Targeting Interleukin (IL) 5 for Asthma and Hypereosinophilic Diseases

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Abstract: Although glucocorticosteroids are still the first line of treatment for chronic asthma, over the last two decades great advances have been made in understanding the pathogenesis of asthma that enabled the identification of new therapeutic targets for asthma treatment. The interleukin (IL) 5: eosinophil axis is a hallmark pathway of allergic inflammation that has received much attention. Indeed, IL-5 is known to regulate eosinophil differentiation, proliferation, priming and activation. Therefore, therapeutic agents targeting IL-5 have been generated. In this review we will discuss the effects of IL-5 on eosinophils and outline the signaling mechanism involved in IL-5-mediated effects. Furthermore, recent results from clinical trials targeting IL-5 in asthma and hypereosinophilic syndrome will be discussed and an overview of newly developed patents aimed to target IL-5 will be reviewed.

Keywords: IL-5, eosinophils, asthma, hypereosinophilic syndrome.

INTRODUCTION

Interleukin (IL) 5 belongs to the hematopoietic growth factor family of cytokines [1]. While IL-5 was initially described as a T cell replacing factor that is capable to induce B cell differentiation into immunoglobulin-secreting cells [2], the biological effects of IL-5 are best characterized and likely the most potent on eosinophils [3, 4].

IL-5 is predominantly produced by T helper type 2 cells in response to various stimuli including innate immune triggers (e.g. parasite and viral infections) or in settings of allergic responses [5-7]. Nevertheless and in addition to T cells, various other cells can produce IL-5 including mast cells, eosinophils, NK cells, NKT cells and most recently described, adipose tissue-associated c-Kit$^+$ Sca-1$^+$ lymphoid cells [1, 8].

IL-5 has been "traditionally" attributed significant roles in allergic diseases that affect various organs such as the airways (e.g. asthma), skin (e.g. atopic dermatitis) and esophagus (e.g. eosinophilic esophagitis (EE)) [4, 9]. Allergic diseases are characterized with elevated IgE levels, T helper type 2 (Th2) cells and prominent tissue eosinophilia [9]. In these settings, IL-5 has been shown to synergize with eotaxins to mediate eosinophil accumulation in the tissues and consequent tissue damage or excessive repair processes [10, 11]. Recent studies have indicated a role for IL-5 (and therefore for eosinophils) in bacterial clearance in polymicrobial sepsis induced by Cecal Ligation and Puncture as well as in experimental models of colitis [12, 13].

In this review we will discuss the role of IL-5 in eosinophil biology and its potential as a therapeutic target in various eosinophil-related disorders including asthma and hypereosinophilic syndromes.

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IL-5 INDUCED SIGNAL TRANSDUCTION

In order to elicit its biological effects, IL-5 exerts signaling *via* a unique signaling complex, which includes the IL-5 receptor (R) α chain and a common β chain (βc) that is shared with IL-3 and granulocyte-macrophage colony stimulating factor (GM-CSF) [3, 14-16]. The specificity for IL-5 signaling is achieved by the α-chain that binds IL-5 with low affinity [3]. Upon dimerization with the βc-subunit a high-affinity receptor is formed. While the βc-subunit is not required for cytokine binding, it has a critical role in IL-5-induced signal transduction. Given the shared receptor complex between IL-5, IL-3 and GM-CSF, IL-5 displays significant functional homology with IL-3 and GM-CSF [3], though the latter are not "eosinophil-specific" as multiple myeloid cells express that α-subunit for their receptor including macrophages, mast cells and basophils.

The α- and βc-subunits of the IL-5 receptor do not contain kinase domains in their cytoplasmic tail. Therefore, initiation of the signaling cascade is dependent on the phosphorylation of multiple kinases including Janus kinase 1 (JAK1) and 2 [17-22], Src-homology 2 (SH2)/SH3 bearing signaling proteins such as Vav [23, 24], Src-homology-domain-containing transforming protein (Shc) [25, 26], Bruton tyrosine kinase (Btk) [23, 27], Lyn kinase, phosphoinositide 3-kinase (PI3K) [26], signal transducer and activator of transcription (STAT) 1, -3 and -5 [18]. Importantly, IL-5Rα chain is constitutively associated with JAK2 even in the absence of IL-5 stimulation whereas; JAK1 is constitutively associated with the βc-subunit [21]. Both JAK1 and JAK2 are further activated following IL-5 stimulation (Fig. **1**). Indeed, the signaling cascade elicited by IL-5:JAK2:STAT5 is necessary for IL-5-induced effects in both eosinophils and B cells [3, 20, 28].

