

Therapeutic effects of antibodies to interleukin-5

Cuss FM. Therapeutic effects of antibodies to interleukin-5.
Allergy 1998; 53: 89–92. © Munksgaard 1998.

F. M. Cuss

Schering-Plough Research Institute,
Kenilworth, NJ, USA

Key words: allergy; asthma; interleukin-5;
monoclonal antibodies.

Dr F. M. Cuss
Schering-Plough Research Institute
2015 Galloping Hill Road
Kenilworth, NJ 07033
USA

There is considerable, albeit circumstantial, evidence for a role of interleukin-5 (IL-5) in asthma and other inflammatory disorders where eosinophils are prominent. An appreciation of the potential importance of IL-5 has arisen from the study of animal models, measurements of IL-5 in human and animal tissue, and an understanding of the essential role of IL-5 in the generation and maturation of eosinophils in the bone-marrow compartment. When stimulated to leave the bone-marrow, eosinophils accumulate in certain inflamed tissues including the lungs of patients with asthma, the nasal cavity of patients with allergic rhinitis, and the skin of patients with atopic dermatitis. Inhibition of the action of IL-5, by reducing its systemic concentrations (e.g., anti-IL-5 monoclonal antibody), blocking its association with its receptor, or inhibiting downstream cell signaling, could provide a novel therapy for eosinophilic diseases. In animal studies, anti-IL-5 monoclonal antibodies produce a significant reduction of markers of allergic inflammation and are now being assessed in clinical trials. Since the IL-5 receptor is expressed in man only on eosinophils and basophils, IL-5 inhibition could be expected to achieve therapeutic benefit without severe immunosuppression.

IL-5 biosynthesis and cell signaling

IL-5 is produced by several immune cells, including T cells, mast cells, and eosinophils. Allergic inflammation is associated with elevated expression of TH2 cytokines, specifically IL-4 and IL-5, in allergic diseases of the lungs, nose, skin, and gastrointestinal tract. In the lungs, for instance, the activation of CD4⁺ T cells and T-cell expression of IL-4 and IL-5, but not interferon-gamma (IFN- γ) mRNA, has

been demonstrated in bronchial biopsies and in bronchoalveolar lavage (BAL) fluid of both allergen-challenged atopic asthmatic (1) and nonatopic asthmatic patients (2). Increased levels of IL-5 are also seen in the BAL fluid of allergic subjects after segmental antigen challenge and are correlated with levels of eosinophil-derived proteins (3). The major source of IL-5 in allergic disorders is most likely to be T cells (4, 5), although immunohistochemical and *in situ* hybridization studies show that numerous cells, including mast cells and eosinophils, can synthesize IL-5 (6). Autocrine production of IL-5 by eosinophils may contribute to their own recruitment and activation, and to chronic eosinophil inflammation.

Strategies aimed at inhibiting IL-5 biosynthesis in allergic disease need to consider the contributions of different IL-5-producing cell types and the major mechanisms regulating IL-5 production in each cell type. Studies of cytokine synthesis in the presence of cyclosporin A or protein synthesis inhibitors indicate that cytokines are independently regulated. Glucocorticoids are potent inhibitors of IL-4 and IL-5 secretion, but are much less potent for IFN- γ secretion *in vitro* (7, 8). The mechanism for this differential efficacy of steroids for various cytokines is unknown but would have the effect, in the treatment of asthma, of reversing the exaggerated TH2 response (9, 10), including IL-5-induced eosinophilia, that contributes to asthma pathophysiology.

IL-5 exists as a glycosylated homodimer (45–60 kDa), with dimerization being required for full activity (11). IL-5 interacts with a two-chain receptor comprising a unique transmembrane α -subunit and a common transmembrane β -subunit shared by the IL-3 and GM-CSF receptors (12), creating a high-affinity ($K_d=300$ pM) receptor (13). The α -subunit

is restricted to human eosinophils and basophils; thus, IL-5 exerts limited function on human cells (14). The IL-5 receptor, together with the IL-3 and GM-CSF receptors, primarily activate JAK2 kinases in response to ligand binding with subsequent activation of STAT5, a member of a family of transcriptional regulatory proteins (15, 16).

IL-5 in asthma

Airway infiltration by eosinophils is consistently found after antigen administration in sensitive subjects (17, 18), and the most prominent cytokine seen in the BAL fluid of those subjects with late antigen responses is IL-5 (1, 3, 19). When recombinant human IL-5 is delivered as an aerosol to the airways of atopic subjects, eosinophilia is seen in bronchial biopsies and BAL fluid (20).

In patients with asthma, cells from BAL and bronchial biopsies show increased expression of IL-5 compared to samples from nonasthmatic subjects (5, 21). There is a strongly positive relationship between the number of BAL cells expressing IL-5 mRNA, the patient's baseline FEV₁, and the degree of airway responsiveness to methacholine challenge (22). During asthma exacerbations, serum concentrations of IL-5 are significantly higher than during remissions (23). They are also higher in symptomatic than asymptomatic patients (24) and in patients with severe asthma than in those without asthma or those with only mild asthma (23). The therapy of asthma with theophylline or corticosteroids is associated with lower IL-5 concentrations (5, 25, 26).

