Interleukin-5 physiology

Interleukin-5 is a Th2 cell-derived hematopoietic cytokine that plays key roles in the proliferation, maturation, migration and survival of eosinophils as well as some modulatory role in the development and function of basophils and mast cells. It is a homodimeric glycoprotein with a molecular weight of 40–50 kDa [13–16].

Interleukin-5 has commonality of protein structure with other hematopoietic cytokines such as IL-3 and granulocyte-macrophage colony-stimulating factor (GM-CSF) in possessing four α -helices. IL-5 possesses two identical polypeptide chains arranged in four α -helix bundles; three helices are comprised from one chain of the homodimer with a fourth helix from the other chain. Its structure is thought to confer a unique

functional motif for binding to its specific receptor subunit, IL-5R α , and a separate motif for binding to the signalling subunit, β c, of the receptor [17–20].

The IL-5R α subunit polypeptide chain is specific only to IL-5 binding; the β c chain also binds the cytokines IL-3 and GM-CSF via a shared extracellular domain [21–23].

Results of a recent study that used fluorescence resonance energy transfer to evaluate changes in the IL-5R's β c oligomers suggests a sequential ligand binding process beginning with the IL-5-specific α subunit, followed by an IL-5-directed rearrangement of β c subunits as they bind the ligand's α -subunit. The authors hypothesize that IL-5 (and perhaps IL-3 and GM-CSF) is able to direct cellular responses through such a sequential binding process [24].

Cellular sources of interleukin-5

Although eosinophils and basophils express the IL-5R on their surface, they also function as cellular sources of IL-5. CD34⁺ progenitor cells may also produce IL-5, and if these progenitors become an eosinophil or basophil, they may also express the IL-5R. Other cellular sources of IL-5 that do not express the IL-5R include Th2 cells, mast cells, invariant natural killer (NK) T cells and non-B/non-T cells [25–31] (Fig. 1).

Interleukin-5 and eosinophils

Eosinophils are primarily associated with the allergic response, releasing leukotrienes such as C4, D4 and E4, as well as proteins such as eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EDN), eosinophil

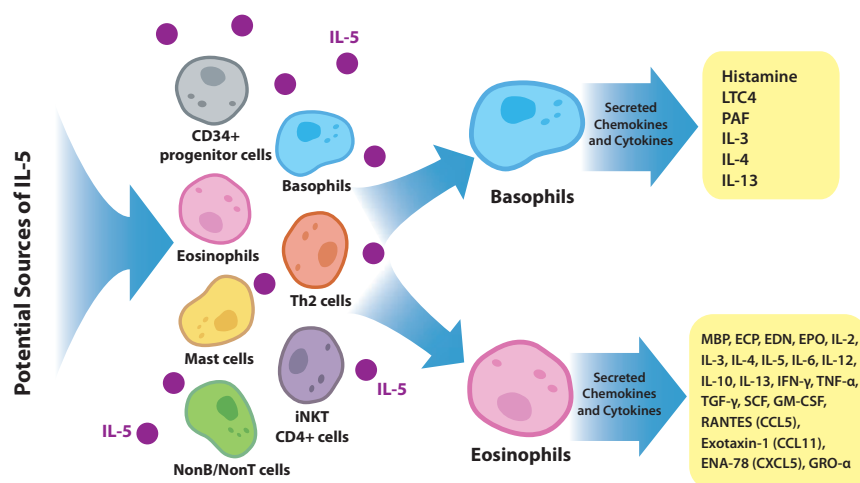


Fig. 1. Sources of IL-5 and its effects on eosinophils and basophils. CCL5, chemokine C–C motif ligand 5; CXCL5, chemokine C–X–C motif ligand 5; ECP, eosinophil cationic protein; EDN, eosinophil-derived neurotoxin; ENA-78, epithelial neutrophil activating peptide-78; EPO, eosinophil peroxidase; GM-CSF, granulocyte-macrophage colony-stimulating factor; GRO, growth-related oncogene; iNKT, invariant natural killer T lymphocyte; LTC4, leukotriene C4; PAF, platelet activating factor; RANTES, regulated upon activation normal T-expressed and secreted; SCF, stem-cell factor.

peroxidase and major basic protein (MBP) and numerous cytokines and chemokines such as IL-1-IL-6, IL-8, IL-10, IL-12, IL-16, GM-CSF, regulated upon activation normal T-expressed and secreted (RANTES), TGF- α , TGF- β , monocyte chemotactic protein 1 and macrophage-inflammatory protein 1 α .

Interleukin-5 is involved in the differentiation and maturation of eosinophils in the bone marrow (probably comprising a pool of readily mobilizable cells in response to allergens) [32–36], in their migration to tissue sites (usually in the presence of an allergic response) [37–39], in the prevention of eosinophil apoptosis [40–43] and, probably, in the ability of eosinophilic precursors to proliferate, differentiate and function independently *in situ* in various tissues, including the lung [5, 44–47].

Studies demonstrating the capacity of IL-5 priming to induce locomotor activity in eosinophils have suggested a significant role for IL-5 in eosinophil localization at inflammation sites [48]. Progressive expression of the IL-5R α subunit on CD34+ bone marrow progenitor cells from mild asthmatics has been shown to occur in response to allergen challenge, suggesting that IL-5R expression by progenitor cells favours eosinophilopoiesis in blood or tissue [28].

Interleukin-5 has been recognized as the cytokine most specific to the eosinophil lineage [46, 49–51] and has been identified across animal models as being a key common denominator in allergic inflammatory pathways [52].

Further support for the concept of a multi-functional IL-5 specificity within eosinophil-mediated allergic diseases comes from studies that identified an autocrine release of IL-5 from eosinophils in asthmatic subjects; the released IL-5 acted on the eosinophils' IL-5Rs to prevent their own apoptosis [40].

Evidence is accumulating to suggest that allergen-driven *in situ* airway hematopoiesis in the direction of CD34+ cells committed to the eosinophil lineage may involve the cysteine–cysteine chemokine receptor-3 (CCR3)/eotaxin pathway as well as IL-5 [53]. Hence, an evolving understanding of this cytokine/chemokine/receptor interaction will be essential in the development of therapeutics to address allergic disease at the tissue level.

Interleukin-5 and basophils

Interleukin-5 also appears to play an interactive role in the development, metabolism and function of basophils and mast cells [15, 54, 55]. Basophils express a number of cytokine receptors such as IL-2R α , GM-CSFR α , IL-3R, IL-4R and IL-5R [56, 57], and produce IL-4 and IL-13 [58]. Augmented production of mediators (such as leukotrienes) in basophils has been observed with IL-5 and GM-CSF priming [59, 60].

Interleukin-5 has been identified as a basophilopoietin; the cytokine stimulated dose-dependent increases in histamine content of HL-60 cells and generated a greater proportion of basophil-containing, histamine-positive, eosinophil-type colonies compared with GM-CSF, IL-3 or G-CSF [61]. In contrast to its direct effect on histamine content in basophils, IL-5 does not affect histamine release or degranulation *per se*, but rather serves as a primer for these basophil-characteristic events, especially when combined with ATPase inhibitors such as thapsigargin [62, 63]. IL-5 has been shown to amplify allergen-induced histamine release from basophils from patients with allergic rhinitis; this effect was comparatively greater with IL-3 than with IL-5 [64]. Similarly, a greater expression of IL-3R than either IL-5R or GM-CSFR has been found on basophils, compared with eosinophils, which expressed the highest level of IL-5R [65].

Mast cells and interleukin-5

Interleukin-5 production by mast cells has been demonstrated in murine allergic models [54] and mast cells are thought to play key roles in the initiation of an inflammatory response characteristic of acute allergic type 1 reactions in humans.

Although IL-5R has been detected in cultured human mast cells [66], more recent studies have failed to confirm expression of IL-5R on resting and activated mast cells [67]. Additional investigations are needed to resolve this question.

Interleukin-5/interleukin-5R interactions

Interleukin-5 conformation and receptor binding

Binding of IL-5 to its receptor subunits involves dynamic conformational changes in binding sites that are specific to the IL-5 cytokine itself and to IL-5R α and β c subunits in terms of amino acid residues, their locations within epitopes and charges. These changes take place during what is essentially a two-phase process beginning with a high-affinity binding of IL-5 to IL-5R α , followed by binding of this activated complex to β c to initiate signal transduction [20, 68–71]. This receptor activation probably involves dimerization of a critical cytoplasmic domain of β c [72].