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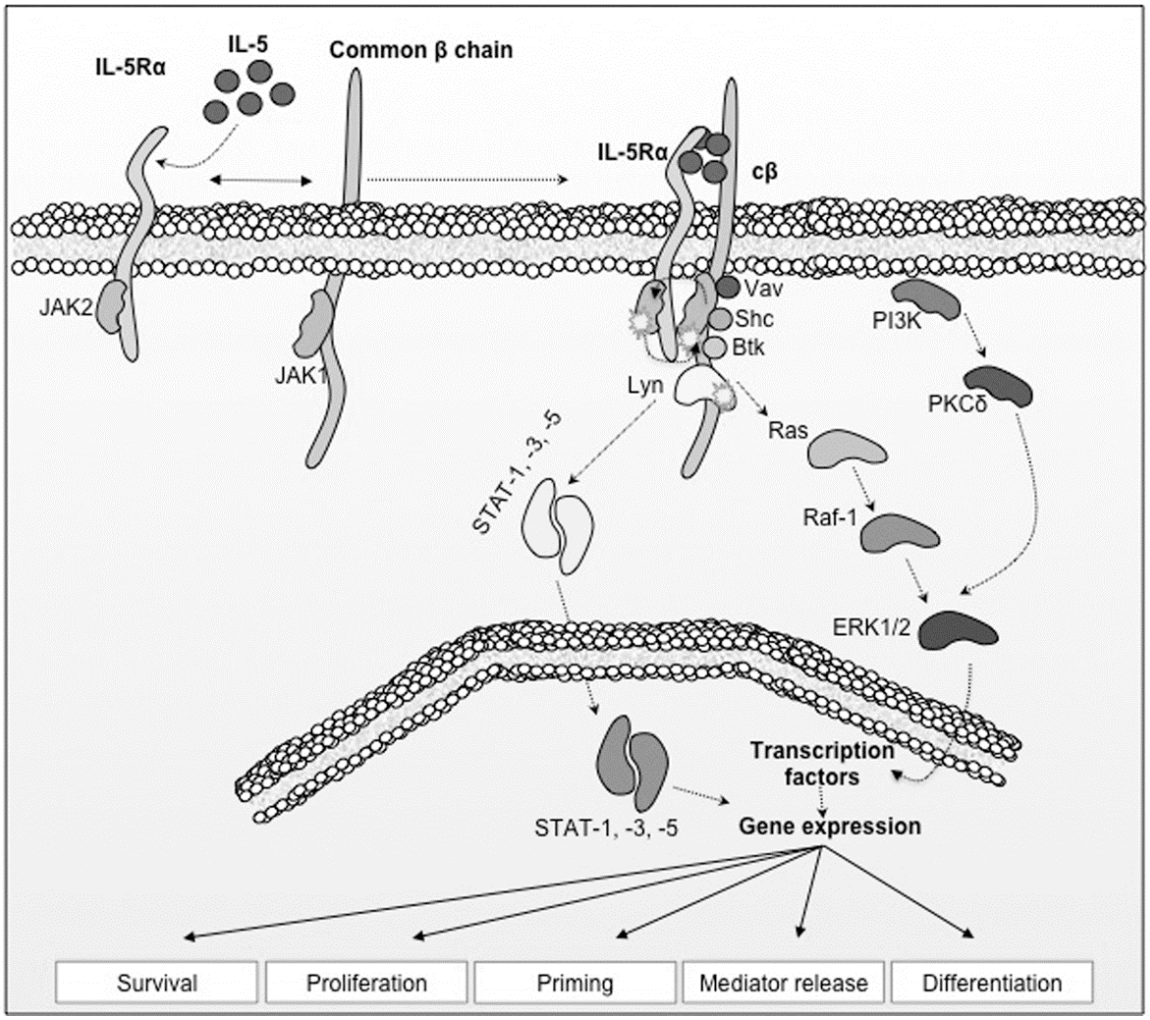

Fig. (1). Mechanism of IL-5 signal transduction

Upon binding to its specific α-chain subunit in the IL-5 receptor, the complex dimerizes with the β chain, which is common to the IL-3 and GM-CS receptors. while the Lyn kinase is constitutively associated with the β-common chain, multiple signaling cascades including the JAK: STAT pathway, the PI3K: PKCδ pathway and the RAs: Raf: ERK pathways are initiated ultimately leading to expression of genetic pathways involved in survival, proliferation, differentiation, priming and mediator release.

