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CYTOKINE MODULATORS AS NOVEL THERAPIES FOR ASTHMA

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■ **Abstract** Cytokines play a critical role in orchestrating and perpetuating inflammation in asthmatic airways and several specific cytokine and chemokine inhibitors are now in development for the treatment of asthma. Inhibition of IL-4 with soluble IL-4 receptors has shown promising early results in asthma. Anti-IL-5 antibody is very effective at inhibiting peripheral blood and airway eosinophils but does not appear to be effective in symptomatic asthma. Inhibitory cytokines, such as IL-10, interferons, and IL-12 are less promising because systemic delivery produces intolerable side effects. Inhibition of TNF-α may be useful in severe asthma. Many chemokines are involved in the inflammatory response of asthma, and small-molecule inhibitors of chemokine receptors are in development. CCR3 antagonists are now in clinical development for the treatment of asthma. Because so many cytokines are involved in asthma, drugs that inhibit the synthesis of multiple cytokines may prove to be more useful. Several such classes of drug are now in clinical development, and the risk of side effects with these nonspecific inhibitors may be reduced by the inhaled route of delivery.

INTRODUCTION

Cytokines play a critical role in the orchestration of chronic inflammation in all diseases, including asthma. Multiple cytokines and chemokines have been implicated in the pathophysiology of asthma (1, 2). There is now an intensive search for more specific therapies in asthma, and inhibitors of cytokines and chemokines figure prominently in these novel therapeutic approaches (3) (Table 1).

STRATEGIES FOR INHIBITING CYTOKINES

There are several possible approaches to inhibiting specific cytokines (4, 5). These include drugs that inhibit cytokine synthesis (glucocorticoids, cyclosporin A, tacrolimus, myophenolate, Th2 selective inhibitors), humanized blocking antibodies to cytokines or their receptors, soluble receptors to mop up secreted cytokines,

TABLE 1 Cytokine modulators for asthma[a]

Anti-cytokines	**Inhibitory cytokines**
Anti-IL-4	IL-1 receptor antagonist
Anti-IL-5	IL-10
Anti-IL-13	IL-12
Anti-IL-9	Interferons
Anti-TNF-α	IL-18
Anti-IL-1	
Chemokine inhibitors	**Cytokine synthesis inhibitors**
CCR2 antagonists	Corticosteroids
CCR3 antagonists	Immunomodulators
CCR4 antagonists	Phosphodiesterase 4 inhibitors
	NF-κB inhibitors
	p38 MAP kinase inhibitors

[a]Abbreviations: IL = interleukin, NF-κB = nuclear factor-κB, MAP = mitogen-activated protein.

small-molecule receptor antagonists or drugs that block the signal transduction pathways activated by cytokines (4) (Figure 1). On the other hand, there are cytokines that themselves suppress the allergic inflammatory process, and these may have therapeutic potential in asthma (6, 7).

INHIBITION OF Th2 CYTOKINES

T helper 2 (Th2) lymphocytes play a key role in orchestrating the eosinophilic inflammatory response in asthma, which suggests that blocking the release or effects of these cytokines may have therapeutic potential. This has been strongly supported by studies in experimental animals, including mice, using deletion of cytokine genes specific to Th2 lymphocytes.

Anti-IL-5

IL-5 plays an essential role in orchestrating the eosinophilic inflammation of asthma (8). In IL-5 gene knockout mice, the eosinophilic response to allergen and the subsequent airway hyperresponsiveness (AHR) are markedly suppressed, which validates the strategy to inhibit IL-5 (Figure 2). IL-5 inhibition has so far been achieved using blocking antibodies. Monoclonal antibodies to IL-5 inhibit eosinophilic inflammation and AHR in animal models of asthma, including primates (9, 10). This blocking effect may last for up to three months after a single intravenous injection of antibody, making treatment of chronic asthma with such a therapy a feasible proposition. Humanized monoclonal antibodies to IL-5 have been developed and a single intravenous infusion of one of these antibodies

