

New millennium: The conquest of allergy

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New directions in allergic diseases: Mechanism-based anti-inflammatory therapies

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Advances in our understanding of allergic inflammation have led to the development of several novel anti-inflammatory drugs that target specific aspects of the inflammatory process. These treatments are based on improvements in existing therapies or on a better understanding of the cellular and molecular mechanisms involved in atopic diseases. Although most attention has been focused on asthma, treatments that inhibit the atopic disease process would have application to all atopic diseases, which often coincide. Specific agents that are now in development for the treatment of allergic inflammation include inhibitors of eosinophilic inflammation (eg, anti-IL-5, CCR3 antagonists, and very late antigen 4 inhibitors), drugs that may inhibit allergen presentation, and inhibitors of T_H2 cells. More general anti-inflammatory approaches include novel corticosteroids, phosphodiesterase inhibitors, and mitogen-activated protein kinase inhibitors. Most of the new therapies in development are aimed at inhibiting or suppressing components of the allergic inflammatory response, but in the future, there are possibilities for development of preventive and curative treatments. (*J Allergy Clin Immunol* 2000;106:5-16.)

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Current therapies for asthma and rhinitis are effective in the majority of patients, but treatment of atopic dermatitis is less satisfactory. There is a need for the development of new treatments that deal with more severe asthma, which is currently not well controlled by high doses of inhaled corticosteroids, and a need for a safe oral medication that would be effective in all atopic diseases because they often occur together. The development of safe and effective oral therapies may depend on the discovery of treatments that are more specific for the atopic disease process to avoid the side effects that may be associated with more general anti-inflammatory drugs, such as corticosteroids.

Abbreviations used

AHR:	Airway hyperresponsiveness
AP-1:	Activator protein 1
cys-LT:	Cysteinyl leukotriene
iNOS:	Inducible nitric oxide synthase
5-LO:	5'-Lipoxygenase
MAP:	Mitogen-activated protein
NF-κB:	Nuclear factor κB
NO:	Nitric oxide
PDE:	Phosphodiesterase
STAT:	Signal transduction activated transcription factor
VLA:	Very late antigen

New therapies for allergic diseases have been developed by improving existing classes of drugs or by discovering new classes of drugs through research. Advances in understanding the molecular mechanisms of atopy have identified several new targets that might lead to novel therapies for allergic diseases in the future.^{1,2} Although most attention has focused on asthma, the atopic disease with the greatest clinical and economic effect, treatments that are effective against the underlying atopic disease process may be effective against all atopic diseases, which commonly occur together. Many new classes of anti-inflammatory drugs are in development, highlighting the fact that there are many possible therapeutic targets in the allergic process.

CORTICOSTEROIDS

Corticosteroids are the most effective currently available treatment for atopic diseases, and high doses of oral corticosteroids control all atopic diseases. However, systemic side effects limit the dose that can be given over long periods, and in the early 1970s, this led to the development of topical steroids, which have local anti-inflammatory effects. Inhaled corticosteroids have dramatically improved the treatment of asthma and are now first-line treatment for persistent asthma in all patients.^{3,4} Nasal steroids are the most effective treatment for allergic rhinitis.⁵ Topical steroids are associated with dermal atrophy, however, and this has restricted the use of corticosteroids in the management of atopic dermatitis. Newer inhaled corticosteroids for asthma, including budesonide, fluticasone propionate, and mometasone furoate, have a high level of anti-inflam-

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matory action with minimal side effects because the swallowed fraction of the drug is largely removed by the hepatic metabolism.³ However, these drugs are absorbed from the lung or nasal mucosa and therefore may have some systemic effects at high doses. There has therefore been a search for corticosteroids that are metabolized locally, so that there is less risk of systemic absorption from the respiratory tract or the skin. However, these *soft steroids*, such as butixocort 21-propionate and tipredane, proved to have poor efficacy in clinical studies because they are metabolized before they exert any anti-inflammatory action. New soft steroids, such as ciclesonide, appear to be more promising,⁶ and corticosteroids that are inactivated in plasma are now in development. Corticosteroids are highly effective in controlling atopic diseases, and there is increasing evidence that if started early, they may prevent some of the irreversible airway narrowing in asthma.^{3,4} However, corticosteroids are not curative, and inflammation recurs when they are discontinued.

Advances in understanding the molecular mechanisms whereby corticosteroids suppress inflammation at a molecular level may lead to the development of safer steroids or drugs that mimic their key anti-inflammatory actions. Corticosteroids bind to a cytosolic glucocorticoid receptor that translocates to the nucleus and binds as a homodimer to DNA to activate genes, including anti-inflammatory genes. However, the major anti-inflammatory effect of corticosteroids is suppression of multiple inflammatory genes, including cytokines, inflammatory enzymes, adhesion molecules, and inflammatory mediator receptors; this is why corticosteroids are so effective in complex inflammatory conditions, such as atopic diseases. Most of the anti-inflammatory actions of corticosteroids can be accounted for by inhibiting transcription factors, such as activator protein 1 (AP-1), nuclear factor- κ B (NF- κ B), and nuclear factor of activated T cells, that regulate inflammatory gene expression.⁷ These effects are mediated, at least in part, by inhibition of core histone acetylation.

