

Review Article

Cytokine and anti-cytokine therapy for the treatment of asthma and allergic disease

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ABSTRACT

Asthma is a chronic inflammatory disorder of the airways superimposed upon structural changes that include an increase in smooth muscle and airway wall remodeling. In addition to a background of chronic mediator release, asthma is characterized by considerable variations in airway function brought about by important interactions with the environment, including allergen, pollutant and virus exposure. At least in mild–moderate disease, cytokines released from Th2 cells appear important in orchestrating the inflammation. The situation in more severe disease is complicated by the superimposition of a Th1 on top of a Th2 response. Until recently, the only controller treatment for chronic asthma has been corticosteroids. However, identification of specific effector molecules in asthma has led to targeting of specific pathways by using cytokines and cytokine inhibitors. Administration of a monoclonal blocking antibody against IgE has been shown to be highly efficacious in severe allergic asthma, but enhancement of Th1 responses or attempts to reduce eosinophils using anti-interleukin-5 monoclonal antibodies have no clinical benefit. In more severe asthma, blockade of tumor necrosis factor- α using the decoy etanercept has revealed efficacy in a small open study supporting the view that Th1, in addition to Th2, pathways are important as the disease adopts a more severe phenotype. Thus, like atopic dermatitis, it is likely that asthma is not a single disease, but a group of disorders that differ in

the relative contribution of specific pathophysiological pathways.

Key words: anti-interleukin-4, anti-interleukin-5, anti-tumor necrosis factor- α , asthma, cytokines.

INTRODUCTION

In 1860, Henry Hyde Salter, a physician at Charing Cross Hospital in London, described asthma as ‘. . . paroxysmal dyspnoea of a peculiar character with intervals of healthy respiration between attacks’.¹ In addition, he first drew our attention to the characteristic inflammation that infiltrates the airways in asthma and the wide range of environmental factors that could provoke attacks. It is clearly established that bronchial asthma is a disorder of the conducting airways that exhibit hyperresponsiveness to a wide variety of endogenous and exogenous stimuli, leading to variable airflow obstruction. The recognition that airway inflammation underlies at least a component of this hyperresponsiveness has received considerable support from the observation that inhaled and oral corticosteroids, which are highly efficacious in controlling asthma, also have a major impact on reducing the airway inflammatory response.² However, asthma differs from other chronic inflammatory disorders, such as rheumatoid arthritis, Crohn’s disease and psoriasis, in exhibiting a characteristic cytokine response dominated by Th2 cytokines, the majority of which are encoded in a small cluster on chromosome 5q32–34.³ It has been suggested that this coordinated regulation of the immune response in favor of Th2 cytokines, which include interleukin (IL)-3, IL-4, IL-5, IL-6, IL-9, IL-13 and granulocyte–macrophage colony stimulating factor (GM-CSF), results from a reduction in the inhibitory influence of Th1 cytokines,

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especially IL-18, IL-12 and interferon- γ , and, as a consequence, results in Th2 polarization of the immune response by default.⁴ This imbalance between Th1- and Th2-type immunity in those destined to become atopic manifests early in life, and possibly prenatally.⁵ A number of explanations has been put forward to explain this dysregulation in the immune response, but an interaction between susceptibility genes and fetal programming by the intrauterine environment appears to be important.^{6,7}

