New targets for drug development in asthma

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Asthma is a chronic inflammatory disease that affects about 300 million people worldwide, a total that is expected to rise to about 400 million over the next 15–20 years. Most asthmatic individuals respond well to the currently available treatments of inhaled corticosteroids and β-adrenergic agonists; however, 5–10% have severe disease that responds poorly. Improved knowledge of asthma mechanisms has led to the recognition of different asthma phenotypes that might reflect distinct types of inflammation, explaining the effectiveness of anti-leucotrienes and the anti-IgE monoclonal antibody omalizumab in some patients. However, more knowledge of the inflammatory mechanisms within the airways is required. Improvements in available therapies—such as the development of fast-onset, once-a-day combination drugs with better safety profiles—will occur. Other drugs, such as inhaled p38 MAPK inhibitors and anti-oxidants, that target specific pathways or mediators could prove useful as monotherapies, but could also, in combination with corticosteroids, reduce the corticosteroid insensitivity often seen in severe asthma. Biological agents directed against the interleukin-13 pathway and new immunoregulatory agents that modulate functions of T-regulatory and T-helper-17 cells are likely to be successful. Patient-specific treatments will depend on the development of discriminatory handprints of distinct asthma subtypes and are probably over the horizon. Although a cure is unlikely to be developed in the near future, a greater understanding of disease mechanisms could bring such a situation nearer to reality.

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Introduction

Asthma is one of the most common chronic inflammatory diseases, affecting about 300 million people worldwide, a total that is expected to rise by an additional 100 million—mainly in children—over the next 15–20 years.[1] Asthma accounts for about one out of every 250 deaths worldwide and has profound health-care costs in terms of emergency room visits and hospitalisations. Asthma also has enormous indirect costs and is one of the leading causes of work and school absenteeism.[2] Most patients with asthma respond well to current treatments; however, 5–10% of patients have severe disease that often fails to respond to conventional therapy; these patients account for more than 50% of the total health-care costs associated with asthma.[3]

By understanding the different types of airway inflammation in various subtypes of asthma, it should be possible to address some of the important questions in asthma research: which triggers or factors underlie airway smooth muscle hyper-responsiveness? What are the processes (genetic or environmental) that underlie different subtypes of asthma? Which aspects of airway remodelling are important in disease subtypes? What are the best biomarkers of disease progression or treatment response? Why are some patients less responsive to conventional therapies than are others? It is now recognised that there are distinct asthma phenotypes[4] and that distinct therapeutic approaches may only impinge on some aspects of the disease process, or at least outcome measures, within each subgroup. Thus, treatments might affect exacerbation rates without altering day-to-day symptoms or lung function, reflecting the fact that distinct cells or mediators in the lung could drive airway hyper-responsiveness or specific inflammatory components, and that treatments directed at a single cell or mediator might only affect a single aspect of disease.[5] The current reductionist approach to understanding the disease has led to the development of drugs that target specific pathways or mediators. In the future, we may need to target and assess several outcome measures and biomarkers simultaneously, and undertake subgroup analyses of the responses obtained when assessing combinations of new drugs.

Current asthma treatments

Initial approaches to treat asthma emphasised the relief of bronchoconstriction with bronchodilators, particularly β_2-adrenergic agonists, but the discovery of airway inflammation as an important pathophysiological component of asthma has led to the use of inhaled corticosteroids as the mainstay of asthma therapy[6]—these drugs are the most effective anti-inflammatory treatment available for asthma. Asthmatic inflammation is characterised by eosinophilia, mast cell infiltration,

Search strategy and selection criteria

We undertook a detailed appraisal of peer-reviewed publications over the past 10 years with the NCBI PubMed website for English language publications with the keywords: "asthma", in combination with "treatment", "novel therapy", "glucocorticoid insensitivity", "new drugs", "steroid-sparing", "severe", "immunomodulation", and "unmet need". We also searched the reference lists of articles identified by this search strategy and selected those we judged relevant. We also had source publications that we have accumulated because of our association with asthma therapy and research in the past 15 years. Review articles are cited to provide readers with more details and more references than this Review has room for. Our reference list was modified on the basis of comments from peer reviewers.