Both IL-5R α and β c subunits contain extracellular fibronectin-III domains, which are characteristically conserved within the class I receptor superfamily (hemopoietin receptor family) [73, 74] in what is sometimes termed a cytokine recognition motif, in terms of IL-5R α [68, 69, 75] as well as β c [20, 75]. These motifs are thought to be specific to certain groups of amino acid residues in IL-5/IL-5R α , which may bind either IL-5 or an antagonist [69, 76, 77], suggesting the

potential to tailor specific molecules for therapeutic intervention.

Signalling pathways

Receptor-specific signalling involving IL-5 and IL-5R is diverse and complex, and it takes place primarily via phosphorylated activation of a variety of intracellular signal transduction pathways that regulate eosinophil differentiation, proliferation and survival. [78]. These signal transduction pathways are catalysed by Janus kinases (JAK) [78], JAK/signal transducer and activator of transcription (STAT) [79, 80], mitogen-activated protein kinase (MAPK) [81–83], the extracellular signal-regulated kinases (ERK1 and ERK2) [38], Lyn tyrosine

kinase [43, 84, 85], phosphoinositide 3-kinase [86] and nuclear factor κ B (NF- κ B) [81–83, 87–89]. IL-5 can also induce JAK-binding protein expression in eosinophils, which constitutes a negative feedback regulatory mechanism of IL-5 signalling [90]. As IL-3, GM-CSF and IL-5 share a common β chain receptor, some of these signalling pathways are also shared among these cytokines. The IL-5 signalling pathways, receptor interactions and effects on biological processes are shown in Fig. 2.

Janus kinases/signal transducer and activator of transcription pathways. The activation of the JAK/STAT pathways is essential for IL-5-dependent signal transduction [91]. IL-5 specifically activates a variety of

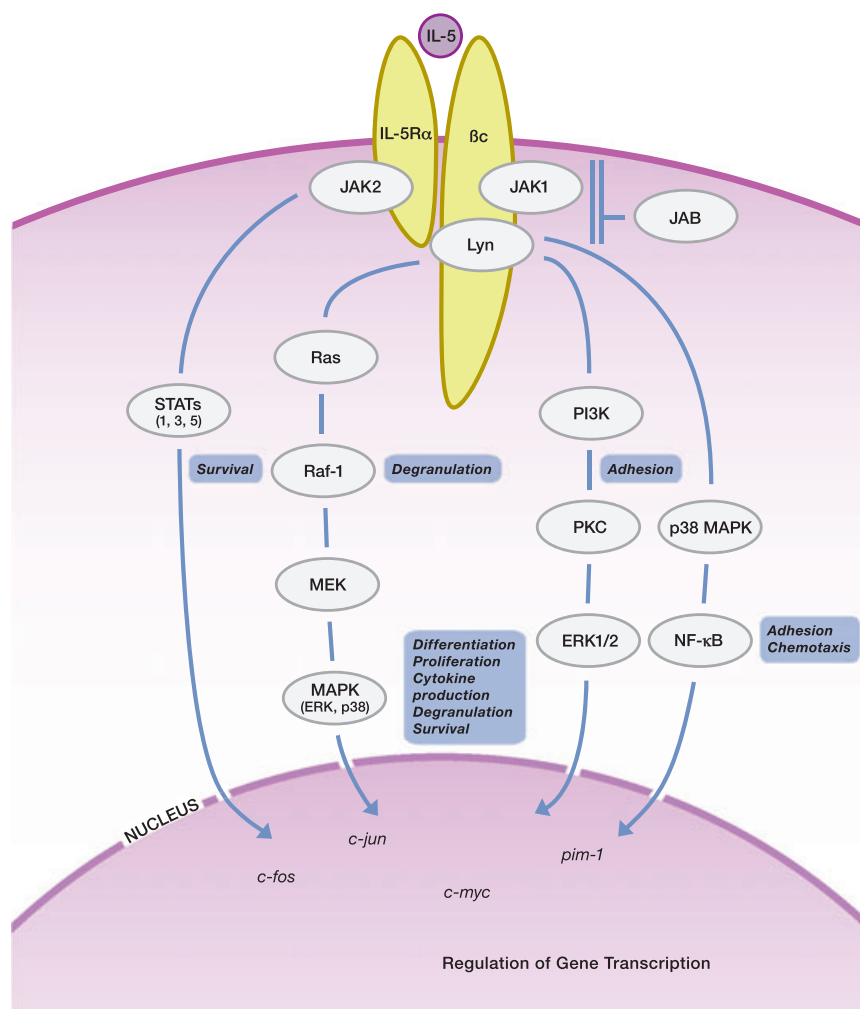


Fig. 2. IL-5 signalling pathways, receptor interactions and effects on biological processes. IL-5 stimulation of eosinophils activates a number of signalling pathways, including JAK/STAT, Ras/MAPK, PI3K/ERK and p38/NF- κ B, ultimately leading to transcription of genes involved in eosinophil differentiation, proliferation and survival. ERK, extracellular signal-regulated kinases; JAB, JAK-binding protein; JAK, Janus kinases; MAPK, mitogen-activated protein kinase; MEK, MAP or ERK kinase; NF- κ B, nuclear factor kappa B; PI3K, phosphoinositide 3-kinase; PKC, protein kinase C; Raf-1, v-raf-1 murine leukaemia viral oncogene homologue 1; Ras, Ras GTPase; STAT, signal transducer and activator of transcription. Adapted from IL-5- and eosinophil-mediated inflammation: from discovery to therapy by T. Kouro and K. Takatsu, 2009, *International Immunology* 21:1303–1309. Copyright 2009 by The Japanese Society for Immunology. Adapted with permission.

pathways that include JAK1 and JAK2 [78, 79]. JAK2 and JAK1 are constitutively associated with IL-5R α and the β c chain, respectively; these associations are independent of IL-5 stimulation. Upon IL-5 stimulation, JAK1 associates with IL-5R α , forming an IL-5R α - β c complex [78, 79]. IL-5 stimulates JAK2/STAT1, JAK2/STAT3 and JAK2/STAT5 pathways in eosinophils [91–93], enhancing gene expression of pim-1 and cyclin D3 [93] (STAT3 and STAT5) or other IL-5-inducible genes (STAT1) [92]. JAK2, along with Lyn and Raf-1 kinases, appear to be important molecules in the suppression of eosinophil apoptosis by IL-5, thus playing a key role in cell survival [43]. Raf-1 also appears to play a central role in β_2 -integrin expression and degranulation of eosinophils [43, 78].

Mitogen-activated protein kinase and related pathways.

The MAPK family includes ERK1, ERK2 and p38, among other protein kinases. These proteins are involved in β c receptor signalling. ERK, which induces *c-fos*, is regulated by Raf-1 through activation of MAP or ERK kinase (MEK) [94]. Likewise, Spred-1 is a negative regulator of ERK activation, and is involved in mediating IL-5-induced eosinophil proliferation. Although both p38 and ERK1/2 MAPK play a key role in eosinophil differentiation, cytokine production (e.g. macrophage-inflammatory protein) and degranulation (e.g. eosinophil-derived neurotoxin release), p38 seems to play a greater role in eosinophil differentiation and cytokine production [95]. p38 MAPK, together with NF- κ B, has also been shown to induce adhesion and chemotaxis of human eosinophils during allergic inflammation [81, 83].

Ras/ERK pathways are members of the MAPK family [96] that are activated by IL-5 and are pivotally involved in eosinophil differentiation, proliferation and survival [97, 98]. IL-5 stimulation appears to alter signal transduction in extracellular chemotactic receptors on eosinophils, enabling interaction with ERK pathways and thus secretion of cytotoxic substances such as leukotriene C4 [99], which are important in asthma pathology.

GTPase dynamin-2 also appears to play a critical role in IL-5-stimulated cell function, specifically by down-regulating IL-5-induced cell proliferation. IL-5 enhances tyrosine phosphorylation of dynamin within the ERK pathways, which in turn induces intracellular disposition of IL-5R via endocytosis, shutting down the ERK signalling pathways [100].

Gene expression of c-myc, c-fos, c-jun and pim-1.

Interleukin-5 enhances the expression of several transcription factors including c-myc, c-fos, c-jun and pim-1. Transcription of these genes together with activation

of the Ras-Raf-1-MEK-MAPK pathway is required for IL-5-induced eosinophil proliferation [101, 102].

Phosphoinositide 3-kinase pathway. Phosphoinositide 3-kinase has been implicated in cell adhesion, including IL-5-induced β_2 -integrin-mediated adhesion of eosinophils to the intercellular adhesion molecule-1 through activation of protein kinase C and downstream phosphorylation of ERK [103].