The Th2-type cytokines are especially associated with allergy and parasite immunity because of their actions on some specific cells known to be involved in the allergic inflammatory response.⁸ Of particular relevance is the role of IgE, which provides the initial trigger for many allergic responses. Maintenance of a persistent Th2 response requires the obligatory presence of IL-4, whereas switching of the lymphocytes from IgM to IgE synthesis requires IL-4 or IL-13 acting in concert with the adhesion molecule CD40 and its ligand, and is augmented by IL-6. Proliferation, maturation and differentiation of eosinophils requires IL-3, IL-5 and GM-CSF, whereas basophils will differentiate in the presence of IL-3 alone.⁹ Human mast cells are dependant upon the mast cell growth factor stem cell factor (SCF; c-kit ligand), which, in the presence of IL-6, IL-9 and transforming growth factor (TGF)- β , results in the full differentiation of mast cells containing the granule tetrameric enzyme tryptase.^{10,11} Finally, IL-4 is required to provide the mast cell with its full complement of cysteinyl and prostaglandin (PG) D₂-generating enzymes, as well as their receptors and the high-affinity receptor for IgE. Based on this network of interacting cytokines that orchestrate the allergic and asthmatic inflammatory responses, effort is now being directed at therapeutic applications to modify allergic disease.^{12,13} Encouragement for believing that a hierarchy of cytokines is involved in the pathogenesis of allergy and asthma has come from the use of cytokines or blocking antibodies or soluble receptors and gene-knock out strategies in mice and the clear efficacy of anti-tumor necrosis factor (TNF)- α in other complex inflammatory disorders, such as rheumatoid arthritis, inflammatory bowel disease, psoriasis and sarcoidosis.^{14–17} One argument against this approach has been the concept of cytokine redundancy. That is, if one cytokine is removed, another takes its place. Based on the experience with other chronic inflammatory disorders, it seems as if some hierarchy exists in the way cytokines interact in complex disease and, as a consequence, taking out cytokines high

up in the hierarchy should be clinically beneficial. The recent promising results of the cytokine T lymphocyte-associated (CTLA)-4 fusion protein in interrupting T cell responses in rheumatoid arthritis by depriving CD80/86 on antigen-presenting cells from interacting with CD28 on T lymphocytes places the T cell high up the hierarchy of cells involved in this disease.¹⁸ Our own work,^{3,19} showing a sentinel role of CD28 on T cells and CD80/86 on antigen-presenting cells in maintaining Th2 responses in allergic asthma creates a real opportunity for the CTLA-4 fusion protein that interrupts this signaling as a new therapeutic for chronic asthma.

USE OF BIOLOGICALS IN THE TREATMENT OF ALLERGY AND ASTHMA

The first novel biological agent that has been introduced for the treatment of asthma and food allergy is a humanized monoclonal antibody targeted to the C_ε3-domain on the heavy chain of human IgE.²⁰ The C_ε3 epitope is involved in the binding of IgE to both the α -chain of the high-affinity receptor Fc ϵ R1 and the low-affinity receptor Fc ϵ R2 (CD23).²¹ Administration of anti-IgE directed to the C_ε3 domain effectively depletes circulating and tissue levels of IgE without cross-linking IgE on the surface of mast cells or basophils.²² Omalizumab, the humanized anti-IgE monoclonal antibody that has now entered the clinic, effectively blocks both the mast cell-dependant early and the inflammatory cell-dependant late asthmatic reaction provoked by inhaled allergen in parallel with a reduction in the influx of eosinophils into the airway lumen.²³ Phase 3 clinical trials in adults and children with allergic asthma have shown clinical efficacy of omalizumab (administered by subcutaneous injection 2–4 weeks) beyond that achieved with inhaled or oral corticosteroids.^{24,25} Another anti-IgE (TNX-901) has also been shown to protect against peanut-induced anaphylaxis.²⁶ Omalizumab is also efficacious in patients with allergic rhinitis and synergises with antigen-specific immunotherapy in the prophylaxis of severe allergic rhinitis (R Djukanovic *et al.*, unpubl. obs., 2004). Regular treatment with omalizumab administered subcutaneously at 2–4 week intervals over 16–32 weeks results in a > 95% reduction in circulating eosinophils, a 70% reduction in sputum eosinophils and an 80% reduction in airway tissue eosinophils in patients with asthma, which is paralleled by loss of IgE and Fc ϵ R1 receptors from mast cells, basophils and dendritic cells in airway tissue. This strongly suggests that a

significant component of the eosinophilia in allergic asthma is dependant upon IgE and that the long-term benefit of anti-IgE therapy may be mediated through loss of the ability for effector cells to bind IgE in addition to depletion of IgE itself.

