

Cytokine-directed therapies for asthma

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Increasing knowledge of the pathophysiologic roles of various cytokines in atopic diseases has provided the basis for the development of novel therapies. Strategic approaches for cytokine inhibition include the blocking of transcription factors that lead to their expression, blockade after their release, cytokine receptor antagonism, and the inhibition of signaling pathways that are activated after cytokine-receptor binding. The proinflammatory cytokines IL-5, IL-4, IL-13, and TNF- α are among the therapeutic targets. Results with a humanized anti-IL-5 have been disappointing. Although successful in markedly reducing circulating eosinophils and in preventing eosinophil accumulation in airways, the humanized anti-IL-5 was unable to affect early or late responses to allergen or to reduce airway reactivity to methacholine challenge in patients with asthma. On the other hand, a soluble IL-4 receptor antagonist has shown clinical benefits for patients with moderate asthma who require daily inhaled corticosteroids. Agents that target IL-13 and TNF- α remain to be evaluated in asthmatic inflammation. The use of cytokines with anti-inflammatory effects may also have therapeutic value. The evaluation of such agents in human beings, including IL-10, IL-12, and IFN- γ , is at a preliminary stage, but so far results have not been encouraging. (*J Allergy Clin Immunol* 2001;108:S72-6.)

Key words: IL-5, asthma, eosinophil, IL-4, cytokine

Multiple cytokines play a role in atopic disease, and increasing knowledge in this area over the last decade has provided the basis for a number of novel therapies.^{1,2} Recent research into the therapeutic potential of inhibiting proinflammatory cytokines in allergic asthma, especially IL-5, is presented here. The use as potential therapies of some cytokines that suppress the inflammatory process in allergy is also briefly reviewed.

STRATEGIC APPROACHES

Several strategies for inhibiting proinflammatory cytokines have been considered. At the most basic level, cytokine synthesis can be inhibited by blocking the transcription factors that lead to the expression of those cytokines. In this context, the potential of nuclear factor- κ B as a target has received considerable attention because it regulates the expression of multiple cytokines

Abbreviations used

COX-2: Insoluble cyclooxygenase
GM-CSF: Granulocyte-macrophage colony-stimulating factor
iNOS: Insoluble nitric oxide synthase
PC₂₀: The provocative concentration of methacholine needed to cause a 20% decrease in FEV₁
TNF: Tumor necrosis factor

involved in asthma. Alternatively, an antisense oligonucleotide can be used to "mop up" the messenger RNA, thus preventing the protein from being produced. Or, synthesis can be blocked in some other way, which is probably the way glucocorticoids operate.

At the next level, cytokines can be inhibited after their release from the cells. This is the approach adopted in cytokine blockade, including the use of humanized antibodies that bind to, and thereby inactivate, cytokines or their receptors and the use of soluble receptors to bind with high affinity to secreted cytokines. Another approach is to use receptor antagonists, but this has proved to be exceptionally problematic because cytokine receptors are extremely complex and difficult to inhibit with small molecules because they interact over large areas.

Another approach is to develop drugs that block the signal transduction pathways with inhibitors of kinases activated by cytokine receptors or to inhibit the transcription factors involved. This is also a difficult approach because, although some exceptions do exist, the signal transduction mechanisms are usually shared by many cytokines, with the specificity provided by the receptor.

With regard to using cytokines that suppress allergic inflammation, the fact that patients with asthma may be deficient in some of those cytokines represents a promising avenue for the exploration of therapies of greater specificity. That they are not bioavailable via oral administration, however, is a problem that would have to be overcome.

INHIBITING PROINFLAMMATORY CYTOKINES

Interleukin-5

IL-5 plays a key role in the airway inflammation of asthma. It is essential for the production, maturation, activation, and survival of eosinophils and is also important in eosinophil chemoattraction.³ The central role of IL-5 among cytokines in the development of eosinophilia has been demonstrated in many *in vivo* models. Pretreatment with anti-IL-5 monoclonal antibodies has been shown to suppress allergen-induced airway eosinophilia in guinea pigs and monkeys.^{4,5} In a cynomolgus monkey model of allergic asthma, only small increases in the

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number of eosinophils were found in the bronchoalveolar lavage after antigen challenge when the animals were pretreated with an IL-5 monoclonal antibody, and no increase in airway reactivity was observed.⁵ This blocking effect continued for up to 3 months after a single injection, making treatment of chronic asthma with such a therapy a feasible goal in human beings. Importantly, the fact that IL-5 knockout mice are normal in both behavior and survival is encouraging because it suggests that inhibiting IL-5 would not be dangerous.³