GATA-1. GATA-1 may be involved in early differentiation and eosinophil commitment from progenitors [104]. An apparent synergy between the corticosteroid budesonide and IL-5 has been reported, whereby progenitor differentiation was enhanced in the presence of both. A previously unidentified GATA-1 pathway is thought to be involved in this enhancement of differentiation [105].

Possible Th1/Th2 cytokine synergy. Th1 cytokines such as IFN- γ might act synergistically with IL-5 or GM-CSF via the p38 MAPK, ERKs and other pathways to induce eosinophil functions associated with tissue injury, such as degranulation and superoxide generation [41, 106, 107]. Furthermore, expression of the CD69 antigen on cord-blood eosinophils is not only induced by IL-5 and IL-3 but also by IFN- γ via activation of the JAK2 pathway [108] or through the STAT1 pathway [109]. Although IFN- γ and IL-5 both inhibit eosinophil apoptosis, IL-5 does so via up-regulation of bcl-2 expression, whereas IFN- γ does not function via this mechanism [41].

These results suggest that the Th1 cytokine IFN- γ may produce some of the same effects as IL-5 on eosinophils via some of the same signalling pathways, but that IL-5 and other Th2 cytokines probably have more of a direct bearing on eosinophil production and anti-apoptotic effects than do Th1 cytokines.

Synergism of eotaxins/cysteine–cysteine chemokine receptor-3 with interleukin-5

Eotaxins are a largely eosinophil-specific group of chemokines that signal exclusively through CCR3, a high-affinity receptor that promotes transit of eosinophils and basophils into sites of tissue inflammation [110, 111]. CCR3 is expressed on eosinophils, basophils, mast cells, Th2 cells, macrophages and airway epithelial cells. Eotaxins are produced at sites of allergic inflammation [112–115] and are actively involved in the mobilization and trafficking of eosinophils from the vasculature to the tissues [116].

Eotaxins are important in regulating eosinophilia in asthma [117] and in eosinophilic esophagitis (EoE)

[118], playing a cooperative role with IL-5 and CCR3 in maturation and mobilization of eosinophils.

To date, three main eosinophil-selective eotaxins (1, 2 and 3) have been identified [119–121], and they all act through CCR3 receptors. Although these chemokines have similar eosinophil functional properties, their sequence alignment is only in the 30% range. The differential roles of these eotaxins are not yet clearly understood.

Eotaxin-2 appears to work with IL-5 in the lung to regulate IL-13 production and airway hyperresponsiveness (AHR) [122]. Eotaxins-1, -2 and -3 have been identified as important in the pathogenesis of EoE. Importantly, increased eotaxin-3 levels appeared to be critical in the differentiation of EoE from gastro-oesophageal reflux disease (GERD). This study also observed significantly increased expression levels of eotaxins-1 and -2, in addition to IL-5 and CCR3 [123]. An up-regulated CCR3/eotaxin-3 pathway has also been reported in paediatric EoE, along with increases in intracellular lymphocyte IL-5 expression and higher percentages of CD4⁺ T cells expressing IL-5, which correlated with numbers of eosinophils in oesophageal biopsies [124].

Interleukin-5 as mediator of homeostasis and disease

Animal models

The vast majority of data describing the function of IL-5 *in vivo* are derived from studies using function-blocking antibodies, mice deficient in IL-5 or the IL-5R α chain and mice that express elevated levels of IL-5. Although IL-5 has been identified in the 1980s as a growth and differentiation factor of murine B cells, human B cells do not express IL-5R α and do not respond to IL-5. However, eosinophils are considered the dominant cell type in both species that are at least partially dependent on IL-5 and respond to IL-5 with increased expansion, differentiation, survival and activation. Tissue eosinophilia is a hallmark of a number of human pathologies, such as asthma, AD, helminth infections and rhinitis (reviewed in detail below) that are associated with elevated IL-5 levels as a consequence of enhanced Th2 inflammation. Therefore, and for the purpose of this review, we mainly focus on *in vivo* data that describe the consequences of IL-5/IL-5R modulation on eosinophil biology during homeostasis and in animal models of disease.

Homeostasis. During homeostasis, IL-5 contributes to, but is not required for, eosinophil growth and differentiation. IL-5-deficient mice have mature eosinophils in the bone marrow and peripheral blood, although in reduced numbers. Likewise, systemic overexpression of

IL-5 results in pronounced eosinophilia in blood and tissues [50]. In addition to IL-5, IL-3 and GM-CSF are two particularly important cytokines that regulate eosinophil development, providing an explanation for the presence of mature eosinophils in IL-5 gene-deficient mice. Other than regulating mature eosinophil numbers in mice, IL-5- or IL-5R α -deficient mice show reduced numbers of B-1 cells in peritoneal washouts and B-1 cell-derived IgA⁺ cells in the lamina propria [125, 126]. The administration of a neutralizing anti-IL-5 antibody has a similar effect on B-1 cell numbers in adult mice [127]. In IL-5R α -deficient mice, these cells also express lower levels of the co-stimulatory molecule CD40, a cell surface receptor that is required for efficient T cell-dependent responses.

Taken together, these data demonstrate an important, although somewhat redundant role of IL-5 in the homeostatic regulation of survival, differentiation and activation of B-1 and T cells, the two main cell types that express IL-5R α in the mouse. Although human B cells do not express IL-5R α and do not respond to IL-5, data derived from mouse models describing the consequences of IL-5 inhibition on regulating eosinophil biology are most useful to predict similar effects in human pathologies that are characterized by elevated IL-5 levels and eosinophilic inflammation.

Allergic airway inflammation. Aberrant mucosal eosinophilic inflammation of the lungs is considered a hallmark of asthma and has been found to be associated with elevated levels of IL-5 in bronchial biopsies from patients with asthma [128]. IL-5 overexpression in the lung epithelium of mice has been associated with increased eosinophilic inflammation, AHR and mucus hyper-secretion [46, 49–51]. IL-5 mRNA is up-regulated in the bronchial mucosa upon allergen challenge [129], and IL-5 concentrations correlate with clinical features of asthma [130]. Studies in mice have demonstrated a role for IL-5 in models of allergic airway inflammation. Sensitized IL-5- or IL-5R α -deficient mice are protected from mucosal eosinophilia, AHR and peribronchial fibrosis upon challenge with inhaled allergen [131–133]. Similar data were obtained in mice, guinea-pigs and non-human primates treated with a neutralizing anti-IL-5 mAb before allergen inhalation, whereas allergen inhalation in IL-5 transgenic mice resulted in marked accentuated eosinophilic inflammation and AHR [132, 134–136]. In addition, studies in mice deficient in eosinophils have identified a role for eosinophils in tissue remodelling and AHR [137, 138], further supporting a dominant role for IL-5 in orchestrating eosinophilia and associated consequences in models of allergic mucosal inflammation.

Infections of the airways with respiratory viruses of the *Paramyxoviridae* family, such as respiratory

syncytial virus (RSV), can result in exacerbations in asthmatics and have been considered a major cause for the development of asthma during early childhood [139]. Indeed, elevated levels of IL-5 and eosinophilia are often associated with primary RSV infections. In two recent experiments, Phipps et al. [140] demonstrated accelerated clearance of RSV in IL-5 transgenic hyper-eosinophilic mice, whereas higher viral titres were detected in mice deficient in eosinophils. These studies provide evidence for a role of IL-5 and eosinophils in promoting virus clearance.

Human diseases

Although use of murine animal models may be helpful in providing insights to human eosinophilic diseases, it is important to avoid the pitfalls of overinterpretations of cross-species findings [141]. Several differences between mouse and human eosinophil biology have been documented. First, eosinophil migration in response to select chemokines is divergent in human and mouse eosinophils [142]. Second, mouse eosinophils tend to degranulate to a lesser degree than human eosinophils [143]. Third, the murine orthologs of human ECP and EDN released during degranulation are >50% divergent compared with their human counterparts [144], which may result in altered biological activities. Finally, both murine IL-5 and IL-5R α have

only a 70% amino acid sequence homology with human IL-5 and IL-5R α , respectively [145, 146]. Using IL-5 mouse/human chimeric molecules *in vitro*, it has been found that murine IL-5 can bind human IL-5R, but not the converse [147]. The potential clinical implications of the above differences remain to be determined.