EFFECT OF TH1 CYTOKINES ON ASTHMA

The inhibitory effect of IFN- γ on Th2 responses *in vitro* and in animal models suggests that it may be of use in the treatment of allergic disease. However, when administered by intravenous injection, IFN- γ had no significant effect on airway eosinophils and clinical responses in patients with corticosteroid-dependant asthma.²⁷ Mouse-recombinant (mr) IL-12 administered intraperitoneally to ovalbumen-sensitized and inhalation-challenged mice resulted in a marked reduction in the influx of eosinophils into the airway lumen, compatible with its modifying effect via production of IFN- γ acting to inhibit Th2 responses.²⁸ Human-recombinant (h) IL-12 administered on four separate occasions to a group of allergic asthmatic patients produced a progressive overall reduction in circulating numbers of eosinophils, but this inhibitory effect lasted only 4–7 days after each injection.²⁹ After four injections of incremental doses of hrIL-12 over a period of 22 days, the circulating eosinophil count fell to a nadir of 85% of the starting baseline and was accompanied by a 50% reduction in sputum eosinophils following allergen provocation. However, when compared with placebo, hrIL-12 failed to influence either the early or late allergen-provoked asthmatic reactions or the accompanying increase in bronchial hyperresponsiveness. Intravenous hrIL-12 was associated with a number of side-effects, including fever, a general feeling of malaise and, in some subjects, cardiac arrhythmia, which almost certainly will preclude its use as a future therapeutic.²⁹

TARGETING IL-5

Interleukin-5 is a Th2 cytokine that is essential for the formation and priming of eosinophils for mediator secretion and the level of this cytokine has been shown to increase in patients with active asthma.³⁰ In patients with asthma, IL-5 mRNA expression is increased both in bronchial biopsies and in bronchoalveolar lavage (BAL) cells, and increased release of IL-5 protein has been detected in airway tissue, BAL and sputum following allergen challenge of sensitized asthmatic patients.⁹

Inhalation of IL-5 is associated with a sputum eosinophilia and increased airway non-specific responsiveness. In mice whose gene for IL-5 had been deleted, allergen sensitization and challenge failed to elicit eosinophilia and, in some but not all experimental models, this was accompanied by blockade of airway non-specific hyperresponsiveness (AHR).³⁰ In naturally *Ascaris suum*-sensitized non-human primates, 6 months treatment with a blocking anti-IL-5 monoclonal antibody (TRFK-5) resulted in a marked reduction in AHR to histamine, with an effect being apparent as early as 24 h after the first anti-IL-5 injection. Based on this and a large number of animal studies, a humanized monoclonal antibody against IL-5 has been generated (SB240563; mepolizumab) that was a fully humanized IgG1 kappa antibody expressed in Chinese hamster ovary (CHO) cells.³¹ Mepolizumab exhibited high-affinity binding to human IL-5 ($K_d = 4.2$ pmol/L), inhibited the binding of IL-5 to the IL-5 receptor α chain and profoundly suppressed human eosinophil differentiation, the proliferative response of a human IL-5 receptor-expressing cell-line and neutralized the proeosinophilic activity of hrIL-5 in macaque monkeys as well as the IL-5-dependent eosinophilia.³¹ When administered at a dose of either 2.5 or 10 mg/kg as a single intravenous injection to mildly asthmatic subjects, mepolizumab produced a profound and long-lasting reduction in circulating eosinophil numbers to almost undetectable levels, with the high dose exhibiting efficacy on eosinophils lasting up to 16 weeks after a single injection.³¹ In those who had received placebo, inhaled allergen provocation caused an increase in circulating eosinophils over a period of 7 days; however, this proeosinophilic effect of allergen challenge was totally inhibited by anti-IL-5 treatment. Following allergen challenge, anti-IL-5 treatment at the 10 mg/kg dose reduced sputum eosinophils by > 70%, which remained at this reduced level up to day 30 post injection. Despite these changes in circulating and airway luminal eosinophils, the early and late asthmatic reaction to allergen provocation was not affected by mepolizumab at either dose, nor was there any change in baseline or post allergen challenge non-specific AHR.³¹