Following recruitment and activation of the aforementioned proximal kinases/adaptor molecules, the mitogen activated protein kinase pathway (MAPK) is activated and various reports have shown that the Ras-ERK pathway is important for IL-5 dependent cell differentiation, proliferation and survival [3, 29].

Interestingly, recent studies demonstrate an important role for the IL-5Rα-selective binding adaptor protein syntenin in eosinophilopoiesis [30]. In fact, syntenin forms complexes with multiple IL-5Rα chains and stabilizes the IL-5-induced oligomeric receptor complex [31]. Identification of IL-5-specific adaptor molecules is important since such proteins may be targeted as a therapeutic approach.

IL-5, EOSINOPHILIA AND AIRWAY INFLAMMATION

The role of IL-5 in eosinophilic airway inflammation has been well documented [4]. Although IL-5 is not an effective chemoattractant for eosinophils, it is a potent "priming" agent for eosinophil chemotaxis to other chemoattractants such as CCL5 (RANTES), CCL11 (Eotaxin) and platelet-activating factor (PAF) [32, 33]. Furthermore, IL-5 can increase the expression of adhesion molecules on eosinophils and on endothelial cells and thus amplify eosinophil transmigration from the blood vessels into the tissue [34, 35]. Finally, IL-5 can either prime for or directly activate eosinophil mediator release and cytotoxicity [12, 36], the latter may be important as eosinophils may be responsible for some of the tissue damage that is observed in the asthmatic lung or tissue damage that may be observed in hypereosinophilic syndromes especially the heart [4, 9, 37, 38].

Animal studies have confirmed the essential role of IL-5 in eosinophil functions. Mice that harbor a constitutively active IL-5 gene display elevated numbers of eosinophils in the blood, gastrointestinal tract, liver and other lymphoid organs [39, 40]. Moreover, mice that constitutively express IL-5 in the lung epithelium display a dramatic accumulation of peribronchial eosinophils and striking pathologic changes including the expansion of bronchus-associated lymphoid

tissue (BALT), goblet cell hyperplasia, epithelial hypertrophy, and focal collagen deposition [41]. These changes were also accompanied by eosinophil infiltration of the airway lumen [39]. Of note, the gene targeted transgenic mice featured increased airway hyperresponsiveness (AHR) to methacholine in the absence of aerosolized antigen challenge. These data indicate that local expression of IL-5 in the lung tissue may induce development of AHR and eosinophilia.

In addition to the aforementioned animal studies, the effects of IL-5 on IgE production, airway inflammation and development of airway hyperresponsiveness has been studied in allergen challenged *Il5*$^{-/-}$ mice [42, 43]. In accordance with the studies using transgenic mice, allergen-challenged *Il5*$^{-/-}$ mice showed a significant reduction in lung eosinophilia and prevented allergen induced AHR but still harbored increased IgE levels and Th2 cytokine expression in the lungs [42, 43].

Administration of anti-IL-5 antibody (TRFK-5) to allergen-challenged mice has provided insight into the potential of pharmacologically targeting IL-5 in asthma. Indeed and as expected, treatment of mice with anti-IL-5 did not alter IgE production and the consequent generation of Th2 cytokines but had significant effects on airway eosinophilia and AHR [44-46]. This study as well as others indicated that monoclonal antibody treatment neutralizing IL-5 might be beneficial for asthma therapy.

Besides the proposed roles for IL-5 and eosinophils in the acute inflammatory responses of asthma, IL-5 and eosinophils have been attributed a role in chronic airway inflammation and in remodeling [47]. Studies aimed to examine the role of IL-5 in airway remodeling showed that *Il5*$^{-/-}$ mice had a significant reduction in the number of eosinophils, which was paralleled by a similar reduction in the number of transforming growth factor (TGF) -β^{+} cells, suggesting that eosinophils are a significant source of TGF-β in the remodeled airway [47]. Indeed, eosinophils express TGF-β, are capable to induce fibroblast proliferation and targeting eosinophils in a model of chronic-established asthma using a bispecific antibody fragment reduced the levels of TGF-β that was correlated with eosinophil levels [47, 48]. Furthermore, allergen-challenge induced significantly higher levels of bioactive lung TGF-β cells in wild type mice compared with *Il5*$^{-/-}$ mice. In accordance with this study, Tanaka *et al* show that chronic allergen-exposed IL-5 receptor-null mice display substantially decreased fibrosis, whereas fibrosis is markedly accentuated in IL-5 transgenic animals [11]. Moreover, treatment of wild-type mice with neutralizing anti-IL-5 antibody, administered before each allergen challenge, prevented subepithelial and peribronchial fibrosis. Collectively, these findings demonstrated that eosinophils are involved in allergen-induced subepithelial and peribronchial fibrosis probably by producing a fibrogenic factor, TGF-β and that these effects may be therapeutically targeted by agents targeting IL-5.