Systemic side effects of corticosteroids are largely mediated through DNA binding, however, so that it may be possible to dissociate the anti-inflammatory effects mainly mediated by transcription factor inhibition from their side effects.⁸ Several dissociated corticosteroids have now been synthesized, and a separation between transactivation (DNA binding) and transrepression (transcription factor inhibition) has been demonstrated in gene reporter systems and in intact cells in vitro.⁹ Whether this will translate to in vivo differences has not yet been determined, and because all corticosteroids have to bind to a single class of glucocorticoid receptor, a large separation of effects may not be possible. Identification of the major targets for corticosteroid action, such as NF- κ B, or acetylation and deacetylation of specific core histones may be a more promising approach for the future.

MEDIATOR ANTAGONISTS

Many inflammatory mediators are involved in atopic diseases, and in asthma over 50 different mediators have

been identified.¹⁰ This implies that inhibitors of single mediators would be unlikely to be of major clinical benefit, yet some mediator antagonists have been found to be useful in treating atopic diseases, suggesting that some mediators may play a more dominant role or may be upstream of a complex mediator cascade.

Antihistamines

Histamine H₁-receptor antagonists have long been used in the treatment of atopic diseases.¹¹ Older antihistamines that caused sedation, such as promethazine and chlorpheniramine, have now been replaced by a new generation of antihistamines that are much less likely to sedate, such as loratadine and fexofenadine. These drugs are effective in the treatment of rhinitis and reduce itch in atopic dermatitis but have no clear benefit in asthma.¹² New antihistamines, such as cetirizine, ebastine, and azelastine, have been claimed to have additional antiasthma effects that are not mediated through the H₁-receptor blockade, including an inhibitory effect on eosinophil chemotaxis, adherence to endothelial cells, and recruitment into the airways after allergen challenge.¹³

Antileukotrienes

Antileukotrienes are the first novel class of therapy introduced for asthma in the last 30 years and have attracted considerable interest. Cysteinyl leukotrienes (cys-LTs), generated from the rate-limiting enzyme 5'-lipoxygenase (5-LO), are potent bronchoconstrictors and inducers of plasma exudation and may promote eosinophilic inflammation.¹⁴ 5-LO inhibitors (zileuton) and cys-LT receptor (cys-LT₁) antagonists (pranlukast, zafirlukast, and montelukast) have been developed for the treatment of asthma and possibly other atopic diseases. In challenge studies they reduce allergen-, exercise-, and even irritant-induced asthma, as well as a number of other challenges. In clinical trials they clearly improve asthma symptoms and lung function and reduce the need for rescue bronchodilator treatment.¹⁴ A major advantage is that they are effective orally, and thus far they do not appear to have serious class-specific effects. However, they are less effective in asthma than low doses of inhaled corticosteroids, although some patients (even those with severe disease) may have useful clinical improvement. It is not yet possible to predict which patients respond best, although there is some evidence that patients with aspirin-sensitive asthma do well, as predicted by studies showing increased cys-LT production in these patients.¹⁵ It is possible that genetic polymorphisms in the 5-LO or LTC₄ synthase pathways or in cys-LT₁ receptors might predict better responders in the future.^{16,17} Antileukotrienes may have efficacy in allergic rhinitis, although recent clinical studies have shown relatively little benefit compared with nasal corticosteroids.¹⁸

Tryptase inhibitors

Mast cell tryptase has several effects on airways, including increasing responsiveness of airway smooth muscle to constrictors, increasing plasma exudation,

potentiating eosinophil recruitment, and stimulating fibroblast proliferation. Some of these effects are mediated by activation of the proteinase-activated receptor proteinase-activated receptor 2. The tryptase inhibitor APC366 is effective in a sheep model of allergen-induced asthma¹⁹ but was only poorly effective in asthmatic patients.²⁰ More potent tryptase inhibitors and proteinase-activated receptor 2 antagonists are now in development.

Other mediator inhibitors

Several other specific inhibitors of inflammatory mediators have now been tested in asthma and rhinitis with disappointing effects, including thromboxane synthesis inhibitors and receptor antagonists, platelet-activating factor antagonists, and kinin antagonists. Inhibitors of other mediators relevant to atopic diseases are in development. There is increased production of nitric oxide (NO) in asthma and rhinitis, with evidence for increased expression of inducible NO synthase (iNOS). NO may contribute to vasodilatation and plasma exudation and has been implicated in eosinophil recruitment and survival.²¹ Selective inhibitors of iNOS are now in development but have not yet reached clinical trials in asthma. Endothelins have been implicated in bronchoconstriction, smooth muscle hyperplasia, and fibrosis, with effects mediated by endothelin A and endothelin B receptors.²² Mixed endothelin antagonists have now been developed but have not yet been tested in asthma; if their major effect is to inhibit airway remodeling, they may prove difficult to test in short-term clinical studies, however.

CYTOKINE MODULATORS

Multiple cytokines have been implicated in the pathophysiology of atopic diseases, although some cytokines play a more critical role in atopic inflammation.²³ There are several possible approaches to inhibiting specific cytokines. These range from drugs that inhibit cytokine synthesis (corticosteroids, cyclosporin A, and tacrolimus), humanized blocking antibodies to cytokines or their receptors, soluble receptors to mop up secreted cytokines, receptor antagonists, or drugs that block the signal transduction pathways activated by cytokines (Fig 1). There are some cytokines that suppress the allergic inflammatory process, and these may also have therapeutic potential.