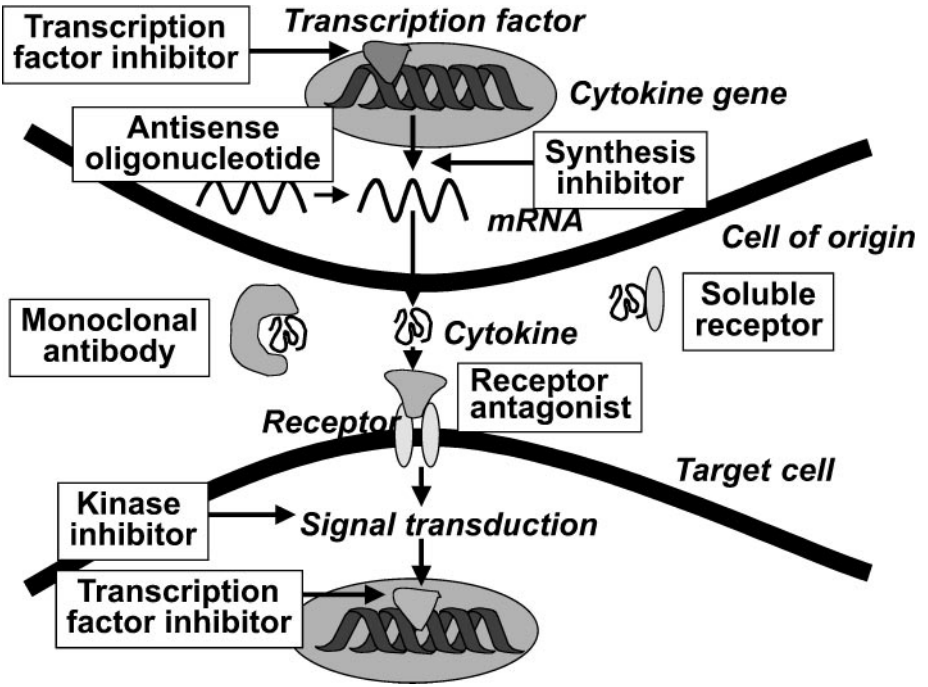

Figure 1 Strategies for inhibiting cytokines.

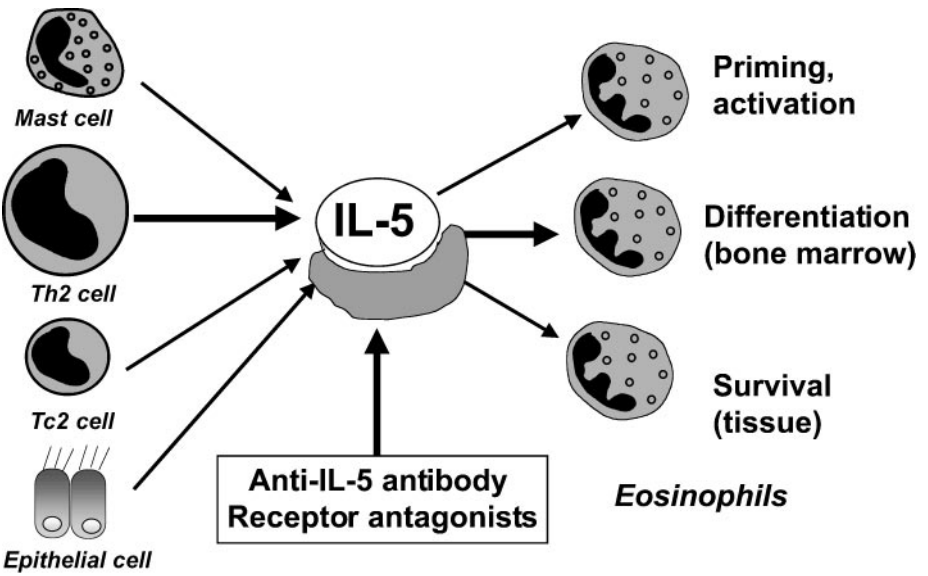

Figure 2 Inhibition of interleukin-5.