In human beings, IL-5 introduced into the airways of patients with asthma induces significant airway eosinophilia,⁶ and inhaled IL-5 causes eosinophilia-induced sputum and bronchial hyperresponsiveness.⁷ Considering these effects, the blocking of IL-5 presents a logical strategy for asthma therapy. Humanized monoclonal antibodies to IL-5 have been developed, and a double-blind placebo-controlled study has shown that a single injection can reduce blood eosinophils for several weeks and prevent eosinophil recruitment into the airways after allergen challenge.⁸ This placebo-controlled study investigated the effects of 2 doses of humanized anti-IL-5 (2.5 mg/kg and 10 mg/kg)⁸ in mildly asthmatic patients over a longer period, during which there were 2 allergen challenges. At the lower dose, blood eosinophils were reduced but began to recover around day 30. The higher dose, however, induced a sharper reduction of eosinophils to levels below normal, where they remained for the entire 16-week period. A similar decrease was seen in sputum eosinophils after allergen challenge in the antibody-treated patients, and they rose as expected in patients receiving placebo. This indicates that the antibody performs consistently in animals and in human beings in preventing eosinophils from accumulating in the airways after allergen challenge. No effect, however, was seen on either the early or the late response to inhaled allergen. Moreover, IL-5 antibody treatment failed to reduce airway reactivity to methacholine challenge in these patients.

The employment of monoclonal antibodies to inhibit IL-5 appears to be the most feasible strategy. IL-5 receptor antagonists have proved difficult to develop commercially; blocking the signal transduction pathways is difficult because no unique pathway exists for the IL-5 receptor.

Interleukin-4

IL-4 presents a promising target for asthma therapy because it is critical for the synthesis of IgE by B lymphocytes as well as for the differentiation of T_H2 cells. Among its more important effects in allergic asthma, IL-4 promotes both the growth and the recruitment of eosinophils to the airways by up-regulating endothelial vascular-cell adhesion molecule expression. Animal studies have demonstrated that anti-IL-4 antibodies ablate allergic airway hyperresponsiveness, a feature that has not been demonstrated with anti-IL-5 antibodies.⁹

Humanized IL-4 antibodies are currently in development, but the approach that has been the most developed is the use of soluble receptor antagonists. As demonstrat-

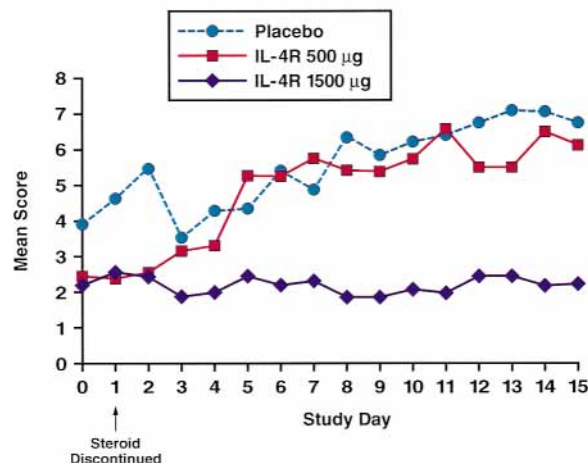


FIG 1. Total asthma symptom score. The total asthma symptom score remained stable through week 2 in the IL-4R 1500 µg group (solid diamonds), but increased in both the placebo group (solid circles) and in the IL-4R 500 µg group (solid squares) (1500 µg vs placebo, $P = .048$; 1500 µg vs 500 µg, $P = .008$), despite abrupt withdrawal of corticosteroid requirements. Day 0 equals baseline in an average of prestudy values. (Adapted with permission from Borish LC, Nelson HS, Lanz MJ, et al. Interleukin-4 receptor in moderate atopic asthma: a phase I/II randomized placebo-controlled trial. *Am J Respir Crit Care Med* 1999;160:1816-1823. Official Journal of the American Thoracic Society. © American Lung Association.)

ed by Borish et al¹⁰ on allergens, IL-4 receptor antagonists administered by nebulizer have clinical benefit for patients with moderate asthma who require daily inhaled corticosteroids. Patients who received a single dose of soluble IL-4R maintained stable symptom scores after the abrupt withdrawal of inhaled corticosteroids (Fig 1).¹⁰ A particular advantage of this preparation is that the effects of a single inhalation appear to persist for a week.