Anti-IL-5

IL-5 plays a key role in orchestrating the eosinophilic inflammation of asthma (Fig 2).²⁴ Blocking antibodies to IL-5 inhibit eosinophilic inflammation and airway hyperresponsiveness (AHR) in animal models of asthma, including primates. This blocking effect may last for over 3 months after a single injection, making treatment of chronic asthma with such a therapy a feasible proposition. Humanized mAbs to IL-5 have been developed, and a single injection reduces blood eosinophils for over 3 months and prevents eosinophil recruitment into the airways after allergen challenge.²⁵ However, this treatment has no effect on the early or late response to allergen

challenge or on AHR, suggesting that eosinophils may be less important for these responses than previously believed. Similar findings have previously been reported in mice in which anti-IL5 antibodies reduced eosinophil responses to allergen but not AHR, whereas AHR is reduced by anti-CD4 antibody that depletes helper T cells.²⁶ Long-term clinical studies are now in progress, particularly in patients with more severe disease. Non-peptide IL-5 receptor antagonists would be an attractive alternative, and there is a search for such compounds by using molecular modeling of the IL-5 receptor chain and through large-scale throughput screening.

Anti-IL-4

IL-4 is critical for the synthesis of IgE by B lymphocytes and to the development of T_H2 cells. IL-4 receptor-blocking antibodies inhibit allergen-induced AHR, goblet cell metaplasia, and pulmonary eosinophilia in a murine model.²⁷ Inhibition of IL-4 may therefore be effective in inhibiting allergic diseases. Soluble IL-4 receptors are in clinical development as a strategy to inhibit IL-4. Nebulized soluble IL-4 receptors (altrakincept) have shown clinical efficacy in patients with moderate asthma and were effective when given as a once weekly nebulization in preventing the deterioration in asthma after reduction in inhaled corticosteroids.²⁸ Both IL-4 and IL-13 are inhibited by a double mutant of IL-4 (Arg121Asp, Tyr124Asp, BAY 169996),²⁹ and this double mutant inhibits the inflammatory response to allergen in mice.³⁰ IL-4 and the closely related cytokine IL-13 signal through a shared surface receptor that activates a specific transcription factor, signal transduction activated transcription factor (STAT-6), and deletion of the *STAT6* gene has a similar effect to *IL4* gene knockout.³¹ This has led to a search for inhibitors of STAT-6, although it will be difficult to deliver these intracellularly. An endogenous inhibitor of STATs, suppressor of cytokine signaling, is a potent inhibitor of IL-4 signaling pathways and offers a new therapeutic target.³²

Anti-IL-13

There is increasing evidence that IL-13 in mice mimics many of the features of asthma, including AHR, increased IgE, and mucus hypersecretion,³³ and induces the secretion of eotaxin from airway epithelial cells.³⁴ IL-13 signals through the IL-4 receptor chain but may also activate different intracellular pathways,³⁵ and therefore it may be an important target for the development of new therapies. A soluble IL-13R α 2-Fc fusion protein, which blocks the effects of IL-13 but not IL-4 has been used successfully to neutralize IL-13 in mice in vivo.³³ The IL-13R α 2-Fc fusion protein markedly inhibits the eosinophilic inflammation, AHR, and mucus secretion induced by allergen exposure. IL-13 is expressed in asthma to a much greater extent than IL-4, indicating that it may be a more important target.³⁶ This suggests that development of IL-13 blockers, such as a humanized IL-13 antibody or the IL-13R α 2 protein, may be a useful approach to the treatment of established allergic diseases.³⁷

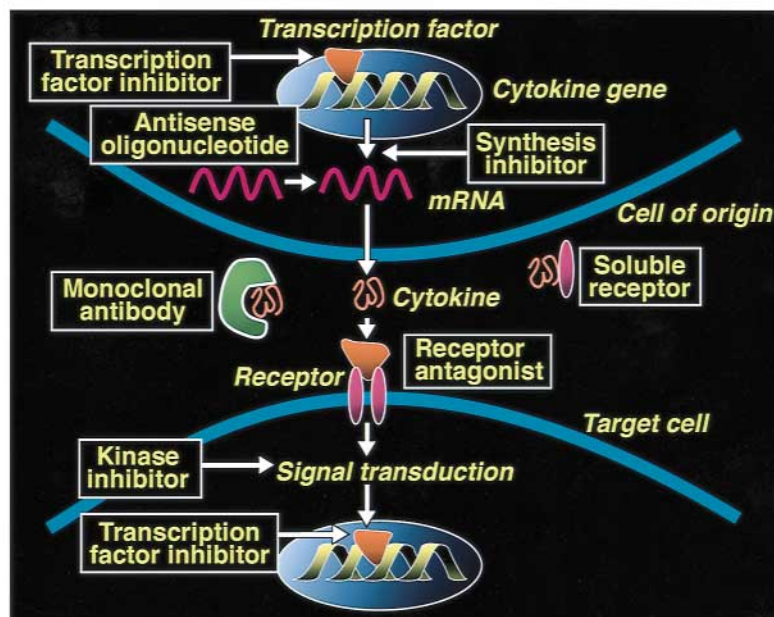


FIG 1. Inhibition of cytokine synthesis. Several strategies are available to inhibit the production or effects of cytokines. Reprinted by permission from *Nature Supplement* 402:B31-8, copyright 1999 Macmillan Magazines Ltd.