Because allergen provocation represents only part of the asthmatic phenotype, the effect of anti-IL-5 treatment on clinical asthma has been investigated. In one small study³² involving patients with severe asthma, the anti-IL-5 humanized monoclonal antibody SCH 55700 was investigated. This anti-IL-5, like mepolizumab, was efficacious

in a non-human primate model of allergic airway inflammation. When administered to patients with severe asthma, SCH 55700 was shown to have no significant effect on lung function or other clinical end-points of asthma control, despite again producing a profound reduction in circulating eosinophil numbers. Of interest, however, was a significant effect of anti-IL-5 on asthma exacerbations. A second, larger clinical study has been conducted on 362 patients, using the anti-IL-5 monoclonal antibody mepolizumab, which was administered by subcutaneous injection on three occasions, 4 weeks apart, at a dose of 250 mg (120 patients) or 750 mg (116 patients) and compared with placebo (126 patients; P Flood-Page *et al.*, unpubl. obs., 2004). The asthmatic patients in this study had a mean baseline forced expiratory volume in 1 s (FEV₁) of 2.5 L (68% predicted) with 25% reversibility and remained symptomatic, despite the use of over 700 µg beclomethasone dipropionate (BDP) and an average of four puffs per day of the short-acting α_2 -adrenergic receptor agonist salbutamol. Over 85% of patients were atopic and had elevated serum total IgE levels. At both the high and low dose, mepolizumab produced a profound reduction in circulating eosinophils of > 90%, which was paralleled by a similar dose-dependant reduction in sputum eosinophils. As observed in the earlier studies, the effect of mepolizumab on circulating sputum eosinophils was long lasting, with little change in eosinophil numbers 12 weeks after the last dose of mepolizumab. However, despite these marked reductions in circulating and airway lumen eosinophils, anti-IL-5 treatment had no effect compared with placebo in a range of clinical asthma end-points, including symptoms, lung-function and α_2 -adrenergic receptor agonist use. However, again at the high dose, there was a suggestion anti-IL-5 reduced the number of severe asthma exacerbations but, in this study (P Flood-Page *et al.*, unpubl. obs., 2004), which was not powered for this end-point, this failed to reach statistical significance, possibly due to the small number of exacerbations that occurred during the trial period.

To seek an explanation for the lack of efficacy of IL-5 on clinical outcome measures of asthma, Flood-Page *et al.*³³ conducted a study of mepolizumab in mild asthmatic patients who underwent endobronchial and bone marrow biopsies. Airway eosinophils and eosinophil granule major basic protein (MBP) staining was determined in bronchial biopsies and in marrow aspirates. Physiological indices, including FEV₁, methacholine responsiveness (measured as the PC₂₀ methacholine) and

eosinophils in blood and sputum, were followed over 12 weeks. The anti-IL-5 treatment had an almost identical effect on circulating and sputum eosinophils, as was observed in previous studies, but, at week 20 with active treatment, only reduced the tissue eosinophil numbers by 55% and bone marrow eosinophils by 52%. There was no significant effect on FEV₁, bronchial hyperresponsiveness or other clinical asthma outcome measures. A further extension of this biopsy study³⁶ has shown that, despite incomplete depletion of airway tissue of eosinophils, the anti-IL-5 therapy had profound effects in reducing staining of the lamina reticularis for tenascin-c, perlecan and lumican, proteoglycans known to contribute towards airway remodeling in chronic asthma.^{35,36} One possible explanation for the positive effect of anti-IL-5 in reducing immunostaining for matrix molecules in the biopsies is the capacity of IL-5 to drive epithelial and fibroblast responses independently of eosinophils. Whatever the explanation for these observations, it is of concern that a substantial decrement of eosinophils in airway tissue of asthmatics comparable to that achieved with inhaled and oral corticosteroids had no effect discernable on outcome measures of chronic asthma, including airway hyperresponsiveness.³⁷