In addition to animal studies, IL-5 mRNA in activated T cells is elevated in lung tissue of patients suffering from bronchial asthma [5, 49]. Furthermore, IL-5 was found to be elevated in the bronchoalveolar lavage (BAL) fluid, induced sputum and serum levels of asthmatics [49, 50]. Notably, IL-5 serum levels correlate with peripheral blood eosinophilia and asthma severity [37, 50]. Comparative differential cytokine expression between severe and moderate asthmatics revealed no difference in IL-5 (and eotaxin) levels whereas IL-4 and IFN-γ levels displayed differential expression pattern [5, 6]. Similar to studies that were conducted in mice, experiments in human eosinophils suggest a role for eosinophils in fibrosis and remodeling [51, 52]. Anti-IL-5 treatment has been effective in significantly reducing the expression of tenascin, lumican, and procollagen III in the bronchial mucosal reticular basement membrane when compared with placebo. A possible mechanism for these effects of anti-IL-5 may be due to reduced numbers and percentage of airway eosinophils expressing mRNA for TGF-β1 and the concentration of TGF-β1 in BAL fluid was largely reduced [53].

In attempt to define the involvement of IL-5 and eosinophils in asthma onset (i.e to define whether eosinophils are bystanders or a cause of allergic airway disease) genetic polymorphisms of *Il5*, *Il5rα* and asthma have been assessed. Recent studies have shown no positive association between polymorphism of *Il5* or *Il5rα* and asthma. Nevertheless, a significant association was found between *Il5* and *Il5rα* polymorphysms and eosinophilia [54]. Variations in *Il5rα* (and *Ccr3*) showed no influence on asthma onset. Given that the role of the eosinophil in asthma pathogenesis has been controversial [55, 56], it is likely that targeting eosinophils with anti-IL-5 will be controversial as well. This controversy is probably due to the fact that anti-eosinophil or anti-IL-5 based treatments will be affecting only one of the multiple pathways for asthma development, which includes other cells and mediators not affected by the eosinophil-IL-5 pathway. Importantly, the interpretation of this data does not mean that IL-5 and/or eosinophils are not targets for asthma therapy as will be illustrated below.

THERAPEUTIC REAGENTS TARGETING IL-5

Asthma is a chronic inflammatory disorder of the airways consisting of intermittent episodes of airway obstruction, wheezing and lung inflammation (www.ginasthma.org). Current treatments are based on prevention of risk factors (allergen avoidance, irritants, infections) and a stepwise approach to pharmacological treatments in order to achieve and maintain well-controlled patients. Controller medications (topical and systemic glucocorticosteroids, leukotriene modifiers, anti-IgE) are used to diminish the inflammatory disorder and are used alone or in combination between them or with reliever drugs (such as short and long acting β2-agonists, inhaled anticholinergics and theophylline). Notably, reliever drugs are used to treat acute exacerbations but regular use of β2-agonists is not recommended.

Developing new drugs for asthma and HES consists of three major approaches: a) Improvements in existing classes of effective drugs; b) development of new compounds; c) development of novel compounds based on serendipity (e.g. drugs that were effective in other diseases) [57]. While much effort is being invested in improving existing effective drugs, strategies to target IL-5 as a mode of therapy fall in the second category for new drug development. In the next few

paragraphs we will outline some novel approaches that target IL-5 or its signaling components.

IL-5 ANTAGONISTS

1. Anti-IL-5 Antibodies

The best therapeutical agents targeting IL-5 are anti-IL-5 specific antibodies. To date, two humanized monoclonal antibodies against human IL-5 have been used in clinical trials. Reslizumab (Ception therapeutics) is a humanized rat monoclonal antihuman IL-5 antibody of the IgG4/κ subtype, which neutralizes IL-5 by binding to amino acids 89–92 [58], and mepolizumab (GlaxoSmithKline Pharmaceuticals), a humanized mouse monoclonal anti-human IL-5 antibody of the IgG1/κ subtype that blocks binding of the human IL-5 to the α-chain of the IL-5 receptor complex expressed on the eosinophils [59]. Notably, the affinity of mepolizumab for human IL-5 is approximately 20-fold greater than that of reslizumab (Kd 4.2 pM vs 81 pM) [59, 60]. Mepolizumab has a half-life of 13-19 days [59, 60], and displays long biological activities, as observed by the significant decrease in peripheral blood eosinophil levels persisting for 3 months in 76% of subjects after the final mepolizumab infusion [61].