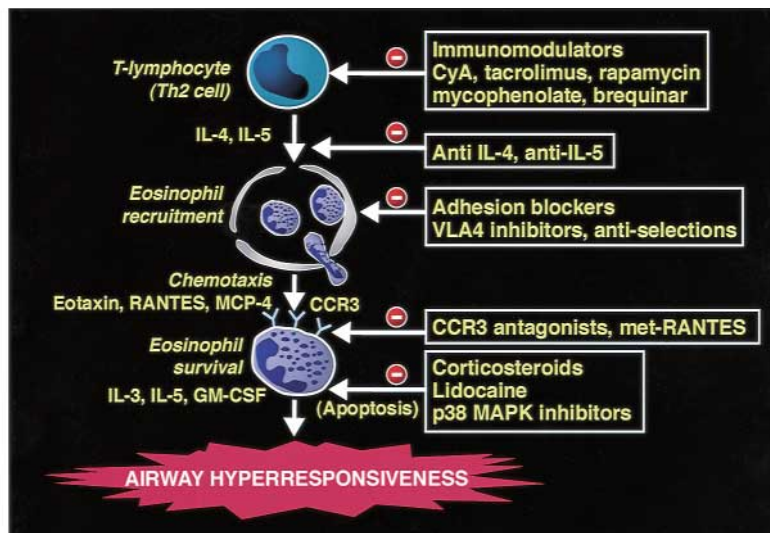


FIG 2. Inhibition of eosinophilic inflammation. Several strategies are possible to inhibit eosinophil inflammation in tissues, including immunomodulators, inhibitors of driving cytokines (IL-4 and IL-5), inhibition of critical adhesion molecules (VLA-4, selectins, and intercellular adhesion molecule 1), blockade of chemokine receptors on eosinophils (CCR3), and induction of apoptosis. MCP, Monocyte chemoattractant protein. Reprinted by permission from *Nature Supplement* 402:B31-8, copyright 1999 Macmillan Magazines Ltd.

Anti-IL-9

IL-9 is produced by T_H2 cells and appears to have an amplifying effect on the expression of IL-4 and IL-5.³⁸ This suggests that IL-9 may be a useful upstream target in asthma, and humanized monoclonal IL-9 antibodies are now in clinical development.

Anti-TNF

TNF- α is expressed in asthmatic airways and may play a key role in amplifying atopic inflammation through the activation of NF- κ B, AP-1, and other transcription factors. In rheumatoid arthritis and inflammatory bowel disease a blocking antibody to TNF- α (eg, infliximab) has produced

remarkable clinical responses, even in patients who are relatively unresponsive to steroids.³⁹ TNF antibodies or soluble TNF receptors (eg, entanercept) are a logical approach to asthma therapy, particularly in patients with severe disease. There is also a search for small molecule inhibitors of TNF, of which the most promising are inhibitors of TNF- α -converting enzyme because these could be given orally. Other new anti-inflammatory treatments, including phosphodiesterase 4 (PDE4) inhibitors and p38 mitogen-activated protein (MAP) kinase inhibitors are also effective in inhibiting TNF- α release from inflammatory cells.

Chemokine inhibitors

CC chemokines, such as RANTES, monocyte chemoattractant protein 3, monocyte chemoattractant protein 4, and eotaxin, may play a critical role in the recruitment of eosinophils in atopic patients, and all of these chemokines act on a common receptor, the CCR3 receptor, that is expressed predominantly on eosinophils and some T_H2 cells.⁴⁰ An antibody to human CCR3 blocks the chemotactic response of human eosinophils to all chemokines.⁴¹ The modified chemokine met-RANTES similarly blocks CCR3 receptors and inhibits eosinophil chemotactic responses to several chemokines.⁴² Chemokine receptors are G protein-coupled receptors with the typical 7-transmembrane spanning segments and are therefore of a simpler structure than the receptors for other cytokines. Nonpeptide inhibitors of CCR3 receptors are now in clinical development, and theoretically, they should be relatively safe because the distribution of CCR3 receptors is so restricted.

Anti-inflammatory cytokines

Some cytokines have anti-inflammatory effects in allergic inflammation and therefore have therapeutic potential.⁴³ Although it may not be feasible or cost effective to administer these proteins as long-term therapy, it may be possible to develop drugs that increase the release of these endogenous cytokines or activate their receptors and specific signal transduction pathways. IL-1 receptor antagonist binds to IL-1 receptors and blocks the action of IL-1 β . In experimental animals it reduces AHR,⁴⁴ and clinical studies are underway.⁴⁵

IL-10 is a potent anti-inflammatory cytokine that inhibits the synthesis of many inflammatory proteins, including cytokines (TNF- α , GM-CSF, IL-5, and chemokines) and inflammatory enzymes (iNOS) that are overexpressed in asthma.⁴⁶ Indeed, there may be a defect in IL-10 transcription and secretion from macrophages in asthma that may increase the intensity and duration of the inflammatory response.⁴⁷ In sensitized animals IL-10 is effective in suppressing the inflammatory response to allergen,⁴⁸ suggesting that IL-10 might be effective in atopic diseases. Recombinant human IL-10 has been shown to have some benefit in controlling inflammatory bowel disease, where similar cytokines are expressed, and is given as a weekly injection.⁴⁹ In the future, drugs that activate the unique signal transduction pathways activated by the IL-10 receptor or drugs that increase

endogenous production of IL-10 may be developed. In mice drugs that elevate cyclic adenosine monophosphate increase IL-10 production, but this does not appear to be the case in human cells.⁵⁰ Specific allergen immunotherapy may increase local IL-10 production.⁵¹