The use of a signal transduction blocker is also an attractive approach because IL-4 uses a unique transcription factor, STAT-6, and the deletion of the STAT-6 gene produces results similar to those of IL-4 gene knockout.¹¹ However, development of such a drug would be difficult. A potent endogenous inhibitor of IL-4 signaling pathways, suppressor of cytokine signaling, has been recently described and represents a new therapeutic target.¹²

Interleukin-13

IL-13, a cytokine with marked similarities to IL-4, is closely linked with IgE production and is a potent inducer of eotaxin secretion from the airway epithelial cells.¹³ In mice, it can induce many of the features of asthma, including airway hyperresponsiveness and mucus secretion, independently of eosinophilic inflammation.¹⁴ Studies by the Wills-Karp group have demonstrated that these features can be ablated by a high-affinity soluble fusion protein that binds to and neutralizes IL-13 without inhibiting IL-4.¹⁴ This soluble receptor antagonist is cur-

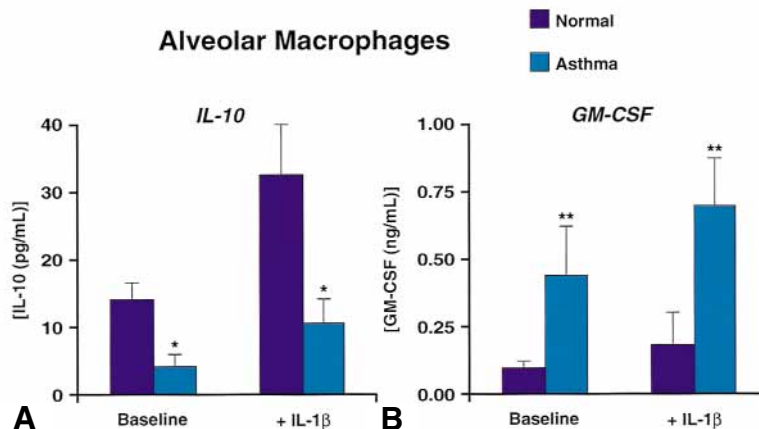


FIG 2. Reduced production of IL-10. **A**, IL-10 protein concentration from alveolar macrophages at baseline and after stimulation with IL-1 β . Data are shown as mean $\pm$ SEM. For GM-CSF (**B**), the levels of release from asthmatic subjects were higher than those from normal subjects at baseline and after stimulation with IL-1 β . * $P < .05$, ** $P < .01$. (Adapted with permission from John M, Lim S, Seybold J, et al. Inhaled corticosteroids increase interleukins-10 but reduce macrophage inflammatory protein-1 α , granulocyte-macrophage colony-stimulating factor, and interferon-gamma release from alveolar macrophages in asthma. *Am J Respir Crit Care Med* 1998;157:256-62. Official Journal of the American Thoracic Society. © American Lung Association.)

rently under development and is expected to go into clinical studies. In addition, STAT-6 inhibitors would inhibit IL-13 as well as IL-4 since they signal through the same transduction pathway.

Tumor necrosis factor- α

TNF- α may have an important amplifying effect on asthmatic inflammation. TNF- α production is increased in asthmatic airways, and because it amplifies the expression of many of the inflammatory genes that are overexpressed in asthma, it may affect disease severity. Moreover, when inhaled by normal subjects, TNF- α has been shown to induce airway hyperresponsiveness.¹⁵

TNF- α monoclonal antibodies (infliximab) are already being used as treatment for inflammatory bowel disease and rheumatoid arthritis, for which they are effective, even in cases of severe corticosteroid-resistant disease. A soluble TNF receptor (etanercept) is also available for treating rheumatoid arthritis, and is as effective as the TNF- α monoclonal antibody.¹⁶ Small molecules that can inhibit TNF would be more desirable. These include TNF- α -converting enzyme inhibitors known as TACE inhibitors. Phosphodiesterase-4 and p38 mitogen-activated protein kinase inhibitors are also extremely potent inhibitors of TNF- α production, but they are not specific and they inhibit multiple cytokines.

CYTOKINES WITH ANTI-ALLERGIC INFLAMMATION PROPERTIES

Interleukin-10

IL-10 has unique functional effects, a number of which are relevant to asthma. It inhibits the production of several proinflammatory cytokines such as IL-1 β , TNF-

α , and GM-CSF, as well as chemokines, particularly eotaxin and RANTES, that could be important for eosinophil recruitment.¹⁷ It also inhibits the inflammatory enzymes, such as insoluble nitric oxide synthase (iNOS) and insoluble cyclooxygenase (COX-2), potentially blocks IL-5, inhibits T_H2 cytokines, and reduces allergen responses by acting at several points in the allergen antigen presentation. Finally, it boosts anti-inflammatory mediators like IL-1 receptor antagonists and tissue inhibitors of matrix metalloproteinase, which can counteract matrix metalloproteinases.