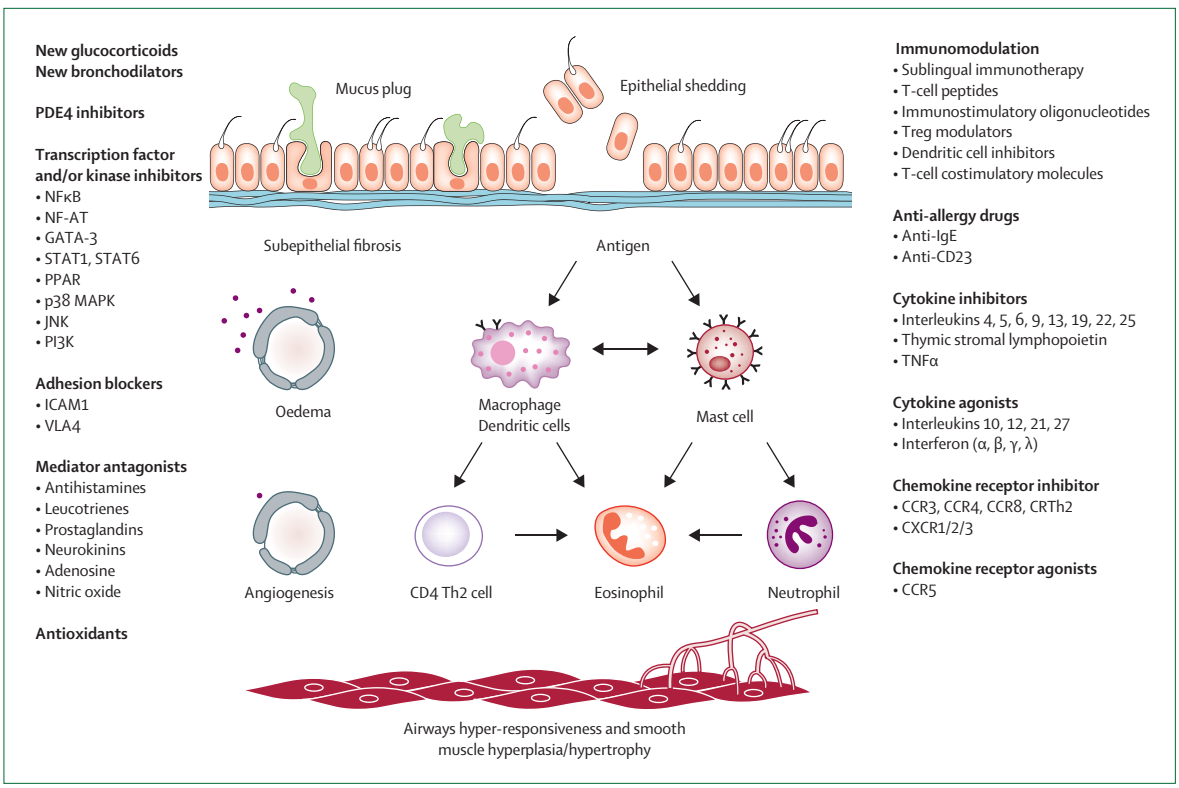

Figure 1: **Potential targets for new asthma drugs**
Even before asthma becomes symptomatic, exposure to an allergen produces structural changes to the airways, including subepithelial fibrosis and epithelial denudation. Inhaled allergens encounter antigen-presenting cells (dendritic cells, macrophages) that line the airways and mast cells which release mediators, such as histamine. The antigen-presenting cells migrate to the lymph nodes, where they induce the proliferation and activation of CD4 T lymphocytes (Th2 cells). These cells express a variety of cytokines and chemokines, under the control of intracellular kinases and downstream transcription factors, which lead to the differentiation, migration, and pathobiological effects of eosinophils. These inflammatory processes lead to pathological effects, such as airway hyper-responsiveness and airway remodelling including vasodilatation and angiogenesis. Neutrophils are increasingly important in severe asthma. As such asthma is a highly complex disease that involves many inflammatory cells, mediators, inflammatory proteins, and intracellular pathways. ICAM=intercellular adhesion molecule. JNK=c-Jun N-terminal kinase. MAPK=mitogen activated protein kinase. NFκB=nuclear factor κB. NF-AT=nuclear factor activated T cells. PDE=phosphodiesterase. PI3K=phosphoinositide-3 kinase. PPAR=peroxisome proliferator-activated receptor. STAT=signal transducer and activator of transcription. TNF=tumour necrosis factor. Treg=T-regulatory cells. VLA=very late antigen.