IFN- γ inhibits T_H2 cells and should therefore reduce atopic inflammation. In sensitized animals nebulized IFN- γ inhibits eosinophilic inflammation induced by allergen exposure.⁵² Administration of IFN- γ by nebulization to asthmatic patients did not significantly reduce eosinophilic inflammation, however, possibly because of the difficulty in obtaining a high enough concentration locally in the airways.⁵³ Interestingly, allergen immunotherapy increases IFN- γ production by circulating T cells in patients with clinical benefit⁵⁴ and increased numbers of IFN- γ -expressing cells in nasal biopsy specimens of patients with allergic rhinitis.⁵⁵

IL-12 is the endogenous regulator of T_H1 cell development and regulates the balance of T_H1 and T_H2 cells.⁵⁶ IL-12 administration to rats inhibits allergen-induced inflammation⁵⁷ and inhibits sensitization to allergens. IL-12 releases IFN- γ but has additional effects on T-cell differentiation. Recombinant human IL-12 has been administered to human subjects and has several toxic effects that are diminished by slow escalation of the dose.⁵⁸ In asthmatic patients IL-12 administration reduces circulating eosinophils but does not inhibit allergen responses or reduce AHR.⁵⁹ In addition, side effects, such as malaise, are common, thus precluding the use of systemic IL-12 for treatment of atopic diseases. In mice administration of an IL-12-allergen fusion protein results in the development of a specific T_H1 response to allergens with an increase in specific IgG2, rather than the normal T_H2 response with specific IgE formation.⁶⁰ This suggests the possibility of using IL-12 to provide a more specific immunotherapy, which might even be curative if applied early in the course of the atopic disease.

NEW ANTI-INFLAMMATORY DRUGS

There has been an intensive search for anti-inflammatory treatments that are as effective as corticosteroids but with fewer side effects. Apart from developing corticosteroids with a greater therapeutic ratio, other approaches have been to identify different classes of anti-inflammatory drugs.

PDE4 inhibitors

PDEs break down cyclic nucleotides that inhibit cell activation, and at least 9 families of enzymes have now been characterized. Theophylline has been used for a long time in asthma therapy and is a weak nonselective PDE inhibitor. PDE4 is the predominant family of PDEs in inflammatory cells, including mast cells, eosinophils, T lymphocytes, macrophages, and structural cells, such as sensory nerves and epithelial cells.⁶¹ This has suggested that PDE4 inhibitors would be useful as an anti-inflammatory treatment in atopic disease, particularly because there is some evidence for overexpression of

PDE4 in cells of atopic patients.⁶² In animal models of asthma, PDE4 inhibitors reduce eosinophil infiltration and AHR responses to allergen.⁶¹ Several PDE4 inhibitors have been now tested in asthma but with disappointing results. One PDE4 inhibitor, CDP840, had a marginal inhibitory effect on the late response to allergen but is not being further developed.⁶³ However, most of the PDE4 inhibitors so far tested clinically have had unacceptable side effects, particularly nausea and vomiting. These are the same side effects that have limited the use of theophylline, at least as a bronchodilator.

Several approaches might overcome these problems. It is possible that vomiting is due to inhibition of a particular subtype of PDE4. At least 4 human *PDE4* genes have been identified, and each has several splice variants.^{61,64} This raises the possibility that subtype-selective inhibitors may be developed that may preserve the anti-inflammatory effect, while having less likelihood of side effects. PDE4D appears to be of particular importance in inflammatory cells, such as T lymphocytes and eosinophils, and may be a more specific target,⁶⁵ and subtype-selective PDE4 inhibitors are now in development. There has recently been interest in PDE7 as a therapeutic target because antisense oligonucleotides that inhibit PDE7 expression inhibit the activation of T lymphocytes.⁶⁶ No selective PDE7 inhibitors have so far been reported, but high throughput screening is underway.

Transcription factor inhibitors

Transcription factors, such as NF- κ B and AP-1, play an important role in the orchestration of asthmatic inflammation,^{67,68} and this has prompted a search for specific blockers of these transcription factors. NF- κ B is naturally inhibited by the inhibitory protein I κ B, which is degraded after activation by specific kinases, particularly I κ B kinase β . Inhibitors of I κ B kinase β or the proteasome, the multifunctional enzyme that degrades I κ B, would thus inhibit NF- κ B, and there is a search for such inhibitors. There are some naturally occurring inhibitors of NF- κ B, such as the fungal product gliotoxin,⁶⁹ although this compound is too toxic for clinical application. There are concerns that inhibition of NF- κ B may cause side effects, such as increased susceptibility to infections, which has been observed in gene disruption studies when components of NF- κ B are inhibited.⁶⁷

Cyclosporin A and tacrolimus inhibit T-lymphocyte function by blocking the transcription factor nuclear factor of activated T cells by blocking activation of calcineurin.⁷⁰ This results in suppression of IL-2, IL-4, IL-5, and GM-CSF and therefore therapeutic potential in atopic diseases.

MAP kinase inhibitors

There are 3 major MAP kinase cascades, and there is increasing recognition that these pathways are involved in chronic inflammation and the expression of multiple inflammatory genes.⁷¹ There has been particular interest in the p38 MAP kinase pathway that is blocked by a novel class of drugs, the cytokine suppressant anti-inflammato-

ry drugs, such as SB203580 and RWJ67657. These drugs inhibit the synthesis of many inflammatory cytokines, chemokines, and inflammatory enzymes. Interestingly, they appear to have a preferential inhibitory effect on synthesis of T_H2 compared with T_H1 cytokines, indicating their potential application in the treatment of atopic diseases.⁷² Furthermore, p38 MAPK inhibitors have also been shown to decrease eosinophil survival by activating apoptotic pathways.⁷³ Whether this new class of anti-inflammatory drugs will prove to be safe in long-term studies remains to be established. However, as with corticosteroids, topical application is a possibility to limit toxicity. Inhibitors of the other major MAP kinase pathways (ERK and JNK) have also now been developed.