It is clear that further clinical studies need to be performed with highly selective agents that deplete the airways of eosinophils before any final conclusions can be drawn about the role of this cell in asthma. In this regard, a recent bronchial biopsy study using humanized anti-IgE (omalizumab) administered to mild asthmatic patients similar to those used in the anti-IL-5 biopsy studies, resulted in an 80% reduction in both sputum and tissue eosinophils, but without any significant improvement in bronchial hyperresponsiveness. This further questions the previous widely held view that eosinophils are fundamental to the pathogenesis of chronic bronchial hyperresponsiveness in asthma. More recently, airway eosinophils have been attributed to playing a greater role in the pathogenesis of asthma exacerbations and also cough-variant asthma (eosinophilic bronchitis).^{38,39} However, the previously accepted role of the eosinophil in maintaining the chronic disordered airway function characteristic of asthma is now being questioned. It has been suggested that, in addition to IL-5, activation of the CCR3 receptor on eosinophils by chemokines such as eotaxins, RANTES and monocyte/macrophage chemotactic peptide (MCP) 3 may be required to draw eosinophils into the lung and that antagonism of both IL-5 and CCR3 receptors maybe necessary to totally deplete the airways of

these cells.³⁷ The CCR3 antagonists are in clinical development, as are blocking antibodies against eotaxin. Their clinical evaluation in asthma is eagerly awaited.

INTERLEUKIN-4

Interleukin-4 and its homolog IL-13, which shares some but not all of its effects, are major therapeutic targets in asthma on account of their actions on T cells, B cells and on structural elements in the airway, including epithelial cells promoting the production of goblet cells and fibroblasts in augmenting airway remodeling.⁴⁰ Interleukin-4 also stabilizes the expression of vascular cell adhesion molecule (VCAM)-1, which is involved in the recruitment of eosinophils. The soluble IL-4 receptor α (sIL-4R α) acts as a natural IL-4 antagonist.⁴¹ In two small studies, inhalation of a single dose of recombinant human sIL-4R (altrakincept) has been shown to be significantly more effective than placebo at inhibiting the decline of baseline lung function and reducing the deterioration in asthma symptoms in patients with moderate–severe disease when this treatment was administered over a period of 2 weeks.^{42,43} Twelve, once-weekly inhalations of sIL-4R was safe and more effective than placebo in preventing deterioration of asthma when glucocorticosteroids were discontinued. However, in two, so far unpublished, large phase III clinical trials in moderate asthma, altrakincept failed to show efficacy and, as a consequence, altrakincept has not been further developed. Part of the difficulty with this recombinant human protein was its rapid proteolytic degradation in the airways and a lack of understanding of the dose needed to optimally neutralize IL-4 in the airways.

A mouse soluble IL-4 mutant has been shown to be an antagonist for both IL-4 and IL-13 *in vitro* via the formation of an unproductive complex with IL-4R α .⁴⁴ Treatment of mice with this antagonist completely inhibited the humoral immune response to allergen and the subsequent development of disordered lung function on allergen challenge.⁴⁴ Similarly, BAY 16–9996 administered subcutaneously, as a human IL-4 double mutein, was effective in reducing airway inflammation and bronchial hyperresponsiveness in a primate model of asthma.⁴⁵ This human recombinant protein was safe and well tolerated in mild to moderate asthmatic subjects and further clinical trials are in progress. Human and humanized blocking IL-4 monoclonal antibodies are also currently in clinical development.

Interleukin-13 has recently assumed considerable prominence as a major cytokine involved in airway inflammation and remodeling in asthma. The IL-13 α 2 receptor has a much higher affinity for IL-13 than its α 1 counterpart, which is responsible for the intracellular signaling effects of IL-13.⁴⁶ When administered to mice prior to sensitization, soluble IL-13 α 2 has a profound effect in modifying the airway inflammatory and remodeling responses with repeated aerosol antigen challenge.⁴⁷ Interleukin-13 remains a highly relevant new target for the treatment of chronic severe asthma, using recombinant human IL-13 α 2 receptor as a molecule decoy or blocking monoclonal antibodies.⁴¹ Both approaches are in clinical trial.