2. IL-5 mRNA Cleavage by Ribozymes

This invention relates to ribozymes, or enzymatic RNA molecules that are designed to cleave mRNA encoding for IL-5. Ribozymes that cleave IL-5 mRNA represent a novel therapeutic approach to various inflammatory disorders one of which is asthma. The invention features use of ribozymes to treat chronic asthma by inhibiting the synthesis of IL-5 in lymphocytes and preventing the recruitment and activation of eosinophils.

IL-5 RECEPTOR ANTAGONISTS

1. Anti-IL-5Rα Antibodies

Recently, MedImmune, Inc. and BioWa, Inc. have announced the licensing of BIW-8405 a monoclonal antibody directed to target the IL-5R [62-63]. BIW-8405 has been developed utilizing a distinct technology that was previously developed at BIoWa termed Potelligent™. This technology platform is used for the development of antibody-dependent cellular cytotoxicity (ADCC) enhanced antibodies. Therefore, the anti-IL-5R antibody will aim to directly deplete eosinophils. In addition to its role in direct depletion of IL-5R-bearing cells, the antibody will likely neutralize the effects of IL-5 on eosinophils as well in case optimal ADCC is not achieved.

2. Peptides that Alter IL-5R Dimerization

An alternative approach to target IL-5 signaling has been recently described. England BP *et al*. have designed peptides that specifically bind to the extracellular domain of the human IL-5Rα chain but share no primary sequence homology to IL-5 [64]. The active form of the peptide is a disulfide-cross-linked dimer that forms spontaneously in solution. Gel filtration analyses, receptor-binding studies, and analytical ultracentrifugation reveal that the dimeric peptide binds simultaneously to two receptor alpha chains in solution. The functional antagonism produced by the bivalent interaction of the dimeric peptide with two IL-5R alpha chains represents a distinctive mechanism for the antagonism of cytokines that use heteromeric receptors [65]. Consequently, a set of N-terminally truncated peptides derived from the 19-amino acid peptide (termed AF18748) demonstrated that the first five amino acids of the peptide do not contribute to receptor binding activity [66]. The shortened peptide blocked IL-5-dependent adhesion of eosinophils and had no effect on stimulation by IL-3, granulocyte-macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor (TNF)-alpha or fMet-Leu-Phe [66]. One drawback of this approach is that the shortened peptides were reported to rapidly brake down *in vivo* due to cleavage of a single chain of the dimer. However, peptide breakdown did not correlate with loss of biological activity. Therefore, the pharmacological properties of such future avenue of therapy should be well examined in further studies. Interestingly, despite its high potency antagonizing the human IL-5 receptor, the aforementioned peptides were unable to antagonize the activity of IL-5 on mouse B13 cells, or on canine eosinophils, indicating high specificity for the human IL-5 receptor. An additional approach to neutralize the effects mediated by IL-5 is by use of peptide-derived antagonists such as those described in [67, 68]. In addition, molecular modeling of the IL-5Rα-chain and large-scale, high-throughput screening was used to discover YM-90709, a relatively selective inhibitor of IL-5 receptors [69]. YM-90709 (2,3-dimethoxy-6,6-dimethyl-5,6-dihydrobenzoindolizino[2,3-b]quinoxaline), has been reported to inhibit the binding of IL-5 to its receptor on human eosinophils and eosinophilic HL-60 clone 15 cells without affecting the binding of granulocyte-macrophage colony-stimulating factor (GM-CSF) to its receptor on the same cells. Moreover, intravenous injection of YM-90709 resulted in the inhibition of allergen-induced eosinophilia, into the bronchoalveolar lavage fluid (BALF) of Brown-Norway rats without affecting peripheral blood or bone marrow leukocytes, with ED50 values of 0.32 mg/kg and 0.12 mg/kg, respectively [69].