Tyrosine kinase inhibitors

Syk kinase is a protein tyrosine kinase that plays a pivotal role in signaling of the high-affinity IgE receptor (Fc ϵ RI) in mast cells, and in syk-deficient mice, mast cell degranulation is inhibited. This suggests that syk might be an important potential target for the development of mast cell-stabilizing drugs.⁷⁴ Syk is also involved in antigen receptor signaling of B and T lymphocytes and in eosinophil survival in response to IL-5 and GM-CSF,⁷⁵ and therefore syk inhibitors might have several useful beneficial effects in atopic diseases. Another tyrosine kinase, lyn, is upstream of syk, and an inhibitor of lyn kinase, PP1, has an inhibitory effect on eosinophil and mast cell activation.⁷⁶⁻⁷⁸ Because lyn and syk are widely distributed in the immune system, there must be concerns about the long-term safety of selective inhibitors, however. This may necessitate topical administration.

Cell-adhesion blockers

Infiltration of inflammatory cells into tissues depends on adhesion of circulating inflammatory cells to endothelial cells before migration to the inflammatory site.⁷⁹ This depends on specific glycoprotein adhesion molecules, including integrins and selectins, on both leukocytes and endothelial cells, which may be upregulated or show increased binding affinity in response to various inflammatory stimuli, such as cytokines or lipid mediators. Monoclonal antibodies that inhibit these adhesion molecules may prevent inflammatory cell infiltration. Thus an mAb to intercellular adhesion molecule 1 on endothelial cells prevents the eosinophil infiltration into airways and the increase in bronchial reactivity after allergen exposure in sensitized primates,⁸⁰ although this has not always been found in other species.⁸¹ The interaction between very late antigen (VLA) 4 and vascular cell adhesion molecule 1 is important for eosinophil inflammation, and humanized antibodies to VLA-4 (41) have been developed (Fig 2).⁸² Small molecule peptide inhibitors of VLA-4 have subsequently been developed that are effective in inhibiting allergen-induced responses in sensitized sheep.⁸³ Inhibitors of selectins, particularly L-selectin on the basis of the structure of sialyl-Lewis^x, inhibit the influx of inflammatory cells in response to inhaled allergen in sensitized sheep⁸⁴ and inhibit adhe-

sion of human eosinophils *in vitro*.⁸⁵ Although blocking adhesion molecules is an attractive new approach to the treatment of inflammatory disease, there may be potential dangers in inhibiting immune responses leading to more frequent infections or risks of neoplasia.

IMMUNOMODULATORS

Nonselective immunosuppressants

T lymphocytes may play a critical role in initiating and maintaining the inflammatory process in atopy through the release of cytokines that result in eosinophilic inflammation, suggesting that T-cell inhibitors may be useful in controlling asthmatic inflammation. The nonspecific immunomodulator cyclosporin A reduces the dose of oral steroids needed to control asthma in patients with severe asthma,⁸⁶ but its efficacy is limited,⁸⁷ and side effects, particularly nephrotoxicity, limit routine clinical use. The possibility of using inhaled cyclosporin A is now being explored because in animal studies the inhaled drug is effective in inhibiting the inflammatory response in experimental asthma.⁸⁸ Immunomodulators, such as tacrolimus (FK506) and rapamycin, appear to be more potent and may be less toxic. Topical tacrolimus appears to be effective in atopic dermatitis and is well tolerated.⁸⁹ Novel immunomodulators that inhibit purine or pyrimidine pathways, such as mycophenolate mofetil, leflunomide, and brequinar sodium, may be less toxic and therefore of greater potential value in long-term therapy.⁹⁰

One problem with nonspecific immunomodulators is that they inhibit both T_H1 and T_H2 cells and therefore do not restore the imbalance between these T_H1 and T_H2 cells in atopy. They also inhibit suppressor T cells (T_C1 cells) that may modulate the inflammatory response. Selective inhibition of T_H2 cells may be more effective and better tolerated, and there is now a search for such drugs.

T_H2 cell inhibitors

Nonselective T-cell suppressants, such as cyclosporin A, may be relatively ineffective in asthma because they inhibit all types of T cells indiscriminately. $CD4^+$ T cells have been implicated in asthma, and a chimeric antibody directed against $CD4^+$ (keliximab) that reduces circulating $CD4^+$ cells appears to have some beneficial effect in asthma,⁹¹ although long-term safety of such a treatment might be a problem. Furthermore, there is increasing evidence that $CD8^+$ cells (T_C2 cells), through the release of IL-5 and other cytokines, might also be involved in atopic diseases, particularly in response to infections with viruses.⁹² There has been a search for selective inhibitors of T_H2 cells by identifying features that differentiate T_H1 and T_H2 cells. The transcription factor GATA-3 appears to be of particular importance in murine and human T_H2 cells^{93,94} and may be a target for selective immunomodulatory drugs. However, an argument against strategies to control atopic disease by targeting T_H2 cells is that chronic stimulation (by exposure to allergen) results in cells that are relatively resistant to immune suppression.⁹⁵

SPECIFIC ANTIALLERGIC DRUGS

Although corticosteroids are very effective in controlling atopic diseases, there are continuing concerns about systemic side effects when high doses are needed. This has prompted a search for more selective anti-inflammatory agents that would selectively target the atopic disease process.

