IL-5-directed approaches in the treatment of eosinophil-driven disease

Richard Marshall and Isidore Faiferman

Eosinophils are involved in the initiation and propagation of diverse inflammatory responses, including antigen presentation and the release of proinflammatory cytokines, chemokines, lipid mediators and cytotoxic granules (1-4) (Fig. 1). Tissue damage and dysfunction resulting from secretion of these pleiotropic mediators is a common pathogenic component in several diseases, including atopic asthma (5), eosinophilic bronchitis (6), nasal polyposis (7), atopic dermatitis (8, 9), eosinophil-associated gastrointestinal disorders (10) (eosinophilic esophagitis [EE], eosinophilic dermatitis (8, 9), eosinophil-associated gastrointestinal syndromes (HES) (11).

Eosinophils are involved in the initiation and propagation of diverse inflammatory responses that may result in tissue damage and dysfunction. A number of diseases such as eosinophilic bronchitis, hypereosinophilic syndrome, eosinophilic esophagitis and eosinophilic gastroenteritis are currently considered to be predominantly eosinophil-driven. In atopic disease, their role has been less clear. Eosinophils are critically dependent on the cytokine interleukin-5 (IL-5), for their maturation in bone marrow, and also influence eosinophil migration and survival. Recent clinical data with monoclonal antibodies (mAbs) directed against IL-5 support this assumption. Mepolizumab (SB-240563, GlaxoSmithKline) is a humanized mouse anti-human IL-5 mAb and has been shown to be safe and effective in the treatment of patients with hypereosinophilic syndrome. Most recently, clinical trials in severe asthma and nasal polyposis have also reported positive data increasing our understanding of the role eosinophils play in these disorders.

The targets

Eosinophils are involved in the initiation and propagation of diverse inflammatory responses, including antigen presentation and the release of proinflammatory cytokines, chemokines, lipid mediators and cytotoxic granules (1-4) (Fig. 1). Tissue damage and dysfunction resulting from secretion of these pleiotropic mediators is a common pathogenic component in several diseases, including atopic asthma (5), eosinophilic bronchitis (6), nasal polyposis (7), atopic dermatitis (8, 9), eosinophil-associated gastrointestinal disorders (10) (eosinophilic esophagitis [EE], eosinophilic gastroenteritis) and hypereosinophilic syndromes (HES) (11).

Interleukin (IL)-3, IL-5 and granulocyte-macrophage colony-stimulating factor (GM-CSF) are involved in regulating eosinophil development. Of these, IL-5 is most specific to eosinophils; IL-5 is the major hematopoietin responsible for eosinophil growth and differentiation (1-4). In humans, IL-5 activity appears to be restricted to the eosinophil-basophil lineages. The IL-5 receptor complex expressed on the cell surface comprises two components: the α-chain specific for IL-5, and the β-chain, which is the signal-transducing unit. IL-5 receptor activation also modulates basophil function, especially histamine release (12). Thus, IL-5 inhibition should attenuate many eosinophil and basophil activities without affecting the function of other cells of the immune system (13), potentially limiting any safety issues, and making it an attractive drug target.

Two humanized monoclonal antibodies (mAbs) that bind to human IL-5 have been developed and tested in clinical trials. Mepolizumab (SB-240563, GlaxoSmithKline) is a humanized mouse monoclonal anti-human IL-5 antibody of the IgG1/K subtype that binds to human IL-5 with high affinity and specificity, thus preventing IL-5 binding to the IL-5 receptor complex α-chain on the eosinophil cell surface (14). Reslizumab (Ception Therapeutics) is a humanized rat monoclonal anti-human IL-5 antibody of the IgG4/K subtype that neutralizes IL-5 by binding to amino acids 89–92 (15). Clinical development of mepolizumab is ongoing in hypereosinophilia, severe asthma and nasal polyposis; reslizumab has recently entered phase II/III for eosinophilic esophagitis in children. Other approaches in early development include a series of monoclonal antibodies directed against IL-5Rα (MEDI-563, MedImmune), an IL-5-based vaccine (Cytos Biotechnology) and an antisense oligonucleotide to the common β-chain of the IL-5 receptor (Topigen).

Pharmacology

Mepolizumab displays cross-reactivity with cynomolgus monkey IL-5; thus, these animals were used as the in vitro model for assessment of the pharmacology and long-term safety of mepolizumab. Studies in rats and mice were conducted using an anti-rat IL-5-specific mAb by this antibody cross-reacted with mouse IL-5.