Another facet of IL-10 that is of considerable interest concerns the evidence that IL-10 production in patients with asthma is defective.¹⁸ We have studied alveolar macrophage production of IL-10 in patients with mild asthma and found that it was significantly reduced, as compared with normal control subjects, both at baseline and after stimulation by IL-1 β , as a result of decreased gene expression.¹⁹ A defect in IL-10 production in patients with asthma may also explain why macrophages from asthmatic patients produce increased levels of inflammatory cytokines. GM-CSF production is greater at baseline and after stimulated production with IL-1 β in asthmatic patients, which may plausibly be the result of the reduced presence of IL-10 to inhibit it (Fig 2).

It is hypothesized that the early response occurs when the macrophage, which is the main source of IL-10 in the airway (rather than the T cell), is affected by inflammatory stimuli, and also through the activation of transcription factors such as nuclear factor- κ B, and switches on the genes of inflammatory proteins, including iNOS, COX-2, GM-CSF, and RANTES—all of which are increased in asthmatic patients. In the late response, occurring some hours after the initial inflammatory burst, IL-10 is produced, which then switches off the inflammatory response. In patients

with asthma, however, this switch is defective, and they therefore experience greater levels of inflammation.

There is, in fact, a correlation between IL-10 deficiency and asthma severity, and it can be countered with treatment. Corticosteroids—and probably also theophylline and phosphodiesterase-4 inhibitors—increase IL-10, and that may be one of the mechanisms by which these agents suppress inflammation.

If patients with asthma have a defect in IL-10, treating them with IL-10 to restore the balance is a logical approach. Animal studies have demonstrated that IL-10 can inhibit allergic responses.²⁰ Moreover, recombinant human IL-10 has proved to be effective in the control of inflammatory bowel disease, in which similar cytokines are expressed.²¹ Future drugs may be developed that increase endogenous production of IL-10 or that activate the signal transduction pathways activated by the IL-10 receptor.

Interleukin-12

Considerable research interest has focused on the IL-12 cytokine because of its importance in determining the T_H1 phenotype while suppressing T_H2 cells, an effect that is largely mediated by the release of IFN- γ . The result of the suppression of T_H2 cells is the inhibition of eosinophilic inflammation and IgE production. Researchers in The Netherlands reported that there was a defect in the production of both IL-12 and IFN- γ in circulating cells of humans with asthma.²²

In mice, the administration of IL-12 was shown to blunt allergen-induced inflammation and airway hyperresponsiveness.²³ However, in early studies of the effect of recombinant human IL-12 in patients with advanced renal carcinoma, severe toxicities were produced, including the deaths of 2 patients as a result of unexpected cardiac arrhythmia.²⁴ It was subsequently found that gradual dose escalation diminished these effects. Consequently, that approach was used in a placebo-controlled study in which patients received progressively greater doses of IL-12 after allergen challenge.²⁵ Eosinophil levels declined in a dose-related fashion, but then recovered when the IL-12 was discontinued. No effect, however, was observed on airway hyperresponsiveness, on the early or late response to allergen, or on PC₂₀. Moreover, although IL-12 reduced eosinophils after allergen challenge, it was not as effective as was the IL-5 antibody in completely preventing sputum eosinophilia. Finally, every patient who received IL-12 experienced side effects, mainly a flu-like illness. Such side effects are almost certainly caused by IFN- γ release.

Although IL-12 is thus not a likely candidate for the treatment of asthma, an interesting study by Kim et al explored the results of giving IL-12 linked with allergen as a fusion protein, ovalbumin-IL-12.²⁶ The rationale for constructing a fusion protein was to minimize nonspecific responses, driving immune responses in an ovalbumin-specific manner. A specific reduction in IgE directed to ovalbumin was, in fact, produced and was associated with a specific increase in the production of IgG-2a, an immunoglobulin that may underlie the protective effect of this immunotherapy.

Thus, although it is clear that IL-12 is not as effective as IL-5 antibodies—nor is it safe—if IL-12 nevertheless could be directed by linking it to allergen, the response to specific allergens could be blocked.

Interferon- γ

IFN- γ has been shown to inhibit eosinophilia. In current studies, it has no effect on asthma when delivered in nebulized form, but it might be effective in other doses.

CONCLUSIONS

Anti-IL-5 antibodies are highly effective in the long-term reduction of eosinophils. The fact that no effect on airway reactivity has been observed with anti-IL-5 treatment is disappointing, but this approach nonetheless merits further study in patients with symptomatic asthma. Evidence that IL-4 receptor antagonists have some clinical benefit in asthma when administered by inhalation is encouraging and also merits further investigation, especially since administration as a single weekly dose may be possible.

TNF- α inhibitors have not yet been investigated in asthma, but should be studied in severe disease. No clinical benefits have been demonstrated with IL-10 in asthma to date, and the interferons do not appear to be effective. IL-12 does have an antieosinophil effect, but major side effects preclude its use, at least as a systemic therapy.

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