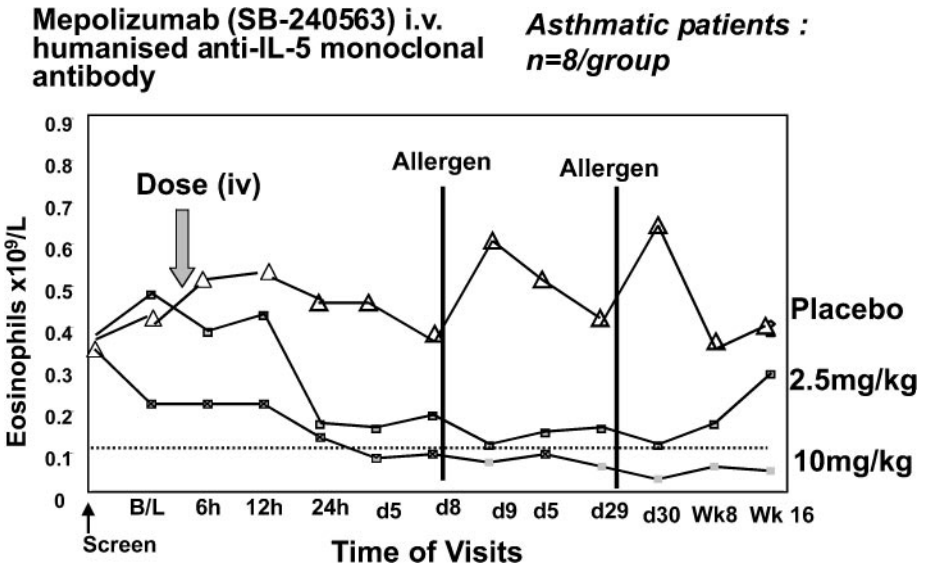

Figure 3 The effect of a humanized monoclonal antibody against interleukin-5 (mepolizumab) on circulating eosinophils in patients with mild asthma (adapted from reference 11).

(mepolizumab) markedly reduces blood eosinophils for several weeks and prevents eosinophil recruitment into the airways after allergen challenge in patients with mild asthma (11) (Figure 3). It is disappointing that this treatment has no significant effect on the early or late response to allergen challenge or on baseline AHR, which suggests that eosinophils may not be of critical importance for these responses in humans (Figure 4). A clinical study in patients with moderate to severe asthma that/by had not been controlled on inhaled corticosteroids therapy has confirmed the profound reduction in circulating eosinophils with anti-IL-5 antibody, but the study reported no significant improvement in either symptoms or lung function (12). In both of these studies it would be expected that high doses of corticosteroids would improve these functional parameters. These surprising results question the critical role of eosinophils in asthma and indicate that other strategies aimed at inhibiting eosinophilic inflammation may also prove to be ineffective.

Somewhat similar findings have previously been reported in studies in mice where anti-IL-5 antibodies reduced eosinophilic responses to allergen but not AHR, whereas AHR was reduced by anti-CD4 antibody that depletes helper T cells (13).

Nonpeptide IL-5 receptor antagonists would be an alternative strategy, and there is a search for such compounds using molecular modeling of the IL-5 receptor α-chain and using large-scale throughput screening. However, the lack of clinical

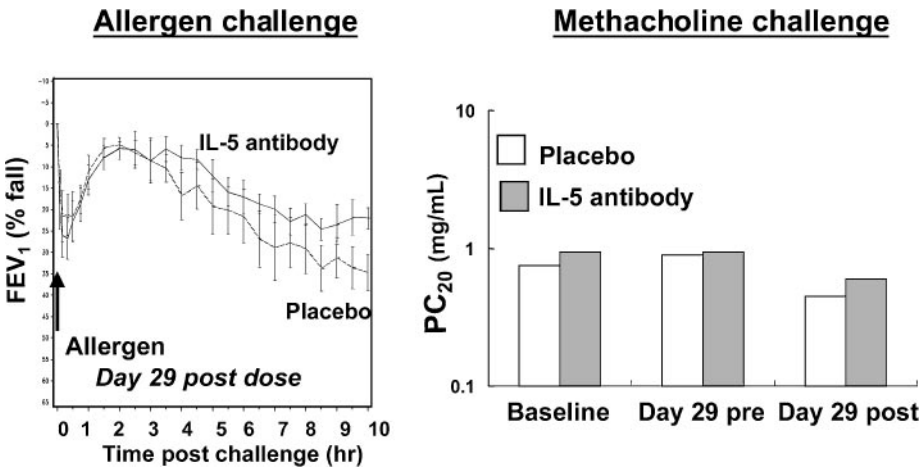

Figure 4 Effect of a humanized monoclonal antibody against interleukin-5 (mepolizumab) on the early and late response to allergen (left panel) and airway hyperresponsiveness (methacholine PC_{20}) (adapted from reference 11).

benefit of anti-IL-5 antibodies has now made this a less attractive approach. It is possible that eosinophils are associated with more chronic aspects of asthma, such as airway remodeling, and in mice a blocking anti-IL-5 antibody prevents the increased collagen deposition in airways associated with repeated allergen exposure (14). Eosinophils may be an important source of transforming growth factor-β in asthmatic airways, resulting in structural changes (15).

Anti-IL-4

IL-4 is critical for the synthesis of IgE by B lymphocytes and is also involved in eosinophil recruitment to the airways (16). A unique function of IL-4 is to promote differentiation of Th2 cells and it therefore acts at a proximal and critical site in the allergic response, making IL-4 an attractive target for inhibition (Figure 5).