The pathophysiology of IL-5 has been extensively investigated in animal models of asthma. Pulmonary eosinophilia and airway hyperresponsiveness are induced by antigen challenge in sensitized mice (27, 28), guinea pigs (29, 30), and monkeys (31), and these effects can be blocked by treatment with monoclonal antibodies to IL-5 (32). IL-5 knockout mice have been used to determine the relevance of IL-5 and of eosinophils both in the generation of these responses and for protection against parasitic infections (28, 33). IL-5-deficient mice do not generate BAL eosinophilia or hyperreactivity after allergic challenge, while their normal counterparts do (28), and when the IL-5-gene is reconstituted the responses return. IL-5 deficient mice maintain baseline levels of circulating eosinophils, but do not mount an eosinophilic response to *M. corti* (a helminthic parasite) infection (33). The worm burden in IL-5-deficient mice and their normal counterparts is the same, suggesting that eosinophils are not involved in controlling the infection. IL-5-deficient mice are healthy and fertile and have normal circulating IgE levels as well as normal T- and B-cell function (33).

It is unlikely that IL-5 has a direct effect on airway function, because instillation of recombinant human or guinea pig IL-5 into normal guinea pig airways has no effect on resting airway function, bronchoconstrictor reactivity, or airway microvascular leakage induced by substance P (34). Expression of IL-5 in lung tissue is critical for the recruitment of eosinophils into the lungs during inflammatory conditions, and it may act together with chemokines such as eotaxin (35).

IL-5 is important in the differentiation of eosinophil precursors in the bone marrow (36). Administration of antigen into the lung results in release of several eosinophil-active cytokines, such as IL-3, GM-CSF, and possibly IL-5 into the circulation (37), and these probably initiate the mobilization of eosinophils out of the bone marrow into the blood which follows antigen challenge.

IL-5 in other allergic diseases

The nasal cavity and its lining are similar in some ways to the airways, and it is likely that IL-5 is also important in inducing the nasal eosinophilia observed in nasal allergies. Antigen challenge in patients with a history of allergic rhinitis causes an increase in IL-5 concentrations in the nasal lavage fluid, and in clinical allergic rhinitis, IL-5 concentrations are correlated with clinical responses (38). Patients treated with a topical corticosteroid during rhinitis show lower concentrations of IL-5 in nasal lavage fluid than those without such treatment (39). Eosinophils are seen prominently in nasal polyps (40), and IL-5 protein concentrations are increased in nasal polyp tissue compared with normal mucosa (41). IL-5 may also be important in atopic dermatitis and other cutaneous disorders that display eosinophilic inflammation such as episodic angioedema, urticaria, and IL-2 capillary leak syndrome (42, 43).

IL-5 production is seen in response to antigen administration in sensitive subjects whether the site is the lung, the nose, or the skin. In diseases where IL-5 is elevated, IL-5 concentrations and disease severity appear well correlated and drop as treatment is instituted and symptoms improve. Therefore, in allergic inflammation and other diseases where IL-5 is a prominent inflammatory mediator, its removal or blockade of its action may be therapeutically effective.

Therapeutic approaches to the inhibition of IL-5

The mechanisms which underlie IL-5 production, release, receptor activation, and cell signaling are theoretically targets for therapeutic intervention.

IL-5 biosynthesis is inhibited by glucocorticosteroids (8, 44) and to some extent by cyclosporin A (45), but the identification of other potent and selective inhibitors has proved difficult. IL-5, once secreted, can be neutralized with anti-IL-5 monoclonal antibodies or soluble IL-5 receptors (46). Selective inhibition of the effects of IL-5 can be achieved with these therapeutic modalities, and they can achieve a very long duration of action (31). An IL-5 receptor antagonist or inhibitors of specific tyrosine kinases or transcription factors induced by IL-5 should also have potential as selective inhibitors of allergic inflammation; however, as yet, there are no reports of such drugs reaching the clinic.

Despite the large number of possible targets for IL-5 pathway inhibition and the considerable efforts of academic groups and the pharmaceutical industry to discover small molecule inhibitors, the most likely therapeutic modality to provide the first clinical "proof of principle" is an anti-IL-5 monoclonal antibody. Monoclonal antibodies have a number of unique properties compared to other proteins. They are extremely selective, have a very high affinity for their target protein, and, if properly engineered, can be used in human therapeutics. Although requiring parenteral administration, recombinant monoclonal antibodies have half-lives of several days in man and therefore require only intermittent administration to maintain therapeutic levels. The administration of murine monoclonal antibodies has been associated with serious reactions such as serum sickness and anaphylaxis as a result of the generation of antimurine antibodies. However, re-engineering to reduce the "murine" content (e.g., by chimerization or humanization) or generation of fully human antibodies has produced proteins which are far less immunogenic. Indeed, severe systemic reactions are very rare when such antibodies are administered. Immune responses, if induced at all, are manifest instead by neutralization and clearance of the therapeutic monoclonal antibody, by shortening of the antibody half-life, and by reduction of the clinical effect. At present, there are many chimeric and humanized monoclonal antibodies in clinical trials for the treatment of several different diseases, and clinical data now being reported suggest that some will prove to be efficacious, safe, and practical.

Animal studies suggest that anti-IL-5 monoclonal antibodies will be safe and effective in allergic diseases, but confirmation of this awaits the results of clinical trials now underway.

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