Preclinical studies in rodent and primate models showed that mepolizumab produced a profound and prolonged (> 3 months) suppression of eosinophil production and prevented antigen-induced lung eosinophilia. Eosinophil counts were taken as a proxy measure of mepolizumab pharmacodynamics. Peripheral eosinophil count decreased in a time-dependent manner following a single subcutaneous injection of mepolizumab. The maximal response (80–95% decrease in eosinophil count compared to baseline) occurred at 3–4 weeks post-dosing. Following 6-monthly repeated administrations of mepolizumab (10 mg/kg) the observed eosinophil counts were persistently < 20% of the baseline levels over the entire 6-month dosing period. The estimated IC50 value of mepolizumab for reducing the levels of circulating eosinophils was 1.43 µg/ml. Multiple dose administrations did not produce the expected degree of reduction as predicted by the pharmacodynamic single-dose model (16).

Pharmacokinetic profile

Following i.v. administration to cynomolgus monkeys, mepolizumab exhibited a relatively low clearance and volume of distribution. Mepolizumab concentration declined in a biexponential manner, with a mean half-life (t1/2) of 12.9 ± 9.4 h. The mean overall terminal t1/2 following intravenous dosing was 13 ± 2 days. After subcutaneous dosing, mepolizumab...
was completely absorbed into the systemic circulation and appeared to decline in a mono-exponential manner with an apparent $t_{1/2}$ of $14.5 \pm 3.8$ days. Maximal concentrations were observed 2 to 4 days postinjection. Studies in monkeys did not reveal any anti-mepolizumab antibody following repeated i.v. or s.c. administration. The mean half-life of mepolizumab given i.v. to patients with asthma was $20 \pm 2.5$ days (16).

**Preclinical animal models**

In a cynomolgus monkey model of IL-5-dependent eosinophilia, s.c. administration of 6 doses (1/day on alternate days) of recombinant human IL-2 produced a profound eosinophilia. This eosinophilia was blocked by $\geq 85\%$ ($p < 0.05$ vs. untreated controls) in monkeys pretreated with mepolizumab (2 i.v. doses of 0.5, 5 or 50 mg/kg at 1 and 29 days before IL-2 administration) (14).

A single 10 mg/kg i.v. dose of mepolizumab in *Ascaris suum*-sensitive monkeys ($n = 8$) significantly decreased the number of eosinophils in blood following *A. suum* challenge at day 1 and week 6 ($p < 0.05$ vs. control-treated animals) and the number of eosinophils in the bronchoalveolar lavage fluid (BALF) at week 3 ($p < 0.05$ vs. control-treated animals) (14). The levels of regulated on activation, normal T expressed and secreted (RANTES) and IL-6, both eosinophil secretion products, in BALF at 3 weeks postdose were also decreased. However, there was no effect on the acute bronchoconstrictor response to *A. suum* antigen challenge (14).

These studies in cynomolgus monkeys indicate that mepolizumab is safe and well tolerated in an *in vivo* animal model, and, by producing long-term reductions in circulating and tissue eosinophils, has therapeutic potential in diseases where overproduction of eosinophils plays an important pathological role.

Experimental murine models of EE suggest a major role for IL-5 in the pathogenesis of this disease. EE can be induced by overexpression of IL-5 in transgenic mice (10) and blocked by neutralizing IL-5 in an allergen-induced murine model of EE (17). Furthermore, mice deficient in IL-5 are protected from induction of experimental EE (17, 18).

**Human clinical studies**

**Asthma**

Initial clinical studies confirmed the safety profile, and the efficacy in reducing circulating eosinophils seen in preclinical species, but called into question the relevance of eosinophils in asthma. Single intravenous infusions of mepolizumab (2.5 mg/kg and 10.0 mg/kg) in patients with mild allergic asthma reduced blood eosinophils by about 85% versus baseline or placebo; this reduction lasted for 16 weeks for the higher dose of mAb used (19). In a subsequent study, patients with mild atopic asthma received mepolizumab 750 mg ($n = 11$) or placebo ($n = 13$) monthly for 3 months. Mepolizumab reduced blood eosinophils by 100% and there was a median reduction...
in BALF eosinophils of 80%. On the other hand, there was a median reduction of only 55% in airway mucosal MBP+ eosinophils. There were no significant changes in FEV₁, or PEFR (peak expiratory flow rate), or in airway basophils, neutrophils, macrophages, mast cells or CD3+ cells (20).