IL-4 blocking antibodies inhibit allergen-induced AHR, goblet cell metaplasia, and pulmonary eosinophilia in a murine model (17). Inhibition of IL-4 may therefore be effective in inhibiting allergic diseases, and soluble IL-4 receptors (sIL-4r) are now in clinical development as a strategy to inhibit IL-4. A single nebulized dose of sIL-4r prevents the fall in lung function induced by withdrawal of inhaled corticosteroids in patients with moderately severe asthma (18). Subsequent studies have demonstrated that weekly nebulization of sIL-4r improves asthma control over a 12-week period (19). Another approach is blockade of IL-4 receptors with a mutated form of IL-4 (BAY 36-1677), which binds to and blocks IL-4Rα and IL-13Rα1, thus blocking both IL-4 and IL-13 (20).

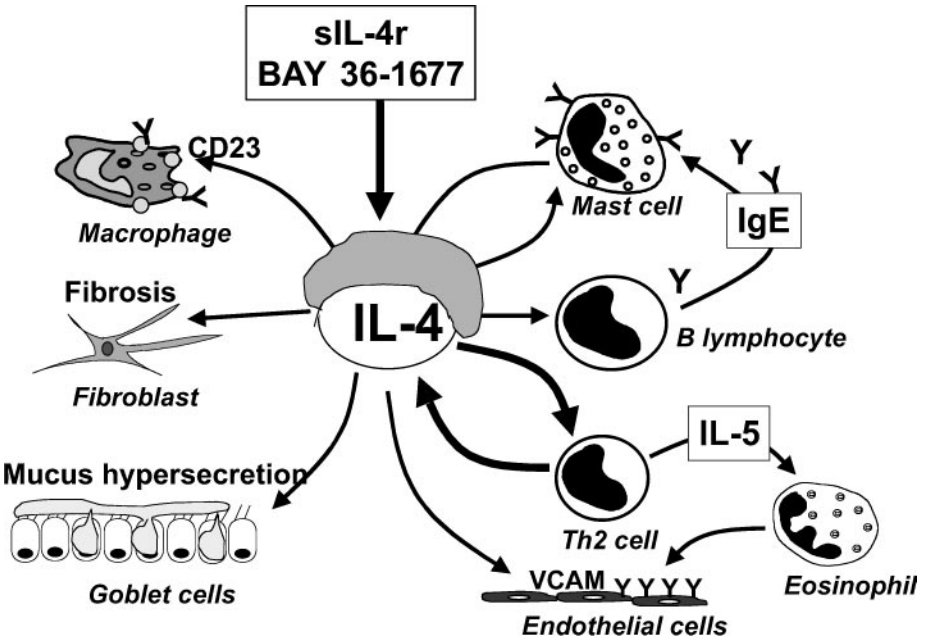

Figure 5 Effects of blocking interleukin-4 in asthma.

IL-4 and the closely related cytokine IL-13 signal through a shared surface receptor, IL-4Rα, which activates a specific transcription factor STAT-6 (21). Deletion of the STAT-6 gene has a similar effect to IL-4 gene knockout (22). This has led to a search for inhibitors of STAT-6; although peptide inhibitors that interfere with the interaction between STAT-6 and JAKs linked to IL-4Rα have been discovered, it will be difficult to deliver these intracellularly. An endogenous inhibitor of STATs, suppressor of cytokine signaling (SOCS-1), is a potent inhibitor of the IL-4 signaling pathway and offers a novel therapeutic target (21, 23).

Anti-IL-13

There is increasing evidence that IL-13 in mice mimics many of the features of asthma, including AHR and mucus hypersecretion, independently of eosinophilic inflammation (24), and potently induces the secretion of eotaxin from airway epithelial cells (25). IL-13 signals through the IL-4 receptor α-chain but may also activate different intracellular pathways via activation of IL-13Rα1 (26), so that it may be an important target for the development of new therapies. A second specific IL-13 receptor, IL-13Rα2, exists in soluble form and has a high affinity for IL-13, thus acting as a decoy receptor for IL-13. Soluble IL-13Rα2 is effective in blocking the actions of IL-13, including IgE generation, pulmonary eosinophilia, and AHR in mice (24). Indeed in the murine model IL-13Rα2 is more effective

than IL-4 blocking antibodies, highlighting the potential importance of IL-13 as a mediator of allergic inflammation. Humanized IL-13Rα2 is now being developed as a therapeutic approach for asthma.