Cromones

Cromones (cromolyn sodium and, more recently, nedocromil sodium) are the most specific antiallergic drugs discovered and have been used in the treatment of all atopic diseases since their discovery in the 1960s. Topical application is effective in asthma, rhinitis, and allergic conjunctivitis, but the effects are less marked than those seen with topical steroids, and they are only effective in mild disease. Their clinical benefit has also been limited by a short duration of action. Cromones appear to have a specific action on allergic inflammation, yet their molecular mechanism of action remains obscure. Although it was initially believed that the primary mode of action of cromones involves inhibiting mast cell mediator release, it has now been demonstrated that they affect several other inflammatory cells and sensory nerves. There is increasing evidence that cromones may act on certain types of chloride channels that are expressed in mast cells and sensory nerves.⁹⁶ Cromolyn phosphorylates a specific cytoskeletal protein moesin in mast cells, indicating a possible mechanism that may inhibit degranulation.⁹⁷ Both cromolyn and nedocromil sodium must be given topically, and all attempts to develop orally active drugs of this type have been unsuccessful, possibly suggesting that topical administration is critical to their efficacy.

The potent diuretic furosemide mimics many of the effects of cromones in inhibiting indirect bronchoconstrictor challenges (allergen, exercise, cold air, adenosine, and metabisulfite) but not direct bronchoconstriction (histamine, methacholine, and prostaglandins) when given by inhalation.^{98,99} The mechanism of action of furosemide is not shared by the more potent loop diuretic bumetanide, indicating that some other mechanism than the inhibition of the $Na^+/K^+/Cl^-$ cotransporter must be involved. This is most likely to involve inhibition of the same chloride channel that is inhibited by cromones. Furosemide itself does not appear to be very effective when given regularly by metered-dose inhaler in asthma,¹⁰⁰ but it is possible that more potent and long-lasting chloride channel blockers might be developed in the future.

Costimulation inhibitors

Costimulatory molecules may play a critical role in augmenting the interaction between antigen-presenting cells and $CD4^+$ T lymphocytes. The interaction between B7 and CD28 may determine whether a T_H2 -type cell response develops, and there is some evidence that B7-2 (CD86) skews toward a T_H2 response (Fig 3). Blocking antibodies to B7-2 inhibit the development of specific IgE, pulmonary eosinophilia, and AHR in mice, whereas anti-

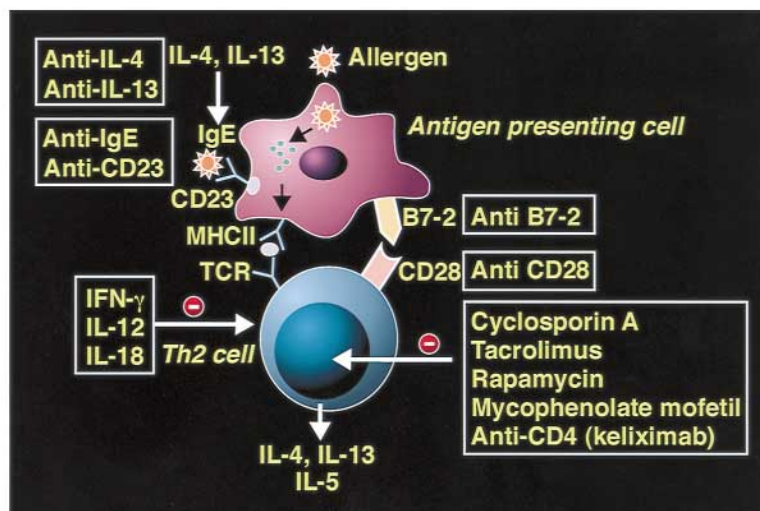


FIG 3. Inhibition of antigen-presenting cells and T_H2 lymphocytes. Therapies are based on inhibition of co-stimulatory molecules (B7-2, CD28) and inhibition of IgE-driven antigen-presenting cells, nonselective immunomodulators, or cytokines that tip the balance away from T_H1 cells toward T_H2 cells (IFN- α , IL-12, and IL-18). TCR, T-cell receptor. Reprinted by permission from *Nature Supplement* 402:B31-8, copyright 1999 Macmillan Magazines Ltd.

bodies to B7-1 (CD80) are ineffective.¹⁰¹ A molecule on activated T cells (CTLA4) appears to act as an endogenous inhibitor of T-cell activation, and a soluble fusion protein construct (CTLA4-Ig) is also effective in blocking AHR in a murine model of asthma.¹⁰² Anti-CD28, anti-B7-2, and CTLA4-Ig all block the proliferative response of T cells to allergen,¹⁰³ indicating that these are potential targets for novel therapies that might be effective in atopic diseases.