In another study, patients with symptomatic asthma of moderate severity, taking ≤ 1,000 µg/day beclomethasone or equivalent, received mepolizumab 250 mg (n = 120) or 750 mg (n = 116) or placebo (n = 126) once a month, for 3 months (week 12), and were followed for an additional 2 months (week 20). There was a statistically significant reduction in blood and sputum eosinophil counts for the duration of the study, including follow-up; there were no changes in FEV₁, PEFR, symptom scores or use of rescue albuterol, compared to placebo. There was a nonsignificant trend (p = 0.065, chi-square test) towards a reduction in exacerbation rates, recorded at weeks 12 to 20, in patient receiving mepolizumab 750 mg (21).

Patients with severe, persistent asthma, received single i.v. infusions of placebo (n = 4) or reslizumab (0.03, n = 2; 0.1, n = 4; 0.3, n = 6; or 1.0 mg/kg, n = 12). Reslizumab dose-dependently reduced blood eosinophil counts. At 1.0 mg/kg the decrease remained significant for 30 days. There were no changes in clinical indices (22).

More recently, two studies have been completed in severe asthma (23) and eosinophilic bronchitis (including asthma; 24), respectively, that support a new role for the eosinophil in the susceptibility to acute exacerbations. The rationale for these studies came from studies suggesting an association between the control of eosinophils in the airway and a reduction in exacerbations (25, 26). The first was a randomized, double-blind, placebo-controlled parallel group of monthly i.v. mepolizumab (750 mg) or placebo for 12 months in 61 subjects with eosinophilic asthma despite high-dose inhaled and/or oral corticosteroids, and a history of recurrent severe exacerbations. The primary outcome was severe exacerbation frequency, defined as episodes of acute asthma requiring treatment with oral corticosteroids. Mepolizumab therapy was associated with a significant reduction in severe exacerbation frequency (2.0 vs. 3.4, relative risk 0.57, 95% confidence interval [CI] 0.32–0.92, p = 0.02) and an improvement in AQLQ (Asthma Quality Of Life Questionnaire) over 12 months of 0.55 (mean difference between groups 0.35, 95% CI 0.08–0.62; p = 0.02). Mepolizumab significantly lowered blood (p < 0.001) and sputum (p = 0.004) eosinophil counts. There was no difference between groups for symptoms, postbronchodilator FEV₁ or airway hyperresponsiveness. In a second smaller study in patients with persistent airway eosinophilia despite oral corticosteroids (including asthma and eosinophilic bronchitis without asthma), mepolizumab protected against exacerbations during steroid withdrawal (24). The very specific effect on exacerbations is intriguing and also contradicts preclinical data suggesting effects on other aspects of the asthma phenotype. It is not entirely clear how eosinophils might predispose to viral exacerbations, but one credible hypothesis supported by in vitro data is that they act as antigen-presenting cells for common respiratory viruses such as rhinovirus.

**Nasal polyposis**

Recent data also points to a role for anti-IL-5 therapy in patients with severe nasal polyposis refractory to steroids (27). Thirty subjects with grades 3–4 nasal polyposis were treated with two monthly i.v. injections of 705 mg mepolizumab (n = 20) or placebo (n = 10). Changes in nasal polyp score and in comparative nasal polyp score were assessed relative to baseline (week 0) at 1 and 2 months post last dose (week 8 and 12).

A significant reduction was observed in nasal polyp score with mepolizumab versus placebo at week 8 (60% vs. 10%, p = 0.011) and week 12 (65% vs. 20%, p = 0.025). Sixty-five percent of subjects on mepolizumab showed a “much better” or “better” comparative nasal polyp score compared to 10% on placebo by week 8 and 70% on mepolizumab versus 20% on placebo at week 12. Over 50% of subjects on mepolizumab demonstrated improvement on blinded assessment of CT scans by three observers at week 8 and the requirement for surgery was also reduced in the group on mepolizumab. It is as yet unknown whether a greater response can be achieved with a longer duration of treatment or whether there are specific individuals who might have a greater response to mepolizumab in this population.