Role of eosinophils in health and disease

The role of eosinophils in health and disease is still evolving. Although much focus has been directed on the role of eosinophils in innate host defence and immune dysregulation, a recent hypothesis termed Local Immunity And/or Remodelling/Repair, the LIAR hypothesis, proposes that the accumulation of eosinophils in various tissues occurs as part of a strategy(ies) to maintain tissue homeostasis in health and disease [148]. Tissue and remodelling may occur in either an immune neutral, Th2-polarized or Th1/Th17-polarized microenvironment. Despite these uncertainties, what is known is that in health, eosinophils – following an eotaxin-1 gradient – migrate predominantly to the gastrointestinal tract and to lesser degrees to the thymus, spleen, lymph nodes and the uterus [149]. In disease states, eosinophils may migrate to a variety of organs such as the nose, lung, oesophagus, heart and skin among others (Fig. 3).

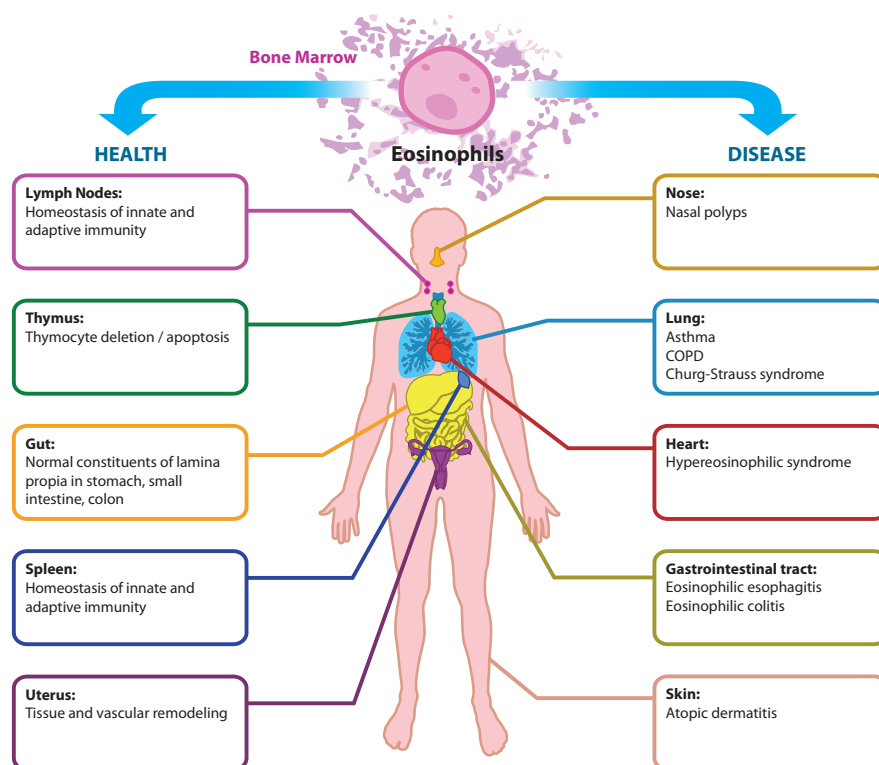


Fig. 3. Role of eosinophils in health and disease. COPD, chronic obstructive pulmonary disease [158].

Interleukin-5 and its receptor have been extensively studied in relation to their role mediating human eosinophil-associated diseases that involve the respiratory, gastrointestinal, circulatory and integumentary systems among others. These diseases with relevant studies referenced in this review are summarized in Table 1.

Asthma. Asthma is a chronic inflammatory disease of the airways. Eosinophils are thought to play a critical role in the pathogenesis and severity of asthma [150], with eosinophilic asthma representing approximately 40–60% of asthmatics [151]. Key roles of IL-5 in the development and trafficking of eosinophils in the pathogenesis of asthma have been extensively studied and reviewed [46, 152]. In the lung, T lymphocytes are the main source of IL-5, with mast cells and eosinophils contributing to the levels of this cytokine. Mast cells release pre-formed IL-5 from granules, whereas T cells secrete IL-5 that is rapidly synthesized [153, 154]. A proportion of CD4⁺ invariant NK T cells may produce large amounts of IL-5 after stimulation by dendritic cells in the presence of IL-2 [25]. Th2 cytokine production including IL-5 is amplified by IL-25 that stimulates Th2 effector memory cells and non-B/non-T cells to markedly increase production of IL-5 [26, 27].

Despite prevalent thinking that eosinophil progenitor maturation and differentiation occur primarily in the bone marrow, there is considerable emerging evidence that differentiation of eosinophils also occurs within tissues having an allergic response at the airway level, such as the airways in atopic asthma. The trafficking of eosinophils in asthma, in the context of bone marrow-blood-tissue transit, has been recently reviewed [152]. Evidence of increased capacity of the bone marrow to produce eosinophils in response to allergen challenge has been demonstrated in asthmatic subjects [35]. Furthermore, in this study, a greater increase in levels of IL-5 mRNA in subjects showing early and late asthmatic responses compared with subjects showing only early responses suggests that an increased capacity of T cells residing in or recruited to the bone marrow following an allergen challenge may be an important source of IL-5 for eosinophil production in dual responders. Thus, to achieve the most clinical response in eosinophilic asthma, it may be important to consider targeting IL-5 in all tissues and organs, where this cytokine exerts its pathogenic effects.

Interleukin-5 and eotaxins in asthma. Eotaxins 1, 2 and 3 are actively secreted by human airway epithelial cells [155]. An increase in the number of CCR3⁺ cells among primitive CD34⁺ and eosinophil lineage-committed CD34⁺, IL-5R α ⁺ progenitor cells has been identified in bone marrow aspirates from dual-responder asthmatics. An accompanying dose-dependent capacity of eotaxin

(but interestingly, not IL-5) to stimulate migration of CD34⁺ progenitor cells *in vitro* has also been observed [34]. This migrational response to eotaxin, however, was primed by pre-incubation of cells with IL-5. Increases in CD34⁺, IL-5R α ⁺ cells (as well as in eosinophils and IL-5) have also been found in sputum of dual-responder asthmatics, suggesting that eosinophil progenitors can migrate to the airway and differentiate *in situ* [45].

Although such a hypothesis argues that survival and function of eosinophils in the airway tissue may not depend on IL-5, at least not exclusively, it further suggests that independent interactions between eotaxin and its receptor, CCR3, may be at least as important as IL-5 in driving tissue histochemical events in asthma [46]. This may partially explain the apparent disconnection between eosinophil targeting and clinical response in asthma. More specifically, the role of airway tissue-derived eotaxins may be pivotal in the allergic response, at least in dual-responder asthmatics.

A synergistic or cooperative effect between IL-5 and eotaxin may in fact be necessary to produce responses such as pulmonary eosinophilia and AHR. Significantly higher levels of both eotaxin and IL-5 have been reported in subjects with acute exacerbations of asthma compared with normal controls or subjects with mild persistent asthma [156], providing evidence of such a cooperative effect between IL-5 and eotaxin in promoting airway eosinophil accumulation during acute asthma exacerbation.

A correlation between IL-5 and eotaxin levels and eosinophil numbers was also seen in the group with mild asthma. This suggests a cooperative effect on airway eosinophilia in less severely affected asthmatic subjects. Furthermore, a negative correlation between both sputum eotaxin and IL-5 and eosinophil apoptosis has been observed, suggesting that both cytokines are involved in the prevention of eosinophil apoptosis, leading to eosinophil accumulation in the asthmatic lung [157].

On the other hand, the role of eosinophils themselves – particularly in the capacity of affecting late asthmatic response and AHR – has undergone a re-evaluation following a study in which mepolizumab (SB-240563), an IgG-K mAb to IL-5, was shown to decrease blood and sputum eosinophil levels, but had no effect on late asthmatic response or AHR [158]. Analysis of the clinical trial milieu of anti-IL-5 mAb therapy again identified a potential disconnect between inhibition of eosinophils and amelioration of the clinical symptoms of asthma [159].

Pre-clinically, eotaxin and IL-5 seem to act synergistically in the differentiation of human CD34⁺ cord-blood progenitor cells into mature eosinophils; these progenitor cells constitutively express CCR3 mRNA.