and activation of T-helper (Th) 2 cells that express interleukins 4, 5, and 13. Structural changes such as goblet cell hyperplasia, airway smooth muscle hypertrophy and hyperplasia, along with subepithelial fibrosis are also present, even in mild disease, although these features may be more prominent in severe disease (figure 1). The functional effects of these structural changes are unclear, but they might contribute to chronic airflow obstruction and reduced airway responses to bronchodilators or inhaled corticosteroids.[7]

Long-term treatment with inhaled corticosteroids reverses airflow obstruction, reduces exacerbations and the need for hospitalisation, and improves quality of life. Inhaled corticosteroids may have also contributed to the reduction in asthma-related deaths over the past few years.[8] Concerns about the long-term detrimental effects of high-dose inhaled corticosteroids therapy include cataracts, osteoporosis in elderly patients, and stunting of growth in children,[9] but there is a high safety margin for most patients on low to moderate doses of inhaled corticosteroids. Compliance with inhaled corticosteroids is poor, possibly due to a misunderstanding of the side-effects and an inability to maintain a daily regime, especially when symptoms are well controlled.[10] However, inhaled corticosteroids may not modify the progression of asthma and are not curative, because asthma symptoms and inflammation recur on discontinuation of treatment.[11]

The effectiveness of inhaled corticosteroids—particularly at low to moderate doses—in controlling asthma and reducing exacerbations is improved by combination with long-acting β_2 agonists and is better than that shown by higher doses of inhaled corticosteroids alone.[12] Thus, combinations of inhaled corticosteroids and long-acting β_2 agonists are used in the treatment of moderate-to-severe asthma[12] and such combinations have now become established as the most efficacious treatment.[1] Because of the rapid action of formoterol compared with salmeterol, it is possible to use the combination of formoterol and budesonide or formoterol and beclomethasone as both maintenance and reliever medication. An alternative approach to asthma control

has been taken with the combination of salmeterol and fluticasone, which has been shown to achieve comprehensive asthma control in most patients.[13] It must be emphasised that long-acting β_2 agonists are not recommended as monotherapy maintenance treatment for asthma.

The need for new therapies

Combinations of inhaled corticosteroids and long-acting β_2 agonists are effective in most (about 90%), but not all, asthmatic individuals.[14] Indeed, even patients whose asthma is apparently well controlled by existing therapies might benefit from more efficacious therapies that are easier to comply with.[11] Surprisingly, telephone surveys of asthmatic patients have reported a high degree of morbidity among patients who report that their asthma was controlled,[11] which could reflect either a lack of compliance or that existing medications are not as efficacious in day-to-day practice as they are in controlled trials. Improved compliance in the mild-to-moderate asthmatic patient could be achieved by the development of safe oral versions of conventional treatments or of new, more efficacious treatments, particularly if these agents altered the course of the disease or pointed towards a cure.

The drive to find new drug targets has led to the introduction of two new classes of anti-mediator agents that are now in clinical practice, namely the leucotriene inhibitors and anti-IgE monoclonal antibodies, which are used in mild-to-moderate and severe allergic asthma, respectively.[1] The development of these drugs reflect the emphasis on specific anti-mediator agents over the past two decades and the discovery that many classes of mediators are seemingly not important in the pathogenesis of asthma. However, there has been a widening of potential targets for asthma treatments, which now include inflammatory and immune cytokines and chemokines, transcription factors, enzymes, and immune cells (figure 1).[4]

Furthermore, different subtypes of asthma occur in children and in adults and even within children and adults,[15] resulting in distinct patterns of treatment sensitivity. A complete analysis of each patient's genome, proteome, and kinome is beyond current technology but could eventually lead to patient-centred rational therapy and highlights why some treatments are only effective in small subsets of patients.

Patients with poorly controlled severe asthma who do not respond well to combination therapy are another major unmet need. One characteristic of this group is relative corticosteroid insensitivity.[7] These patients may have corticosteroid-dependent, or more rarely, corticosteroid-resistant asthma.[7] These corticosteroid-resistant patients are a subset of those patients with severe asthma, but one must note that these terms are not interchangeable because some corticosteroid-resistant patients do not have severe disease and some patients with severe asthma are not treatment-insensitive.[16] The US Severe Asthma Research Program (SARP)[16] and the European Network For Understanding Mechanisms Of Severe Asthma (ENFUMOSA)[17] have highlighted the need to understand the molecular and cellular mechanisms present in severe asthma to enable the identification of novel targets for asthma and to speed up the drug development process.[18] Clarification of the mechanisms that relate to disease subtypes in corticosteroid-resistant patients might not only indicate selective responsiveness to novel therapies in severe asthma but also point to similar subtypes and treatment responsiveness in patients with mild or moderate asthma.