**Atopic dermatitis**

In patients with atopic dermatitis who received two infusions of mepolizumab 750 mg (n = 20) or placebo (n = 23), with 1 week between doses, mepolizumab significantly reduced blood eosinophils versus placebo (p < 0.05). No significant change from baseline in dermal eosinophils was observed with mepolizumab versus placebo. No clinical success, as rated by Physician’s Global Assessment of Improvement (PGA), scoring atopic dermatitis (SCORAD) score, pruritus score, itch score, serum thymus and activation-regulated chemokine (TARC, an objective parameter of disease severity specific for atopic dermatitis) values, or the atopy patch test, was observed. Overall (when including modest or < 50% improvement), a significantly greater improvement in PGA lesion score was seen with mepolizumab versus placebo (72% vs. 42% improvement; p < 0.05) (28, 29).

**Hypereosinophilic syndromes**

One open-label study in four patients with eosinophilic esophagitis administered three mepolizumab i.v. infusions (750 mg/month for 3 months) showed a six-fold decrease in peripheral blood eosinophil levels (p < 0.05), a significant mean decrease in esophageal eosinophilia (from 46 to 6 eosinophils/hpf, p < 0.001), with patients reporting an improvement in clinical outcome and quality of life (30).

Treatment with a single dose of reslizumab in four patients with eosinophilic gastroenteritis significantly reduced both blood eosinophil counts (mean reductions of 70% and 83% at 24 and 48 hours, respectively) and gastrointestinal eosinophils (50–70% reduction in gastrointestinal eosinophils in the majority of patients (3 of 4 patients) (31). This response was sustained for 4 weeks, although at 7 to 8 weeks,
2 subjects had a rebound in their disease (blood eosinophil counts greater than pretreatment counts). Overall symptom scores were not improved. In a second study of 4 patients with eosinophilic gastroenteritis, treatment with a single dose of reslizumab reduced blood eosinophil counts (by \( \geq 30\% \)). Rebound eosinophilia was reported, with peak eosinophil counts occurring between 60 and 90 days posttreatment (32).

In an open-label trial in four patients with HES, mepolizumab improved several disease parameters including peripheral blood eosinophil counts and a variety of clinical manifestations (such as pruritus, skin lesions and low FEV\(_1\)), and quality-of-life measures. Based on these encouraging findings, a multicenter (26 sites worldwide), double-blind, randomized, placebo-controlled, parallel-group trial involving 85 HES patients 18–85 years of age was conducted. This study indicated that mepolizumab had a greater steroid-sparing effect than placebo and was significantly more effective than placebo (1 mg/kg i.v.) were also evaluated in patients with HES (37). In one exploratory study, two of the four patients treated with reslizumab responded with a reduction in peripheral eosinophil counts and an improvement in clinical symptoms. The response lasted for > 30 days, but was associated with a rebound effect (eosinophil counts elevated above baseline levels and an exacerbation of symptoms). This rebound in eosinophil levels appears to be caused by a serum factor (possibly IL-5) that enhances eosinophil survival (32). Since the initial improvement in eosinophil counts and clinical symptoms was promising, five additional doses (monthly doses of 1 mg/kg i.v.) were administered to the two responding patients. The extent of improvement observed decreased with each subsequent treatment. No anti-reslizumab antibodies were apparent.

Mepolizumab has been well tolerated in clinical studies at all doses and all routes of administration (i.v., s.c.). There were few serious adverse events (none considered drug-related), and few withdrawals due to adverse events. Mepolizumab, a humanized monoclonal antibody, did not induce antibody responses in patients with asthma (21). No dose-related trends in laboratory parameters (except eosinophil counts), vital signs or electrocardiograms were observed, and there were no reports of anaphylaxis or serum sickness. One death (cardiac arrest due to dysrhythmia and internal pacemaker/defibrillator failure) occurred 110 days after the first mepolizumab infusion (and 26 days after the last) in a patient with severe HES and a history of multiple cardiovascular comorbidities; this was not considered to be treatment-related (35).

Conclusions

Human clinical trial experience with mAbs directed against IL-5 in patients with asthma was not initially encouraging. However, more recently, new hypotheses generated by emerging data suggest anti-IL-5 may be beneficial in a range of indications including HES, asthma exacerbations and nasal polyposis, particularly when appropriately targeted to the right patient population. The history of this target also highlights the caution that must be applied to the predictive value of preclinical models (in which for example one would have predicted an effect on airway hyperresponsiveness not seen in clinical studies), and the need for biomarkers indicative of key biological processes in the lung for this and other new therapies.

References


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