Table 1. Clinical trials evaluating interleukin-5-mediated diseases

References	Indication/agent tested	Dose	No. of subjects		Primary end-point	Study design	Duration of Exposure	Outcome
			Drug	Placebo				
Straumann 2010 [7]	EoE/mepolizumab	750 mg IV, 2 doses 1 week apart	5	6	Remission (<5 peak eosinophil no./hpf)	Randomized, double-blind, placebo-controlled	8 weeks; non-responders two additional doses 4 weeks apart	Mepolizumab significantly reduced eosinophil numbers in oesophageal tissues; minimal clinical improvement was achieved in a subgroup
Nair 2009 [227]	Asthma/mepolizumab	750 mg IV monthly	9	11	Exacerbations and prednisone-sparing effect	Randomized, double-blind, placebo-controlled, parallel-group	5 months	Mepolizumab allowed a reduction in the dose of prednisone without the development of asthma exacerbations
Halldar 2009 [216]	Asthma/mepolizumab	750 mg IV monthly	29	32	Exacerbations	Randomized, double-blind, placebo-controlled parallel-group	12 months	Significantly fewer severe exacerbations with mepolizumab than placebo over 50 weeks and significant improvement in AQLQ score
Stein 2008 [221]	HES/mepolizumab	10 mg/kg, 3 IV infusions (max 750 mg) monthly	25	0	Peripheral eosinophil counts, plasma IL-5	Open-label	16 weeks	Decreased blood eosinophilia (including CCR3+ cells) and eosinophil activation, increased circulating levels of IL-5/IL-5R α /lymphocyte IL-5 production after mepolizumab; possible endogenous IL-5 autoregulatory pathway.

(continued)

Table 1 (continued)

References	Indication/agent tested	Dose	No. of subjects		Primary end-point	Study design	Duration of Exposure	Outcome
			Drug	Placebo				
Rothenberg 2008 [8]	HES/mepolizumab	750 mg IV every 4 weeks	43	42	Prednisone dose reduction to $\leq$ 10 mg/day for $\geq$ 8 consecutive weeks	Randomized, placebo-controlled, double-blind	36 weeks	Primary end-point reached in significantly higher percentage of mepolizumab subjects vs. placebo; no clinical worsening of HES; eosinophil count $<600/\mu\text{L}$ for $\geq$ 8 consecutive weeks in significantly higher percentage of mepolizumab patients vs. placebo
Busse 2010 [238]	Asthma/benralizumab (MED1-563)	0.0003–3.0 mg/kg IV	44	0	Safety, PK, pharmacologic activity	Open-label, escalating single dose	Single dose per cohort	No serious adverse events, dose-proportional PK, suppression of circulating eosinophils from 24 h post-dose to end of study
Flood-Page 2007 [226]	Asthma/mepolizumab	250 mg IV baseline, weeks 4, 8 750 mg IV baseline, weeks 4, 8	120 116	126	Morning peak expiratory flow, FEV ₁ , daily β_2 -agonist use, symptom scores, exacerbations, QOL, blood and sputum eosinophil counts	Randomized, double-blind, placebo-controlled parallel-group	12 weeks	No statistically significant changes in any clinical end-point; significant reduction in blood and sputum eosinophils in both treatment groups; non-significant trend for decreased exacerbation rates in the 750-mg group.
Oldhoff 2005 [9]	AD/mepolizumab	750 mg IV days 0, 7	18	22	PGA; SCORAD, pruritus scoring, blood eosinophil count, TARC values	Randomized, double-blind, placebo-controlled, parallel-group	Two single doses 1 week apart; 30-day follow-up	No clinical success (PGA, SCORAD, pruritus scoring, TARC values) despite significant decrease in PB eosinophils.

(continued)

Table 1 (continued)

References	Indication/agent tested	Dose	No. of subjects		Primary end-point	Study design	Duration of Exposure	Outcome
			Drug	Placebo				
Klion 2004 [233]	HES/reslizumab	1 mg/kg	4	0	CBC monitored until eosinophil count returned to baseline for 2 months	Open-label	Single dose	Rebound (above baseline) eosinophilia + symptoms in 2/4 patients; decreased eosinophils + no symptom improvement in 1 patient; no change in eosinophils or symptoms in 1 patient
Garrett 2004 [220]	HES/mepolizumab	10 mg/kg IV, 750 mg maximum	4	0	Safety, eosinophils (peripheral/diseased tissue), pulmonary function, QOL	Open-label	Three doses over 12 weeks	Well tolerated; decline in blood and tissue eosinophilia (10-fold in one EE patient), improved clinical and QOL measurements.
Menzies-Gow 2003 [230]	Asthma/mepolizumab	750 mg IV baseline, weeks 4, 8	11	13	Eosinophil counts in blood, BM, and bronchial mucosa biopsy specimens	Randomized, double-blind, placebo-controlled, parallel-group	8 weeks	Significantly reduced terminal differentiation of eosinophils (myelocytes, metamyelocytes, and mature eosinophils) in BM; no significant effect on blood or BM early eosinophil progenitors (CD34+/IL-5R α + cells, eosinophil/basophil CFUs); significantly decreased CD34+/IL-5R α mRNA+ cells in bronchial mucosa.
Kips 2003 [234]	Asthma/reslizumab	0.3 mg/kg IV 1 mg/kg IV	6 12	8	Blood/sputum eosinophil counts, symptoms, pulmonary function tests	Double-blind, randomized, escalating single dose	Single dose, 48-h observation	Baseline FEV ₁ improvement trend that reached significance 24 h after the 0.3-mg/kg dose. No significant changes in other clinical indices of disease activity.

(continued)

Table 1 (continued)

References	Indication/agent tested	Dose	No. of subjects		Primary end-point	Study design	Duration of Exposure	Outcome
			Drug	Placebo				
Flood-Page 2003 [229]	Asthma/mepolizumab	750 mg IV baseline, weeks 4, 8	11	13	Airway, BM, and PB eosinophils, clinical indices of disease activity	Randomized, double-blind, placebo-controlled, parallel-group	8 weeks	Significantly decreased airway, PB, and BM eosinophils; no significant changes in clinical measures (AHR, FEV ₁ , peak flow).
Buttner 2003 [225]	Asthma/mepolizumab	250 mg IV (every 4 weeks) 750 mg IV (every 4 weeks)	7	7	PB leukocytes, eosinophils, lymphocyte subpopulations, T cells, ECP levels	Randomized, double-blind, placebo-controlled, parallel-group	12 weeks	Decreased eosinophils and serum ECP; no change in T cell subsets and T cell cytokine production.
Leckie 2000 [158]	Asthma/mepolizumab	2.5 mg/kg 10.0 mg/kg	8	4	Inhaled allergen challenge, sputum/blood eosinophils, histamine AHR	Randomized, double-blind, placebo-controlled, single dose	Single dose	Decreased blood eosinophils up to 16 weeks and sputum eosinophils for 4 weeks; no significant effect on late asthmatic response or histamine AHR

AD, atopic dermatitis; AHR, airway hyperresponsiveness/hyper-reactivity; AQLQ, Asthma Quality of Life Questionnaire; BM, bone marrow; CBC, complete blood count; CCR3, cysteine-cysteine chemokine receptor-3; CFU, colony-forming unit; ECP, eosinophilic cationic protein; EE, eosinophilic esophagitis; FEV₁, forced expiratory volume in 1 s; HES, hyper-eosinophilic syndrome; hpf, high power field; IV, intra-venously; PB, peripheral blood; PGA, Physician's Global Assessment of Improvement; PK, pharmacokinetics; QOL, quality of life; SCORAD, scoring atopic dermatitis (measurement scale); TARC, serum thymus and activation-regulated chemokine.

Addition of anti-IL-3, anti-IL-5 and anti-GM-CSF antibodies to such cultures produced differentiation of eosinophils independently of these cytokines, provided eotaxin was present. This may explain the eosinophilia in blood and tissues despite the absence of IL-5 [160].

These observations also suggest that the eotaxin/CCR3 interaction may be involved in the clinical symptoms of conditions such as asthma that are driven by tissue-dwelling eosinophils.

Awareness of these emerging interactions involving eotaxins and/or their receptors may be important in a therapeutic context of developing therapeutics targeting the IL-5 pathway.

Atopic dermatitis. Atopic dermatitis is a common pruritic and chronic relapsing inflammatory skin disease. AD typically presents in infancy or early childhood, affecting 20–30% in this age group. Up to 70% tend to remit during adolescence [161]. Patients with AD tend to have a personal or family history of atopy consistent with a Th2 shift in immunity. This history is frequently associated with elevated peripheral-blood eosinophil counts [162, 163]. Although tissue eosinophilia is not always a prominent feature in AD, deposition of eosinophilic granular proteins such as ECP, EDN and MBP are found in most AD skin biopsy specimens [164], suggesting that eosinophil activation and degranulation may be more important in AD than the presence of eosinophils in the dermis of the skin *per se*.