ANTI-TUMOR NECROSIS FACTOR- α

Tumor necrosis factor- α is a new therapeutic target in chronic asthma. The expression of TNF- α is increased in asthmatic airways in proportion to disease severity and inhalation of recombinant TNF- α enhances bronchial hyperresponsiveness and sputum neutrophilia in normal subjects.^{48,49} In patients who have died from asthma, TNF- α expression is increased at both the protein and mRNA levels in a wide number of inflammatory cells, including mast cells, eosinophils, T cells and macrophages. Tumor necrosis factor- α receptors are found on airway monocytes, macrophages, lymphocytes and granulocytes, as well as on mast cells. We have recently shown that TNF- α is able to enhance the release of inflammatory mediators from human lung mast cells through an autocrine mechanism involving the activation of the transcription factor nuclear factor- κ B.⁵⁰ Genetic studies have also revealed that the –308 promoter polymorphism of TNF- α is associated with asthma and its severity,⁵¹ although it is not clear whether this single nucleotide polymorphism is in linkage disequilibrium with the adjacent gene TNF- α .⁵²

It has also been shown that the bronchoconstrictor response to some bronchoconstrictor stimuli (e.g. SO₂ or O₃) is enhanced in subjects positive for the TNF- α –308 allele.⁵³ A recent description of anti-TNF- α therapy using infliximab for rheumatoid arthritis in patients with concomitant severe asthma or chronic obstructive pulmonary disease revealed a marked improvement in the lung disorders in parallel with improvement in the rheumatoid arthritis.⁵⁴ On this basis, we have undertaken a small open study involving 15 patients with severe asthma requiring a mean dose of inhaled corticosteroid

equivalent to 2500 µg/day BDP, a mean daily oral dose of prednisolone 12.1 mg/day, long-acting inhaled α_2 -adrenergic receptor agonists and regular nebulized salbutamol.⁵⁵ After a baseline period, patients were entered into a therapeutic trial of etanercept, a fusion protein consisting of the extracellular ligand-binding portion of the high-affinity human 75 kDa TNF- α receptor linked to the fc-portion of human IgG1. Etanercept serves as a decoy to inhibit both the binding of free TNF- α and TNF- α to cell-associated TNF receptors, thereby rendering extracellular TNF biologically inactive. Subjects received 25 mg etanercept subcutaneously twice a week for a period of 12 weeks and conventional outcome measures of asthma were followed. At the end of the 12 week period, there was a significant increase in both FEV₁ and forced vital capacity in all but one subject and this was paralleled by a sevenfold reduction in the area under the methacholine dose-response curve and a halving of the mean symptom score based on the asthma control questionnaire developed by Juniper *et al.*⁵⁶ These impressive results have stimulated a placebo-control trial to investigate anti-TNF therapy in chronic severe asthma, which is now ongoing. What this small study has shown is that, at the severe end of the disease spectrum, blockade of a 'Th1' cytokine in asthma is highly efficacious and suggests that viewing this disease simply as a Th2 inflammatory response is inadequate, especially as the disease becomes chronic and severe.^{57,58} What is now needed are new studies to investigate the pathophysiology of severe asthma compared with mild or controlled disease. Interventions that are targeted specifically to severe chronic asthma may also include a range of pleomorphic cytokines, such as IL-1 and IL-6, which are known to also be involved in inflammatory joint, bowel and skin disease.

CONCLUDING COMMENTS

Based on the observations so far made with biological agents, which target single molecules in asthma, it is highly likely that asthma is not a single entity, but a series of separate subphenotypes in which individual effector molecules play a dominant role. Examples of this in asthma already exist in sharing the greater efficacy of antileukotrine therapy in patients with aspirin-intolerant asthma, the use of antihuman allergy IgE to treat severe allergic asthma and, based on the small study described above, the potential impact that anti-TNF therapy may

have in patients with chronic corticosteroid refractory disease. A key target for the future would be the identification of biomarkers that reflect these disease subtypes so that anti-asthma therapy can be tailored to the individual patient's need.

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