It has been observed recently that the thickness of the airway epithelium, of the sub-basement membrane, and of the airway smooth muscle in the airways of patients with severe asthma is greater than in those with non-severe asthma.[19,20] These differences were associated with altered expression of markers of epithelial proliferation.[19] Simultaneously, a number of unbiased techniques such as hierarchical clustering of bronchoalveolar lavage cytokine expression,[21] cluster analysis of inflammation and responses to treatment,[22] the analysis of volatile organic components of exhaled breath using an electronic nose,[23] and the analysis of bronchial biopsy samples[24] have supported the possibility of several distinct phenotypes of patients with severe asthma. Targeting nodal points in these clusters may highlight potential novel sites for drug intervention.

Improvement of current therapies

New longer-acting bronchodilators

The long-acting β_2 agonists salmeterol and formoterol have a 12-h bronchodilator effect and, in conjunction with inhaled corticosteroids, improve asthma control and reduce exacerbation rates.[25] Several ultra-long-acting β_2 agonists that are under development, including indacaterol, carmoterol (Chiesi Farmaceutici, Italy), and GSK159797, act for more than 24 h, are fast acting, and are suitable for once-daily dosing.[26] It is also possible to increase the duration of action of inhaled corticosteroids which, in combination with once-a-day fast-onset β_2 agonists, will result in a combination therapy with prolonged action that, although possibly no more efficacious than current drugs, will improve compliance.[26] Although other bronchodilators exist—eg, vasoactive intestinal peptide, neuropeptide analogues, and K^+-channel openers—these drugs have proved difficult to develop because of their potent vasodilator effects.[27]

New corticosteroids with reduced systemic side-effects

Lung absorption of inhaled corticosteroids contributes to the systemic side-effects that are seen with such drugs, particularly with higher doses. Various means to reduce these systemic side-effects have been developed, including systemic or local inactivation, administration of an inactive prodrug that is only converted to active