The importance of IL-5 in the pathophysiology of AD has been documented with increased numbers of cells in the dermis, most probably eosinophils, producing IL-5R α mRNA [165]. IL-4- and IL-5-producing Th2 lymphocytes are increased in the peripheral blood and skin of the acute phase of AD, which results in increased eosinophils in blood. Increased IL-5 is produced by lesional T lymphocytes in chronic AD, resulting in increased eosinophils in the skin of these patients [166]. Although Th2 cytokines predominate in early acute AD, Th1 responses appear to predominate in chronic AD [166]. Dermal eosinophils from lesional AD skin express IL-5 mRNA and protein. These are activated eosinophils expressing the EG2 receptor [167].

Anti-IL-5 (mepolizumab) administered via two intravenous doses of 750 mg to three subjects with eosinophilic dermatitis (and HES) resulted in a significant decrease in blood eosinophil counts and clinical improvement in skin symptoms and lesions within 1–2 weeks [168]. A subsequent study using the same dose of intravenous mepolizumab administered weekly for two doses in a cohort of subjects with AD reduced blood eosinophil counts and improved Physician Global Assessment scores by 17.7%. However, this mild clinical improvement did not reach statistical significance ($P = 0.115$) [9]. The lack of a substantial clinical effect

may have several explanations, including too short a duration of therapy, a suboptimal dose of mepolizumab, histological characteristics of these lesions in adults or a less important role for eosinophils in AD, which, at the disease stage it was studied, may have been less relevant.

Lastly, omalizumab, an anti-IgE mAb, has been shown to improve AD as early as 1 month after therapy with continued augmented clinical improvement plateauing around 9 months of therapy [169]. Although blood IL-5 levels in humans and peripheral-blood IL-5+ T cells *in vitro* have not been found to significantly decrease after omalizumab therapy [170, 171], a study of human dendritic cell-dependent allergen-driven Th2-like cytokine response has demonstrated decreased IL-5 production in the presence of omalizumab [172].

Chronic obstructive pulmonary disease. Chronic obstructive pulmonary disease (COPD) is a condition characterized by partially reversible, progressive airflow limitation with pulmonary and systemic inflammation, which is in part mediated by cytokines and chemokines [173]. COPD includes varying expression of chronic bronchitis (CB) and emphysema phenotypes, and it is defined by a post-bronchodilator forced expiratory volume in 1 s (FEV₁) below 80% of the predicted normal value, and an FEV₁/forced vital capacity ratio below 0.70. [174]. Although airway inflammation in COPD is primarily neutrophilic, studies across the globe have found, using sputum cellular analysis or bronchoalveolar lavage that approximately 30% of patients with COPD have elevated levels of eosinophils in the airways [175–180]. Indeed, sputum eosinophils are increased in the airways of patients with COPD and CB as opposed to those with COPD without CB [181]. These patients are characterized by more frequent exacerbations and more rapid lung function decline than in COPD patients without CB.

Saetta et al. [182] observed airway eosinophilia in asthma and in exacerbations of CB, but only observed increased bronchial mucosal expression of IL-5 in asthma. Zhu et al. [183] identified gene expression of IL-4 and IL-5, as well as the chemokines monocyte chemoattractant protein-4, eotaxin and RANTES in the large airways of healthy non-smokers and in two groups of CB subjects whose FEV₁ suggested COPD: one group of smokers with stable disease (FEV₁ 75% $\pm$ 5 of predicted value) and the other with exacerbations (FEV₁ 61% $\pm$ 8 of predicted value). Zhu et al. concluded that RANTES is responsible for the eosinophilia observed in CB patients with exacerbations and not IL-5.

Zhu et al. recently described increases in numbers of plasma cells and cells expressing genes for IL-5 and IL-4 in bronchial submucosal glands and mucosae of large airways of smokers with CB with or without airway

obstruction (COPD), when compared with asymptomatic smokers. The number of plasma cells was significantly associated with IL-5 mRNA+ cells in gland and subepithelial areas in groups with CB + COPD ($\rho = 0.50$, $P < 0.03$) and with CB alone ($\rho = 0.55$, $P = 0.01$); a similarly significant pattern was observed with IL-4 mRNA+ cells, and both findings suggest a novel role for plasma cells in mucus production [184].

In COPD, the response to oral and inhaled corticosteroids is related to the intensity of the airway eosinophilic inflammation [175, 176] and sputum eosinophilia >3% has been demonstrated to be a good predictor of response to steroids in COPD [177, 178]. A strategy in which inhaled corticosteroids were used to control sputum eosinophilia >3% in COPD resulted in a reduction in the frequency of severe COPD exacerbations requiring hospital admission [180]. Therefore, therapy directed against IL-5 or IL-5R that results in a reduction of eosinophils may prove effective in reducing acute exacerbations of COPD and relieving symptoms in patients who express this phenotype of COPD.

Eosinophil-associated gastrointestinal disorders.

Eosinophilic esophagitis: The umbrella term 'eosinophil-associated gastrointestinal disorders' encompasses a group of clinical entities characterized by inappropriate accumulation of eosinophils in the gastrointestinal tract. The most common disorder in this group is EoE [185], which is a condition characterized by GERD-like symptoms that are refractory to proton pump inhibitor therapy and accompanied by pronounced oesophageal eosinophilic infiltration.

The presence of elevated IL-5 concentrations in the oesophageal mucosa in paediatric [186] and adult [187] patients with EoE has been reported. Of note, these studies identified EoE as a selective Th2 inflammatory response that specifically involves IL-5. T lymphocytes, mast cells and eosinophils all of which are known to produce IL-5 are all markedly increased in oesophageal biopsies from EoE patients [188].

The involvement of various components of Th2-mediated immunity in EoE (including IL-5, CCR3, and eotaxin-3) was recently demonstrated in a study of 12 children with EoE [124]. Subtyping of CD4+ T cells from patients in this study showed significantly higher ratios of both IL-5 : IFN- γ and IL-4 : IFN- γ among active EoE patients and atopic controls compared with normal controls; IL-5 : IFN- γ ratios correlated positively with distal oesophageal eosinophil counts ($r = 0.64$; $P < 0.05$), whereas IL-4 : IFN- γ CD4+ T cell ratios did not [124]. This suggests that eosinophilia in this area is an IL-5-specific Th2 response within an already Th2-skewed immunoreactive environment.

Topical corticosteroid therapy continues to be an important pharmacologic option for both initial and

maintenance treatment of EoE [189]. However, in a 50-week prospective study of adults with EoE treated with 250 μ g swallowed budesonide twice daily, 50% were unable to achieve a histological response (eosinophils $\leq 20/\text{hpf}$) [190], demonstrating a significant unmet need for long-term therapy for this disease.

Hyper-eosinophilic syndrome: HES is a continuum of heterogeneous diseases in which end-organ tissue damage coexists with prolonged, elevated blood eosinophil counts ($>1.5 \times 10^9$ cells/L), and for which other eosinophilic disorders have been ruled out [191–195].

Involvement of IL-5 in HES resides mainly in the lymphocytic variant [196], in which an expanded Th2-derived clonal population overproduces IL-5. Early case reports suggested that IL-5 production by T cells in patients with HES had colony-stimulating effects on eosinophils [197]. A subsequent case report confirmed that such abnormal IL-5 production in HES was due to a Th2 cell clone [198], which was identified as a CD4+ CD3– phenotype [199, 200], and subsequently characterized as the most frequently observed phenotype [192]. Anti-IL-5 therapy has been shown to be effective in the treatment of patients with well-controlled HES [8] and those with the lymphocytic variant of HES, although a subset of the latter subjects failed to respond, presumably due to excess IL-5 that was not completely neutralized by anti-IL-5 treatment [201].

Churg-Strauss syndrome (CSS): It is a rare systemic vasculitis characterized by granuloma formation and severe blood and tissue eosinophilia in patients with long-standing asthma. Chronic eosinophilic pneumonia with asthma has been reported to precede approximately half of all CSS cases [202].