Anti-IL-9

IL-9 is a Th2 cytokine that may enhance Th2-driven inflammation and amplify mast cell mediator release and IgE production (27). IL-9 may also enhance mucus hypersecretion (28). IL-9 and its receptors show an increased expression in asthmatic airways (29, 30). Strategies to block IL-9, which include blocking antibodies, are now in development (31).

INHIBITION OF PROINFLAMMATORY CYTOKINES

Proinflammatory cytokines, particularly IL-1β and tumor necrosis factor-α (TNF-α), may amplify the inflammatory response in asthma and chronic obstructive pulmonary disease (COPD) and may be linked to disease severity. This suggests that blocking IL-1β or TNF-α may have beneficial effects, particularly in severe airway disease.

Anti-IL-1

IL-1 expression is increased in asthmatic airways (32) and activates many inflammatory genes expressed in asthma. There are no small-molecule inhibitors of IL-1, but a naturally occurring cytokine, IL-1 receptor antagonist (IL-1ra), binds to IL-1 receptors to block the effects of IL-1 (33). In experimental animals IL-1ra reduces AHR induced by allergen. Human recombinant IL-1ra does not appear to be effective in the treatment of asthma, however (34).

Anti-TNF

TNF-α is expressed in asthmatic airways and may play a key role in amplifying asthmatic inflammation through the activation of NF-κB, AP-1, and other transcription factors (35). In rheumatoid arthritis and inflammatory bowel disease, a blocking humanized monoclonal antibody to TNF-α (infliximab) and soluble TNF receptors (etanercept) have produced remarkable clinical responses, even in patients who are relatively unresponsive to steroids (36, 37). Such antibodies or soluble TNF receptors are a logical approach to asthma therapy, particularly in patients with severe disease, and clinical trials are now under way.

Because of the problems associated with antibody-based therapies, there is a search for small-molecule inhibitors of TNF. TNF-α-converting enzyme (TACE) is a matrix metalloproteinase-related enzyme critical for the release of TNF from the cell surface. Small-molecule TACE inhibitors are in development as oral TNF inhibitors (38).

ANTI-INFLAMMATORY CYTOKINES

Some cytokines have anti-inflammatory effects on inflammation and therefore have therapeutic potential (6, 7). While it may not be feasible or cost-effective to administer these proteins as long-term therapy, it may be possible to develop drugs in the future that increase the release of these endogenous cytokines or activate their receptors and specific signal transduction pathways.

IL-10

IL-10 is a potent anti-inflammatory cytokine that inhibits the synthesis of many inflammatory proteins, including cytokines [TNF-α, granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-5, chemokines] and inflammatory enzymes (iNOS) overexpressed in asthma (39) (Figure 6). Indeed there may be a defect in IL-10 transcription and secretion from macrophages in asthma (40, 41). In sensitized animals IL-10 is effective in suppressing the inflammatory response to allergen (42), which suggests that IL-10 might be defective in atopic diseases. Specific allergen immunotherapy results in increased production of IL-10 by T helper cells, and this may contribute to the beneficial effects of immunotherapy (43).

Recombinant human IL-10 has proved to be effective in controlling inflammatory bowel disease and psoriasis, where similar cytokines are expressed, and may be given as a weekly injection (44). Although IL-10 is reasonably well-tolerated, there are hematological side effects. In the future, drugs may be developed that activate the unique signal transduction pathways activated by the IL-10 receptor