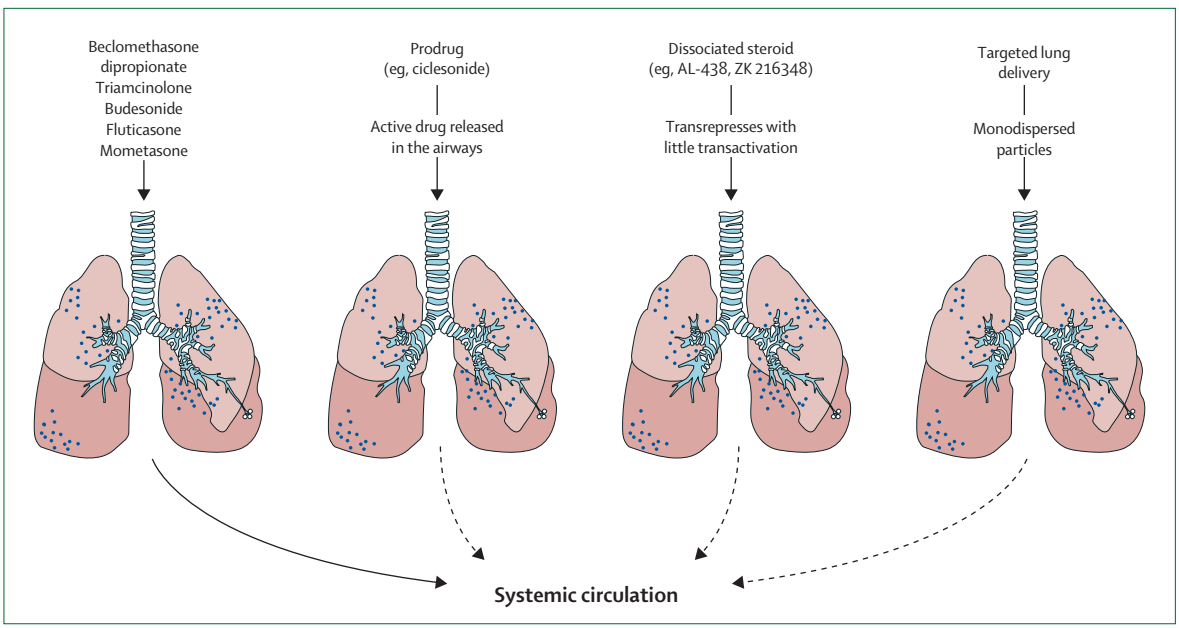

Figure 2: **Approaches to reduce the side-effects of inhaled corticosteroids**
Several approaches are being taken to reduce systemic absorption including the use of pro-drugs such as ciclesonide, which is activated to release the active form des-ciclesonide only in the lungs, and the use of corticosteroids that are able to repress inflammatory gene expression without affecting the induction of corticosteroid-inducible genes, many of which are responsible for the side-effects. Alternatively, it may be possible to deliver monodispersed corticosteroid particles to specific parts of the airway using new delivery devices, thereby reducing the dose of inhaled drug required to obtain the equivalent clinical benefit.

drug in the airways (eg, ciclesonide[28]), or targeted airway deposition using monodispersed particles (figure 2).[29] Another approach has been to develop dissociated corticosteroids that do not activate the mechanisms that lead to side-effects while preserving therapeutic anti-inflammatory effects. Many of the detrimental side-effects of corticosteroids have been linked to transactivation (ie, activation of gene transcription through DNA binding of the activated glucocorticoid receptor) whereas most beneficial effects are associated with transrepression (ie, activated glucocorticoid receptor binding to other transcription factors rather than to DNA sites), although this mechanism requires confirmation in human beings.[9,30,31] Dissociated corticosteroids may be just as effective as conventional inhaled corticosteroids, have a better safety profile, and might even lead to safer oral corticosteroids.[30] Linking the development of dissociated corticosteroids to drugs with a non-steroidal backbone such as AL-438 (Abbott Ligand) and ZK 216348 (Bayer-Schering Pharma) may further improve the therapeutic index, because these drugs will also lack activity at other nuclear hormone receptors which also cause side-effects.[32] Lastly, the addition of an NO-donating group to prednisolone (NCX1015, NiCox Research Institute, Italy) and budesonide (NCX1020, NiCox Research Institute) has resulted in improved corticosteroid efficacy against lipopolysaccharide-induced inflammatory responses and prevented lipopolysaccharide-induced airway hyper-responsiveness, eosinophilia, and neutrophilia in guineapigs. By contrast, the parent compounds had only a small effect on eosinophilia.[33,34] In a similar manner, NO groups have been tagged to β_2 agonists.[35] The NO moiety is probably donated to specific residues within the respective ligand binding domains, thereby affecting receptor function.

Lipid mediator blockade

Many inflammatory mediators have been implicated in asthma and specific inhibitors and antagonists against many of these have been developed (figure 1, table). However, the only mediator antagonists that are currently used in asthma therapy are antileucotrienes, which block cysteinyl-leucotriene receptors or the synthesis of leucotriene.[36] Despite initial promise, inhibitors of other pro-inflammatory mediators, including histamine, prostaglandins, platelet-activating factor, bradykinin, and tachykinin, have proved ineffective.[27]

Cysteinyl-leucotriene receptor antagonists, such as montelukast, improve lung function and asthma symptoms in patients with mild to moderate asthma, but their efficacy is reduced compared with low-dose inhaled corticosteroids. Moreover, such agents are less effective in severe asthma,[37] although a recent study suggests that they may be beneficial in asthmatic patients who smoke.[38] Zileuton, a 5-lipoxygenase inhibitor that blocks the synthesis of cysteinyl-leucotrienes and leucotriene B4, provides similar efficacy to montelukast in asthma. These compounds need to be tested in neutrophilic asthma, because they would also inhibit neutrophilic chemotaxis and activation. Levels of lipoxin A4, an endogenous anti-inflammatory eicosanoid, are reduced in severe