Accumulation of eosinophils in CSS appears to involve Th2 cytokines such as IL-4, IL-13 and IL-5 [203]. Significantly higher levels of IL-4 and IL-5 are secreted by T cell activated peripheral blood mononuclear cells from patients with CSS than from healthy controls [203]. However, reports of increased circulating levels of IL-5 in patients with CSS have been inconsistent [6, 204, 205]. Measureable serum IL-5 concentrations were reported in all patients with CSS ($n = 5$) in one study [6]. Similarly, a case report of CSS in which the patient's plasma IL-4 remained static described elevated levels of IL-5 along with IL-13, serum ECP and blood and urine EDN, until treatment with prednisolone, at which point all markers fell at various rates [205]. In contrast, elevated serum levels of IL-5 correlated positively with blood eosinophilia only in two patients with more severe active CSS in one study [206], and only 30% of patients with active CSS in another study had detectable levels of plasma IL-5 [204]. These findings suggest that eosinophilia in CSS may be associated with other factors in addition to

IL-5. Despite these uncertainties, a pilot study of seven steroid-dependent CSS subjects treated with mepolizumab resulted in decreases in peripheral blood eosinophil counts and a safe reduction in daily oral corticosteroid requirements [207].

Eosinophilic nasal polyposis: Up to 4% of the population suffers from nasal polyps often associated with chronic rhinosinusitis [208]. The causes and pathogenesis of nasal polyps are incompletely understood and probably are multifactorial. Histological studies of nasal polyps have demonstrated that 80–90% contain abundant numbers of eosinophils that are partially activated [209]. Topical glucocorticoids may reduce nasal polyp size and nasal symptoms in some patients, but in others, oral glucocorticoids and/or sinus surgery is required to control the disease. Unfortunately, the 3-year relapse rate has been estimated to be 82% in patients who have nasal polyps that contain high number of eosinophils [210] and this could potentially signal the need for future repeat sinus surgeries in these patients.

Given the high prevalence of eosinophils in the majority of nasal polyps, treating recalcitrant patients with a mAb that antagonizes the IL-5/IL-5R pathway may be justifiable. In a small pilot study using reslizumab, nasal polyp size was significantly reduced

most prominently in subjects that had elevated baseline nasal secretions of IL-5 [211]. However, surface expression of the IL-5R α on eosinophils in nasal polyp tissue has been shown to be reduced [212] and this decreased expression tracked with the activation status of the eosinophil. In addition, Hamilos et al. [213] observed that GM-CSF mRNA and not IL-5 mRNA is increased in subjects with chronic sinusitis and nasal polyps, and that tissue eosinophilia correlates most strongly with GM-CSF and not IL-5. Studies are needed to determine the full extent of the effectiveness of targeting the IL-5/IL-5R pathway in patients with eosinophilic nasal polyps.

Anti-interleukin-5 therapeutic approaches

Clinical trials of therapeutics involving mAbs to IL-5 and its receptor, and other approaches, have been completed or are currently under way at various phases of clinical development. Table 1 summarizes these studies and Fig. 4 depicts the various IL-5/IL-5R approaches.

Although subjects receiving these mAbs have demonstrated consistent reductions in eosinophil numbers in blood and sputum, clinical response in asthma and other allergic diseases have been more variable [214–216].

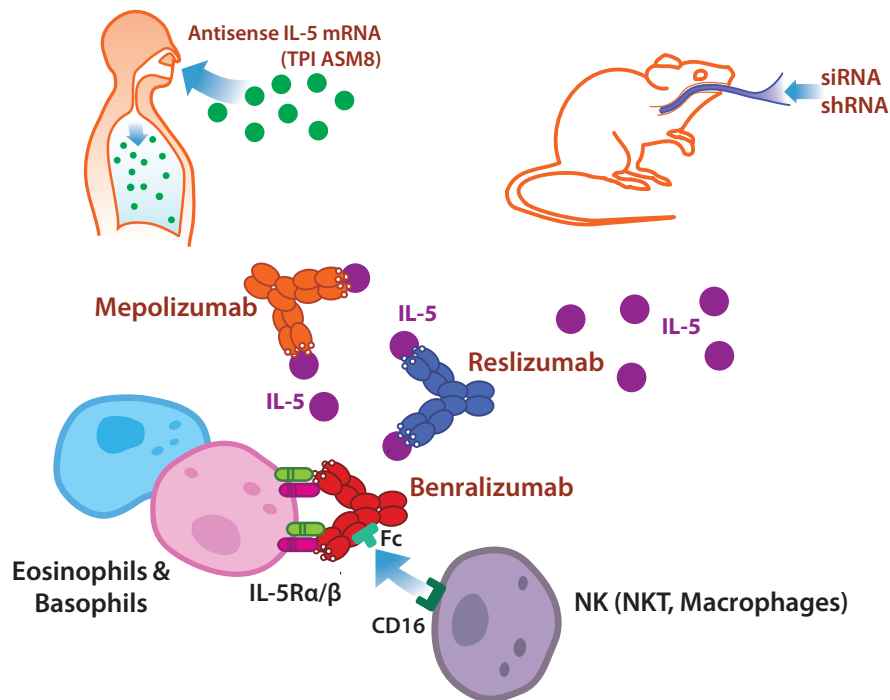


Fig. 4. IL-5/IL-5R targeted therapeutic approaches for eosinophil-associated diseases. Therapeutic approaches targeting IL-5/IL-5R include the anti-IL-5 mAbs mepolizumab and reslizumab, the anti-IL-5R α mAb benralizumab and the antisense IL-5 mRNA TPI ASM8. These agents are being evaluated in clinical studies. shRNA and siRNA directed against IL-5 are being tested in animal models. CD16, cluster of differentiation 16; Fc, fragment crystallizable receptor; NK, natural killer cell; iNKT, invariant natural killer T lymphocyte; shRNA, short hairpin RNA; siRNA, small interfering RNA.

Anti-interleukin-5 monoclonal antibody

Mepolizumab. Mepolizumab (formerly SB 240563) is a fully humanized anti-IL-5 mAb that binds with high affinity and specificity to free IL-5 and thus prevents binding between IL-5 and IL-5R α [217, 218].

Mepolizumab is to date the most widely studied anti-IL-5 mAb in the treatment of asthma and numerous other entities marked by increased eosinophil numbers. A relatively recent review of mepolizumab clinical trials covering various indications also identifies potential pitfalls to therapeutic efficacy [219].

A small study of mepolizumab in patients with HES demonstrated an adequate safety profile for this mAb as well as early evidence of sustained reductions in peripheral eosinophil counts and a corticosteroid-sparing effect in these patients [201, 220]; the latter effect was also observed in *FIP1L1-PDGFR α* -negative patients in a larger study [8].

A recent translational study evaluated clinical use of mepolizumab in a diverse patient population with a variety of eosinophilic disorders including HES and eosinophil-associated gastrointestinal disorders (which were comorbid in three patients). The main findings of this study included identification of an Ig/IL-5 immune complex that was probably responsible for increased blood levels of IL-5 after mepolizumab treatment; this suggests the existence of an endogenous IL-5 autoregulatory pathway. This study also observed sustained reductions in blood eosinophilia and CCR3+ cells, as well as eotaxin-mediated eosinophil shape change *ex vivo* after mepolizumab treatment [221]. A previous study by the same authors demonstrated marked decreases in peripheral and oesophageal eosinophilia in patients with EoE after mepolizumab therapy [222]. In this study and the translational study described above, responsiveness to mepolizumab did not correlate with plasma IL-5 levels [221, 222].

The most recent clinical study of mepolizumab in EoE reported significantly reduced mean numbers of mucosal oesophageal eosinophils (73.2–32.8 eosinophils/hpf active vs. 56.7–52.7 eosinophils/hpf placebo, $P = 0.011$) in addition to reversal of expression of molecules associated with oesophageal remodelling. However, there was no decrease in mean mucosal eosinophils in the duodenum (17.0–17.6 eosinophils/hpf active vs. 16.3–15.9 eosinophils/hpf placebo, NS) nor a change in mucosal mast cell numbers (16.6–15.2 mast cells/hpf active vs. 16.7–16.0 mast cells/hpf placebo, NS) [223]. This difference in effect was thought to be due to lower IL-5R α surface expression in duodenal eosinophils compared with EoE. Unfortunately, like most clinical studies involving this mAb, limited improvement in clinical symptoms was demonstrated [7]. The lack of a clinical effect of mepolizumab on EoE

could be explained by an incomplete depletion of mucosal eosinophils along the upper GI tract to levels considered to be in complete remission (<5 eosinophils/hpf). Another non-mutually exclusive explanation could be the failure to effectively reduce mast cells in the tissue mucosal and/or smooth muscle. In the latter instance, increased numbers of tryptase-positive mast cells have recently been found in the smooth muscle of children with EoE with only low levels of eosinophils in the same tissue [224]. Interestingly, tryptase-positive mast cells in the smooth muscle correlated with the clinical symptom of dysphagia, $r = 0.72$, $P = 0.04$.