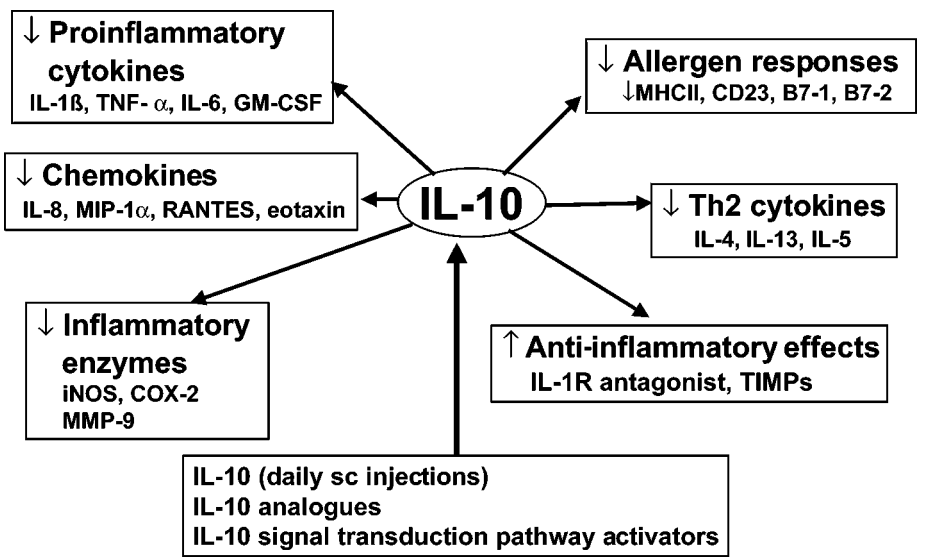

Figure 6 Anti-inflammatory actions of interleukin-10.

or that increase endogenous production of IL-10. In mice, drugs that elevate cyclic AMP increase IL-10 production, but this does not appear to be the case in human cells (45).

Interferons

Interferon-γ (IFN-γ) inhibits Th2 cells and should therefore reduce atopic inflammation. In sensitized animals, nebulized IFN-γ inhibits eosinophilic inflammation induced by allergen exposure (46). Administration of IFN-γ by nebulization to asthmatic patients did not significantly reduce eosinophilic inflammation, possibly due to the difficulty in obtaining a sufficiently high concentration locally in the airways (47). It is interesting that allergen immunotherapy increases IFN-γ production by circulating T cells in patients with clinical benefit (48) and increases numbers of IFN-γ expressing cells in nasal biopsies of patients with allergic rhinitis (49). A preliminary report suggests that IFN-α may be useful in the treatment of patients with severe asthma who have reduced responsiveness to corticosteroids (50).

IL-12

IL-12 is the endogenous regulator of Th1 cell development and determines the balance between Th1 and Th2 cells (51). IL-12 administration to rats inhibits allergen-induced inflammation (52) and inhibits sensitization to allergens. IL-12 releases IFN-γ but has additional effects on T cell differentiation. IL-12 levels released from whole blood cells are lower in asthmatic patients, which could indicate a possible reduction in IL-12 secretion in asthma (53).

Recombinant human IL-12 has been administered to humans and has several toxic effects diminished by slow escalation of the dose (54). In patients with mild asthma, weekly infusions of human recombinant IL-12 in escalating doses over four weeks caused a progressive fall in circulating eosinophils, a reduction in the normal rise in circulating eosinophils after allergen challenge (55) (Figure 7), and a concomitant reduction in eosinophils in induced sputum. However, there was no reduction in either early or late response to inhaled allergen challenge and no reduction in AHR. Furthermore, most of the patients suffered from malaise, and one out of the 12 subjects had an episode of cardiac arrhythmia. This suggests that IL-12 is not a suitable treatment for asthma. In mice administration of an IL-12-allergen fusion protein results in the development of a specific Th1 response to the allergen, with increased production of an allergen-specific IgG2, rather than the normal Th2 response with IgE formation (56). This indicates the possibility of using local IL-12 together with specific allergens to provide a more specific immunotherapy, which might even be curative if applied early in the course of the atopic disease.

IL-18

IL-18 was originally described as an IFN-γ–releasing factor, but it has a mechanism of action different from that of IL-12 (57). IL-12 and IL-18 appear to have a

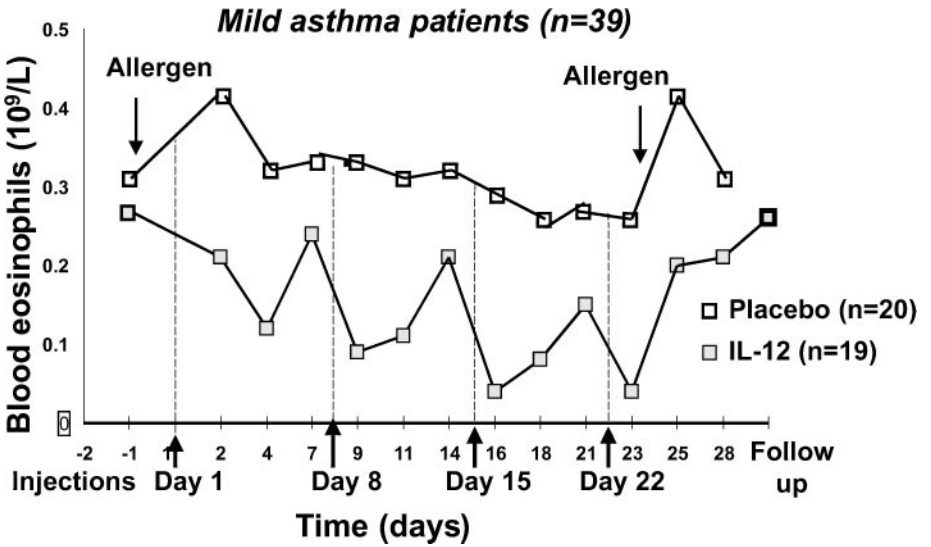

Figure 7 Effect of interleukin-12 on peripheral blood eosinophils in patients with mild asthma (adapted from 55).