3. Antisense Oligonucleotides Targeting IL-5R Chains or IL-5

Antisense oligonucleotides are single-stranded synthetic nucleic acid sequences consisting of 15–25 nucleotides. The antisense effects of these polymers are due to the hybridization with the target mRNA in a sequence-dependent (and therefore specific) complementary manner *via* binding of hydrogen bonds. Most anti-sense oligonucleotides are designed to activate RNase H, which cleaves the RNA moiety of a DNA-RNA heteroduplex, leading to the degradation of the targeted mRNA [70]. An additional mode of operation for antisense oligonucleotides is conformational/steric blockade of the ribosome, with translational arrest. Recently antisense oligonucleotides that are directed against nucleic acid sequences coding for the common sub-unit of the IL-3, IL-5 and GM-CSF receptors (as proposed by Topigen Pharmaceutiques Inc [71, 72]) or to the IL-5 receptor α chain have been proposed. *In vitro* antisense oligonucleotides inhibited the IL-5R βc chain mRNA expression and significantly decreased the level of cell surface IL-5R βc chain protein expression and function on TF-1 and U937 cells. IL-5R βc

chain-targeted oligonucleotides were also able to inhibit TF-1 cell proliferation *in vitro* in the presence of GM-CSF, IL-3, or IL-5 in the culture medium as well as eosinophil survival [73]. Moreover, *in vivo* experiments in rats have shown that eosinophil influx and airway hyperresponsiveness in response to allergen (i.e OVA) challenge were greatly decreased in animals treated antisense oligonucleotides targeting the IL-5R βc. These studies underscore the potential utility of a topical antisense approach, targeting IL-5R βc for the treatment of asthma. Notably, IL-5 is not the sole target that has been proposed for asthma treatment and various other targets (reviewed in [74]) including chemokine receptors (i.e CCR3), IL-4 and/or IL-13 and signaling molecules have been proposed.

TARGETING IL-5 IN ASTHMA

Traditionally, corticosteroids have been the most common drug used for the treatment of immunological and allergic disorders. However, during the last decade, novel biological therapies have been developed targeting specific mediators [75-77], in some trials such therapies have shown the possibility of steroid sparing effects [78, 79], allowing the prevention of the well-known long-term side effects of glucocorticoids [80-82]. Since IL-5 regulates major eosinophil functions including proliferation, maturation, release from bone marrow, blood and tissue levels, activation and survival [83-85]. Thus, targeting IL-5 in allergic disorders such as asthma, atopic dermatitis and EE is one of the obvious goals for the therapy. Initial studies using mepolizumab, have raised doubts as for the ability of anti-IL-5 therapy to be beneficial for asthma [86]. Nevertheless, consequent studies (extensively reviewed by Busse *et al*. [80]) revealed that targeting IL-5 might be effective for long-term treatment of patients with selected eosinophilic disorders such as asthma.

Given that eosinophils are not the sole inflammatory cell involved in asthma pathogenesis or other allergic disorders there is a possibility that, subsets of patients which display-increased eosinophilia will be more susceptible to anti-IL-5-based therapies. Concomitant with this notion, studies using reslizumab (humanized anti-IL-5 antibody, Ception Therapeutics) to treat nasal polyposis showed encouraging results in patients with massive bilateral nasal polyps and high baseline IL-5 [87]; In this phase I, single dose, randomized, double-blind placebo controlled clinical trial, the therapy was well tolerated with no severe adverse events, and peripheral blood eosinophilia significantly decreased in comparison to placebo. Interestingly, 10 subjects demonstrated significant rebound eosinophilia (but not serum eosinophil cationic protein (ECP) levels) by week 24 and 32 after treatment with the 1 and 3 mg/kg dose therapy, respectively. In addition, nasal secretion of ECP and IL-5 were significantly reduced. Finally, individual nasal polyp scores improved in half of the treated patients for 4 weeks. Of specific note is that reslizumab responder patients had increased IL-5 concentrations in nasal secretions at baseline compared with non-responders, and increased nasal IL-5 levels of >40 pg/mL predicted the response to anti-IL-5 treatment. Thus, despite the fact that this trial showed no significant clinical symptom score improvement in the reslizumab treated groups, it would be important to assess the effects of anti-IL-5 therapy in trials designed with a cohort of nasal polyposis patients that have a baseline nasal IL-5 secretion of more than 40 pg/mL.

In agreement with the latter study, two recently published clinical trials from independent centers that have studied the effects of mepolizumab in patients with eosinophilic-asthma have reported significant reduction in lung and blood eosinophils with clinical improvements concerning a lowered rate of asthma exacerbations in mepolizumab-treated patients or allowed prednisone sparing in this specific group of asthmatic patients [88, 89].