In studies of subjects with asthma, mepolizumab has consistently demonstrated reduction in peripheral blood [225] and sputum eosinophils [226–228]. However, reductions in airway eosinophil counts have been less clear, with results ranging from a 55% decrease vs. placebo in a 5-month dosing study [229] to no significant change vs. placebo in a 12-month dosing study [216]. The explanation for this divergence in airway eosinophils is unclear, but could be due to differences in asthma severity, inclusion criteria or a greater placebo effect in the latter study. Mepolizumab has also been shown to reduce mature eosinophil counts in bone marrow [230] of atopic asthmatics, without affecting eosinophilic progenitor numbers. Despite the above biological activities against eosinophils, a large phase 2 study of 362 unselected, moderate asthmatics failed to demonstrate any significant clinical benefits of mepolizumab [226].

The most substantial clinical benefit of mepolizumab therapy in patients with asthma has been reported in two recent studies. Notably, the clinical response in one study of severe refractory eosinophilic asthmatics (sputum $\geq 3\%$) [216] included significantly fewer severe exacerbations than placebo over the 50-week treatment period (2.0 vs. 3.4 mean per subject; 95% confidence interval, 0.32–0.92; $P = 0.02$) and significant Asthma Quality of Life Questionnaire improvements (mean increase from baseline, 0.55 vs. 0.19; mean difference between groups, 0.35; 95% confidence interval: 0.08–0.62; $P = 0.02$), and significantly reduced blood ($P < 0.001$) and sputum ($P = 0.002$) eosinophil counts. However, as with other studies evaluating mepolizumab, there was no significant differences between groups in symptoms, FEV₁ after bronchodilator use or AHR [216]. In another smaller pilot study of uncontrolled, steroid-dependent eosinophilic asthmatics (sputum $\geq 3\%$) [227], treatment with mepolizumab was associated with a significant decrease in the number of sputum and blood eosinophils along with improvement in FEV₁ and asthma control that were maintained for 8 weeks after the last infusion. There were 12 asthma exacerbations in 10 patients who received placebo, nine of whom had sputum eosinophilia at the time of exacerbation. In comparison, only one patient who received

mepolizumab had an asthma exacerbation, and this episode was not associated with sputum eosinophilia ($P = 0.002$). Patients who received mepolizumab were able to significantly reduce their prednisone dose ($P = 0.04$) [227]. In contrast to the Halder mepolizumab study, Nair showed significant improvements in Asthma Control Questionnaire scores and post-bronchodilator FEV₁. One possible explanation could be that in this study, the baseline FEV₁ reversibility of subjects was 28.4% whereas that in the Halder study was only 9.1%. Although improvement in FEV₁ has been a standard benchmark of clinical improvement in asthma, it has been postulated by Pavord et al. [231] that at least for eosinophilic asthma, steroid responsiveness may be more important in predicting a reduction in future asthma exacerbations with mepolizumab therapy than a baseline improvement in FEV₁. Despite subtle differences in the Halder and Nair studies, both studies suggest that the blockade of IL-5 function may offer benefit in severe eosinophilic asthma.

Reslizumab. Reslizumab (formerly SCH55700) is a humanized anti-IL-5 mAb that has been used successfully in the treatment of nasal polyps, reducing polyp size in half of patients studied; baseline concentrations of IL-5 in nasal secretions were predictive of response to reslizumab [232]. A study of a single dose of reslizumab in the treatment of four paediatric HES patients showed that although eosinophil levels fell and clinical symptoms improved, rebound eosinophilia to levels above baseline and return of HES symptoms necessitated reinstitution of reslizumab therapy, with a reduced effect on eosinophil count than in the first dose [233].

Kips et al. [234] conducted the first clinical study to evaluate reslizumab in 18 patients with severe persistent asthma and eight placebo patients. A significant ($P = 0.019$ vs. placebo), but short-lived improvement in FEV₁ was demonstrated, with no other significant differences from placebo. In a 15-week phase 2 study in adults with poorly controlled eosinophilic asthma (high-dose inhaled corticosteroids and sputum eosinophils $\geq 3\%$), the reslizumab group showed a significant improvement in FEV₁ and a non-significant trend in fewer asthma exacerbations (not significant) [235].

Although these are the only published clinical studies to date on the use of reslizumab, a phase 2b/3 study has been completed in 226 paediatric patients with EoE, with mixed results, including an equivocal clinical symptom end-point [236].

Anti-interleukin-5R monoclonal antibody

Benralizumab. Benralizumab (formerly MEDI-563) is a humanized anti-IL-5R α mAb that binds with high affinity

to the alpha chain of the IL-5R to block IL-5 function and induce apoptosis of eosinophils and basophils through antibody-dependent cell-mediated cytotoxicity (ADCC) [67]. By binding to the IL-5R, benralizumab targets the effector cells that are driven by IL-5 (eosinophils/basophils) rather than the numerous cells that only produce IL-5 (Fig. 1). As anti-IL-5 mAbs act by neutralizing free IL-5, it is theoretically possible that cellular sources of IL-5 could in some disease states exceed the ability of anti-IL-5 mAbs to completely neutralize all circulating IL-5.

The structure, binding epitope, affinity for IL-5R α and ADCC function of benralizumab have been recently described in non-clinical studies; the product potently and efficiently depleted peripheral-blood eosinophils and their precursors to less than the limit of detection. In addition, benralizumab showed intense immunohistochemical reactivity in eosinophils, but not mast cells in both bronchial and peripheral human lung biopsy specimens, suggesting that it possesses activity as a potential asthma therapeutic that would be capable of interacting with, and presumably depleting, IL-5R bearing cells at the airway level [67].

Benralizumab was also shown to produce a rapid and nearly complete depletion of peripheral-blood eosinophils that persisted for at least 84 days after single intravenous doses of 0.3–3.0 mg/kg [237]. Benralizumab showed an adequate safety profile and dose-proportional pharmacokinetics (PK) in this phase 1 study. A phase 2 multiple ascending dose safety study using a subcutaneous formulation of benralizumab in adult asthmatics resulted in PK/PD activities similar to those observed with intravenous dosing [238]. Two additional phase 2 studies of benralizumab in adult subjects with asthma are ongoing (ClinicalTrials.gov identifiers: NCT00659659 and NCT00768079).

Other products under development

The drug product TPI ASM8 contains two modified phosphorothioate antisense oligonucleotides designed to inhibit allergic inflammation by down-regulating human CCR3 and the β c chain of IL-3, IL-5 and GM-CSF [239, 240].

A recent summary of presentations at the International Quality & Productivity Center [241] includes clinical results of TPI ASM8. At its highest dose level tested, this compound reduced early and late asthmatic responses by 32% and 49%, respectively [242]. A second study reported a significant reduction in early asthmatic response ($P = 0.04$) and a trend towards reduction in the late asthmatic response ($P = 0.08$) among 17 subjects with mild atopic asthma who received 1500 μ g of TPI-ASM8 once daily for 4 days, compared with placebo [242].

Small interfering RNA molecules directed against IL-5 [241], as well as short hairpin RNAs against eotaxin or IL-5 [243] have shown promising effects in murine models of asthma.

Safety considerations

All of the drugs in development mentioned above aim to inhibit or deplete IL-5-dependent cells, especially eosinophils, that exert pathophysiologic consequences in various tissues or organs in the body. Although eosinophil depletion may improve various disease states where exuberant activity has been observed, it is important to continue pharmacovigilance in current and future studies as theoretical risks of eosinophil depletion have been postulated such as helminth infestations, viral, bacterial and fungal infections and cancer [244].

Future directions

The main obstacle to effective therapy with anti-IL-5 mAbs has been suboptimal amelioration of clinical symptoms despite consistent reductions in blood and tissue eosinophilia. *In vitro* studies of differential effects of IL-5, IL-3, GM-CSF and eotaxin on these cytokines' expression in human tissue eosinophils suggest that variable cross-regulation of the receptors by the cyto-

kine ligands (including up-regulation of IL-3 α by IL-5, IL-3 and GM-CSF) may cause eosinophils at sites of allergic inflammation to have reduced expression of IL-5R. The authors hypothesized that these observations may partially explain the limited clinical benefits observed so far in anti-IL-5 mAb clinical studies [245].

Others have suggested that the use of ADCC may offer more of a novel approach, especially in the milieu of targeting IL-5R rather than IL-5 itself [12]. With continued study, mAbs that specifically target the IL-5R rather than the cytokine itself, and which have demonstrated good eosinophil binding (especially in the lung) [237] and safety/tolerability profiles [246], may be found to offer additional benefit, especially in the treatment of asthma.

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