synergistic effect on inducing IFN-γ release and inhibiting IL-4–dependent IgE production and AHR (58).

CHEMOKINE INHIBITORS

Many chemokines are involved in the recruitment of inflammatory cells in asthma. Over 50 different chemokines are now recognized, and they activate more than 20 different surface receptors (59). Chemokine receptors belong to the seven transmembrane receptor superfamily of G protein–coupled receptors, and this makes it possible to find small-molecule inhibitors, which has not been possible for classical cytokine receptors (60). Some chemokines appear to be selective for single chemokine receptors, whereas others are promiscuous and mediate the effects of several related chemokines (Figure 8). Chemokines appear to act in sequence in determining the final inflammatory response, and so inhibitors may be more or less effective depending on the kinetics of the response (61).

CCR3 Inhibitors

Several chemokines, including eotaxin, eotaxin-2, eotaxin-3, RANTES, and macrophage chemoattractant protein-4 (MCP-4) activate a common receptor on eosinophils termed CCR3 (62). A neutralizing antibody against eotaxin reduces eosinophil recruitment into the lung after allergen and the associated AHR in

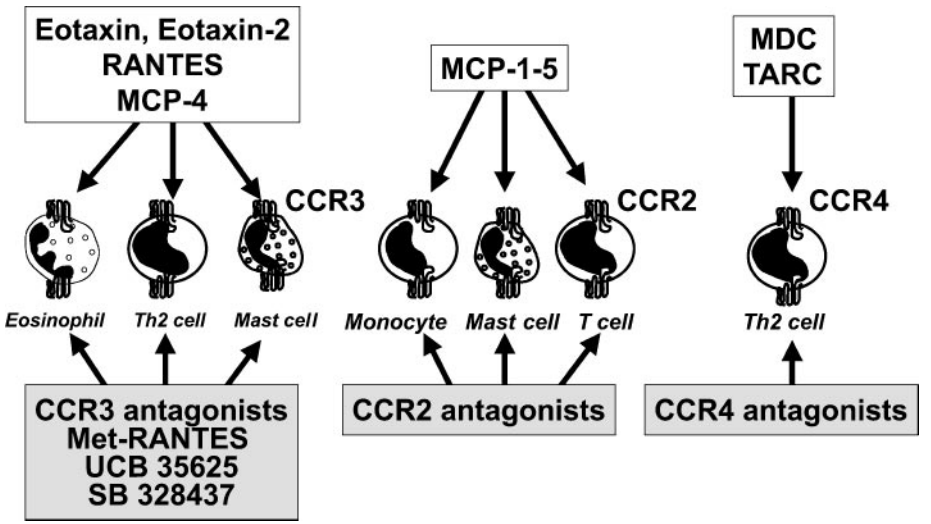

Figure 8 Chemokine receptor antagonists in asthma.

mice (63). There is increased expression of eotaxin, eotaxin-2, MCP-3, MCP-4, and CCR3 in the airways of asthmatic patients and this is correlated with increased AHR (64, 65). Several small-molecule inhibitors of CCR3, including UCB35625, SB-297006, and SB-328437, are effective in inhibiting eosinophil recruitment in allergen models of asthma (66, 67); drugs in this class are currently undergoing clinical trials in asthma. Although it was thought that CCR3 were restricted to eosinophils, there is some evidence for their expression on Th2 cells and mast cells, so that these inhibitors may have a more widespread effect than on eosinophils alone; this would make them potentially more valuable in asthma treatment. RANTES, which shows increased expression in asthmatic airways (68), also activates CCR3 but has effects on CCR1 and CCR5, which may play a role in T cell recruitment. Modification of the N-terminal of RANTES, met-RANTES, has a blocking effect on RANTES by inhibiting these receptors (69).