For example, Nair *et al.* [89] studied the effects of mepolizumab in patients with severe asthma that required oral and inhaled corticosteroids to control their symptoms. The study aimed to define the effects of mepolizumab assessing steroid sparing effects of mepolizumab and assessment of clinical observations for 8 weeks following the last mepolizumab infusion. The primary outcomes of the study showed that asthma exacerbations were significantly different for the mepolizumab-treated group occurring only in one patient, while 10 patients who received placebo ($p=0.002$) developed asthma exacerbations. Importantly, the mepolizumab-treated group was able to reduce prednisone by 84% of the maximum possible reduction compared to only 48% in the placebo group. Furthermore, asthma control questionnaires of mepolizumab-treated patients indicated significant improvement.

Focusing on specific subsets of asthmatic patients, Haldar *et al*. [88] enrolled patients with refractory eosinophilic asthma. In their study cohort, 29 patients were treated for one year with monthly infusions of mepolizumab. Similar to Nair *et al.* they also observed a reduction in the number of severe exacerbations per mepolizumab-treated patients compared to the placebo controls. Of note, mepolizumab did not reduce the recovery time from an asthmatic exacerbation treated with prednisolone; In this study, mepolizumab-treated patients showed a significant reduction in blood and induced sputum eosinophils, in comparison to the placebo control group. Similar to the aforementioned mepolizumab study (i.e. Nair *et al.* [89]) an overall improvement in the score of the quality of life questionnaire was reported by the mepolizumab-treated patients but no significant changes were found between the groups with respect of asthma symptoms, FEV1 after bronchodilators or in the airway hyperresponsiveness.

Of note, a recent press release by Ception Therapeutics using reslizumab for treatment of a cohort of patients with eosinophilic asthma emphasizes the potential of IL-5-targeted therapy in this subset of asthmatic [90]. Reslizumab was reported to significantly improve quality of life questionnaire and had significant changes in FEV1 levels in the reslizumab-treated patients in comparison to placebo controls.

Collectively these recent studies highlight the potential use of anti-IL-5 antibodies and other agents targeting IL-5 in eosinophilic asthma. Nevertheless, from these recent studies it appears that targeting IL-5 alone will not be enough for asthma treatment. This may be related to phenotype of the

eosinophil in the lung tissue and/or bioavailability of the antibody in different compartments.

Studies aimed to target the IL-5Rα chain using BIW-8405 (or MEDI-563) were recently conducted, and a Phase 1, open-labeled, dose-escalating study to evaluate the safety and tolerability of a single intravenous infusion in adults with mild-to-moderate asthma was recently reported. Data from the completed Phase 1 study demonstrated that the antibody was well-tolerated with biologic activity producing substantial and prolonged depletion of blood eosinophils. The antibody is also currently being evaluated in a double-blind, placebo-controlled Phase 1 study to evaluate the safety and tolerability and effects of the antibody on airway eosinophils in adults with asthma.

TARGETING IL-5 IN HYPEREOSINOPHILIC SYNDROMES

In attempt to abolish the development and pathological effects of eosinophils anti-IL-5 directed therapies could prove beneficial in hypereosinophilic syndromes (HES) and eosinophilic gastrointestinal disorders.

HES consists of a diverse group of several heterogeneous disorders, defined by the presence of persistent peripheral blood eosinophilia equal to or greater than 1.5 X 10^9/L, in the absence of a secondary cause of eosinophilia (e.g. parasitic infections), with signs and symptoms of eosinophil-associated end organ involvement and damage [91]. One of the emerging eosinophilic diseases is eosinophilic esophagitis (EE), which is a part of a series of chronic eosinophil-associated gastrointestinal disorders that are being increasingly recognized [92]. EE is defined as a primary clinico-pathological disorder of the esophagus, characterized by esophageal and/or upper gastrointestinal (GI) tract symptoms in association with esophageal mucosal biopsies containing ≥15 intraepithelial eosinophils per high power field in one or more biopsies and absence of pathological GERD as evidenced by a normal pH monitoring study of the distal esophagus or lack of response to high dose proton pump inhibitor medication [93]. Mepolizumab has been shown to be effective in the treatment of EE reducing esophageal eosinophilia by 9 folds and improving clinical outcomes and quality of life [94, 95]. The baseline levels of plasma IL-5 did not affect response to therapy [96].