Anti-IgE

Strategies to block IgE are an obvious approach to the more specific treatment of atopic diseases and are discussed in detail by de Vries (this series). Clinical studies with a humanized murine mAb (E25) directed to the high-affinity IgE receptor (Fc ϵ RI) show reduction in early and late responses to inhaled allergen and eosinophil counts in induced sputum.¹⁰⁴ Recent studies in patients with moderate and severe asthma have demonstrated significant steroid-sparing effects. In patients with severe asthma who require oral steroids, there was a significant reduction in oral steroid requirements, and several patients were able to completely withdraw oral steroids in comparison with placebo.¹⁰⁵ This represents an important advance in therapy. Although injections of antibody may not be feasible for the long-term treatment of mild asthma, this could be a realistic therapy for patients with more severe forms of asthma or atopic dermatitis, in whom high IgE levels may be found.¹⁰⁶

Allergen may also signal through a low-affinity IgE receptor (Fc ϵ R2, CD23), which is expressed on B lymphocytes, macrophages, and eosinophils. An anti-CD23 Fab fragment blocks allergen-induced airway inflammation in mice, suggesting that this might be a therapeutic approach to inhibit allergic responses.¹⁰⁷ However, E25 also results in reduced signaling through CD23, and this may contribute to its clinical efficacy.

PREVENTIVE STRATEGIES

Atopy appears to be due to immune deviation from T_H1 to T_H2 cells, which may arise because of a failure to inhibit the normal T_H2 preponderance at birth. This may be due to an absence of the environmental factors, such as bacterial infections and endotoxins, that stimulate the T_H1 response.

Immunotherapy

Subcutaneous injection of small amounts of purified allergen has been used for many years in the treatment of allergy. It is effective in the treatment of insect venom anaphylaxis and hay fever and may induce prolonged remission¹⁰⁸ but is less effective in asthma. The molecular mechanism of desensitization is still uncertain. Cloning of several common allergen genes has now made it possible to prepare recombinant allergens for injection, although this purity may detract from their allergenicity because most natural allergens contain several proteins. Intramuscular injection of rats with plasmid DNA expressing house dust mite allergen results in its long-term expression and prevents the development of IgE responses to inhaled allergen.¹⁰⁹ This suggests that allergen gene immunization might be a useful specific therapeutic strategy in the future. The major allergens of peanuts, which are responsible for an increasing number of severe anaphylactic reactions, have been cloned. Oral administration of microparticles of DNA complexed to chitosan in mice produces intestinal epithelial expression of the peanut allergen and prevention of anaphylaxis after oral challenge.¹¹⁰

Peptide immunotherapy

Small peptide fragments of allergen (epitopes) are able to block allergen-induced T-cell responses without inducing anaphylaxis.¹¹¹ T cell-derived peptides from cat aller-

gen (Fel d 1) appear to be effective in blocking allergen responses to cat dander¹¹² but may induce an isolated late response to allergen by means of direct T-cell activation.¹¹³

Vaccination

A relative lack of infections may be a factor predisposing to the development of atopy in genetically predisposed individuals, leading to the concept of vaccination to induce protective T_H1 responses to prevent sensitization and thus prevent the development of atopic diseases. BCG vaccination has been associated with a reduction in atopic diseases in Japan,¹¹⁴ but this has not been confirmed in a Swedish population.¹¹⁵ BCG inoculation in mice 14 days before allergen sensitization reduced the formation of specific IgE in response to allergen and the eosinophilic response and AHR responses to allergen, with an increase in production of IFN- γ .¹¹⁶ This has led to several clinical trials of BCG vaccination to prevent the development of atopy. Similar results have been obtained in mice with a single injection of heat-killed *Mycobacterium vaccae*, another potent inducer of T_H1 responses,¹¹⁷ and with *Listeria* species. *Lactobacillus acidophilus* in yogurt, another potential means of tipping back the balance from T_H2 to T_H1 cells, weakly increases IFN- γ formation in adult asthmatic patients.¹¹⁸ Immunostimulatory DNA sequences from bacteria, such as unmethylated cytosine guanosine dinucleotide-containing oligonucleotides, are also potent inducers of T_H1 cytokines. In mice administration of cytosine guanosine dinucleotide-containing oligonucleotides increases the ratio of T_H1 to T_H2 cells, decreases formation of specific IgE, and reduces the eosinophilic response to allergen, an effect that lasts for over 6 weeks.¹¹⁹ These promising animal studies encourage the possibility that vaccination might prevent or cure atopic diseases in the future.

GENE THERAPY

Because atopic diseases are polygenic, it is unlikely that gene therapy will be of value in the long term. However, understanding the genes involved in atopic diseases and in disease severity may identify new molecular targets and may also predict the response to different forms of therapy (pharmacogenetics). Transfer of anti-inflammatory genes may provide specific anti-inflammatory or inhibitory proteins in a convenient manner, and gene transfer has been shown to be feasible in animals by using viral vectors.¹²⁰ Anti-inflammatory proteins relevant to asthma include IL-10, IL-12, and I κ B. Antisense oligonucleotides may switch off specific genes, but there are considerable problems in getting these molecules into cells. An inhaled antisense oligonucleotide directed against the adenosine A_1 receptor has been shown to reduce AHR in a rabbit model of asthma, demonstrating the potential of this approach in treating asthma.¹²¹ Suitable target genes may be *IL4* or *IL5*. Considering the practical problems encountered by gene therapy, this approach is unlikely in the foreseeable future, other than for proof of concept studies.

CONCLUSIONS

Many different therapeutic approaches to the treatment of atopic diseases may be possible, yet there have been few new drugs that have reached the clinic. Topical glucocorticoids are very effective as chronic treatment in atopic diseases and suppress the underlying inflammatory process. An advance in therapy would be the development of more specific antiallergic drugs that lack side effects. If these treatments can be taken orally, this would treat asthma, rhinitis, and eczema, which often coincide. The possibility of developing a cure for atopy is remote, but strategies to inhibit the development of sensitization in early childhood offer such a prospect in the future.

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