CCR2 Inhibitors

MCP-1 activates CCR2 on monocytes and T lymphocytes. And blocking MCP-1 with neutralizing antibodies reduces recruitment of both T cells and eosinophils in a murine model of ovalbumin-induced airway inflammation, with a marked reduction in AHR (63). MCP-1 also recruits and activates mast cells, an effect mediated via CCR2 (70). MCP-1 instilled into the airways induces marked and prolonged AHR in mice, associated with mast cell degranulation. A neutralizing antibody to MCP-1 blocks the development of AHR in response to allergen (70). MCP-1 levels are increased in bronchoalveolar lavage fluid of patients with asthma (71). This has led to a search for small-molecule inhibitors of CCR2.

CCR4 Inhibitors

CCR4 are selectively expressed on Th2 cells and are activated by two chemokines: monocyte-derived chemokine (MDC) and thymus- and activation-dependent chemokine (TARC) (72). Inhibitors of CCR4 may therefore inhibit the recruitment of Th2 cells and thus prevent persistent eosinophilic inflammation in the airways.

OTHER APPROACHES TO CYTOKINE INHIBITION

Although there have been several attempts to block specific cytokines, this may not be adequate to block chronic inflammation in asthma, as so many cytokines are involved and there is considerable redundancy of effects. This has suggested that the development of drugs that have a more general effect on synthesis of cytokines may be more successful. However, these drugs also affect other inflammatory processes, so their beneficial effects cannot necessarily be ascribed to inhibition of cytokine synthesis alone.

Corticosteroids

Corticosteroids are by far the most effective treatments for asthma and part of their efficacy is due to inhibition of inflammatory cytokine expression. This is mediated via an effect on glucocorticoid receptors to reverse the acetylation of core histones linked to increased expression of inflammatory genes (73).

Immunomodulators

Cyclosporin A, tacrolimus, and rapamycin inhibit the transcription factor NF-AT that regulates the secretion of IL-2, IL-4, IL-5, and GM-CSF by T lymphocytes (74). Although it has some reported beneficial steroid-sparing effects in asthma (75), the toxicity of cyclosporin A limits its usefulness, at least when given orally. More selective Th2 inhibitors may be safer for the treatment of asthma in the future. An inhibitor of Th2-cytokines, suplatast tosilate (76), is reported to provide clinical benefit in asthma (77). Cytotoxic (CD8+) T lymphocytes are prominent in COPD, and therefore immunomodulatory drugs may also have a role in this disease.

Phosphodiesterase 4 Inhibitors

PDE-4 inhibitors inhibit the release of cytokines and chemokines from inflammatory cells via an increase in intracellular cyclic AMP (78). Their clinical use in asthma is limited by side effects such as nausea.

NF-κB Inhibitors

NF-κB regulates the expression of many cytokines and chemokines involved in asthma and COPD (79). There are several possible approaches to inhibition of NF-κB, including gene transfer of the inhibitor of NF-κB (IκB); inhibitors of IκB kinases (IKK); NF-κB-inducing kinase (NIK); and IκB ubiquitin ligase, all of which regulate the activity of NF-κB; and the development of drugs that inhibit the degradation of IκB (80). One concern about this approach is that effective inhibitors of NF-κB may result in immune suppression and impair host defenses, since knockout mice, which lack NF-κB proteins, succumb to septicemia. However, there are alternative pathways of NF-κB activation that might be more important in inflammatory disease (81).

p38 MAP Kinase Inhibitors

Mitogen-activated protein (MAP) kinases play a key role in chronic inflammation, and several complex enzyme cascades have now been defined. One of these, the p38 MAP kinase pathway, is involved in expression of inflammatory cytokines and chemokines (82, 83). Nonpeptide inhibitors of p38 MAP kinase such as SB 203580, SB 239063, and RWJ 67657, also known as cytokine synthesis anti-inflammatory drugs (CSAIDS), have now been developed, and these drugs have a broad range of anti-inflammatory effects (84). However, there may be issues of safety as p38 MAP kinases are involved in host defense. However, it is possible that the inhaled route of delivery may reduce the risk of side effects.

CONCLUSIONS

Several drugs that modulate cytokines have now been developed for the treatment of asthma and other allergic diseases. Inhibition of specific Th2 cell–derived cytokines, such as IL-4 and IL-5, has so far proved to be somewhat disappointing in clinical studies. This may reflect the fact that this approach is too selective and therefore not able to suppress the inflammatory process completely. Drugs that have a broader range of actions, such as anti-inflammatory cytokines and small-molecule inhibitors of signal transduction pathways involved in inflammatory cytokine secretion, may be more useful in the future.

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