Recently, the first randomized, double-blind, placebo controlled trial of patients with HES was conducted by Rothenberg and colleagues. Importantly, HES patients in this study were not associated with the constitutively activated tyrosine kinase FIP1L1–platelet-derived growth factor receptor α (*FIP1L1-PDGFRA*). This study evaluated the safety and efficacy of mepolizumab in patients requiring 20 to 60 mg per day of prednisone to maintain a stable clinical status, and aimed at demonstrating the ability of anti–IL-5 to decrease prednisone dependency in HES [78]. Impressively, this international trial revealed a striking ability of anti–IL-5 to decrease prednisone doses, decrease blood eosinophilia, and maintain clinical stability compared with placebo. The primary end point of the study was to obtain the reduction of prednisone dose to 10 mg or less per day for 8 or more consecutive weeks. This was obtained in 84% of the patients as compared with 43% in the placebo group ($p<0.001$), with no increase in clinical activity of HES. Daily prednisone dose was reduced by almost 80% between baseline and week 36, and treatment with prednisone could be stopped in almost 50% of the patients receiving mepolizumab. The secondary end point of the study was to obtain blood eosinophil counts of 600 per microliter or less for 8 or more consecutive weeks, and this was achieved in 95% of the treated group compare with 45% on the placebo ($p<0.001$). Withdrawal due to lack of efficacy was observed in 5 of the 43 (12%) of the mepolizumab treated patients, and treatment failure, defined as worsening of the HES requiring other therapies or increase in prednisone dose (> 60 mg/day) was observed in 9/43 (21%) of the patients receiving mepolizumab. Interestingly mean serum IL-5 levels were below level of detection in most of the patients at baseline, probably due to the prior therapy with glucocorticoids, Nevertheless this suggests that IL-5 levels should not be a criteria for treating HES patients with mepolizumab. Of note, mepolizumab treatment caused an increase in soluble IL-5 receptor levels in some patients.

In addition, a recently multicenter retrospective HES study analyzing clinical characteristics and response to different therapies has showed that anti-IL-5 therapy was effective in 12 of 15 patients that received anti-IL-5 as monotherapy [96].

Collectively, these studies indicate that targeting IL-5 is likely to present a main treatment in non FIP1L1-PDGFRA-HES.

IMMUNOBIOLOGICAL EFFECTS OF MEPOLIZUMAB

Recently a prospectively study done in 25 patients with HES and eosinophilic gastrointestinal disorders aimed for the evaluation of the hematologic and immunologic effects of anti-IL-5 have demonstrated that mepolizumab decrease blood eosinophilia and CCR3+ cells by 20 and 13 fold, respectively, and that this effect was not related to the baseline IL-5 levels [61]. The effects were observed 3 months after receiving the last infusion of mepolizumab, in 76% of the subjects. Not only the number of peripheral blood eosinophils decreased, but also their activation in response to eotaxins. Interestingly the expression of IL-5 receptors alpha was increased in this study but this was not demonstrated in other studies [97], even though there were differences in the experimental protocols. Of note the investigators found an increased IL-5 production by peripheral blood lymphocytes and an increased circulating plasma IL-5 levels, likely due to a circulating long-live IL-5/mepolizumab complex. Concordantly with this observation, eosinophil-deficient mice have shown also an increase in circulating IL-5 [98], supporting the idea of an endogenous autoregulatory pathway involving eosinophils and their IL-5 receptor alpha that regulates IL-5 levels. These findings have clinical significance, since cautious monitoring should be done for weaning anti-IL-5 therapy, to prevent recurrent eosinophilia.

CURRENT & FUTURE DEVELOPMENTS

Treatment with agents designed to block IL-5 or its signaling components seem to be promising for specific

disorders. Nevertheless several key questions need to be addressed in respect with the current anti-IL-5 strategies. For example, determining the ideal dose and dose interval to achieve clinical sustained responses is currently unknown. This is especially important since rebound eosinophilia has been reported after discontinuation of anti-IL-5. In addition, the effects of rebound eosinophilia and their impact on clinical symptoms are yet to be defined. Likewise, the long-term effects of anti-IL-5 targeted therapy are currently unknown. These questions still remain to be answered and will likely be addressed in the near future. Nevertheless, it is likely that therapies targeting IL-5 will be the drug of choice for HES patient subsets and perhaps for subsets of eosinophilic phenotypes of asthmatic patients.

CONFLICT OF INTEREST

The authors have not stated any conflict of interest.

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