	Function	Drug and stage of development
β_2 adrenergic receptor	Ultra-long bronchodilation	Indacaterol (phase II), carmoterol (phase II), GSK159797 (phase II)
Glucocorticoid receptor	Anti-inflammatory	GSK685689 (phase II), GSK870086 (phase II), AL-438 (phase I), ZK 216348 (phase I)
PGD2/CRTh2 inhibitors	Th2 cell recruitment and activation	TM30089, ODC9101 (phase II), AZD1981 (phase II), ramatroban (phase II)
BLT1 antagonist	Mononuclear/granulocyte recruitment	CP-105696 (phase I), LY293111 (phase II, no effect against allergen challenge)
CCL11	Blocks eosinophil recruitment/activation	CAT-213 (preclinical)
CCR3	Blocks eosinophil recruitment/activation	Met-RANTES (phase II, moderate/severe asthma)
CXCR4	Blocks Th2 activation	AMD070, AMD3100, SP01A (all preclinical for asthma, all phase II HIV, AMD3100 phase III for multiple myeloma)
CXCR1/2	Blocks neutrophil recruitment/activation	Repertaxin (preclinical, phase II for graft *vs* host disease)
Interleukin 5	Blocks eosinophil recruitment/activation	MEDI-563 (phase I, severe asthma), mepolizumab (phase II)
Interleukin 12	..	Interleukin 12 (phase II, no effect on lung function, adverse side-effects, not developed further)
Interleukin 10	Endogenous anti-inflammatory agent	Interleukin 10 (preclinical for asthma, approved for psoriasis/Crohn's disease, recruited in 1999 for asthma)
Interferon γ	..	Interferon γ (phase II, no effect on lung function in severe asthma, not developed further)
Interleukin 13	Key driver of asthmatic inflammation	Pitrakinra (interleukin-4/13 mutein), CAT-354, IMA-638 (both in phase II)
VLA4 antagonist	Adhesion molecule blocker	GW-559090, IVL745, CDP323 (CDP323 phase II, not developed)
PDE4	Anti-inflammatory	GSK256066 (phase II)
p38 MAPK	Anti-inflammatory	GSK681323, GSK856553, VX-745, BIRB-796, Ro-320-1195, Scio-469 (all in phase II), SB2439063, RWJ-67657
JNK	Anti-inflammatory	SP600125, CC-401, CNI-1493 (dual JNK/p38 MAPK) (all in preclinical for asthma; CC-401 and CNI-1493 in phase II in rheumatoid arthritis and Crohn's disease)
SYK	Mast cell degranulation, T-cell and B-cell function	Antisense (preclinical), BAY61-3606 (preclinical), R343 (phase I)
IKK2	Anti-inflammatory	AS206868, SC-514, BMS345541, TPCA-1 (all preclinical, MLN0415 [phase I])
CD23	Reduces IgE	Lumiliximab (phase I)
Sphingosine-1 phosphate receptor	Prevents dendritic cell activity	FTY720 (preclinical for asthma, Phase II for multiple sclerosis and transplant rejection)
DP1	Prevents dendritic cell activity	BW245C (preclinical)
VDR	Increased interleukin-10 expression in Treg cells	Vitamin D3 (phase II, steroid sparing)

Table: **Summary of some of the compounds in development for asthma**

asthma[39] and treatment with this compound might be considered as an anti-inflammatory agent for these patients.[39]

Recently, the gene that encodes 5-lipoxygenase-activating protein (FLAP) has been linked to risk for myocardial infarction, stroke, and restenosis of the coronary artery. FLAP inhibitors showed some efficacy in early clinical trials in asthma but were not developed commercially because of efficacy and safety concerns, but new safer FLAP inhibitors such as DG031 (DeCode Genetics, Iceland) could be useful in patients with distinct genetic or phenotypic backgrounds, especially as a steroid add-on therapy in the treatment of asthma exacerbations.[40]

Prostaglandin PGD2, produced by mast cells, acts mainly through the G-protein-coupled receptor DP2 (also known as CRTh2, chemoattractant receptor-homologous molecule expressed on Th2 lymphocytes) to mediate Th2 recruitment and activation.[41] Ramatroban, a thromboxane receptor antagonist and a partial DP2 antagonist, is used to treat perennial rhinitis in Japan[41] and more selective and potent DP2 antagonists are being developed for asthma and allergic rhinitis. For example, TM30089 (7TM Pharma, Denmark) is effective in animal models of asthma and a once daily oral molecule, ODC9101 (Oxagen, UK), is now in phase IIa clinical trials for asthma.[41]

Leucotriene B4, acting through its receptor BLT1, is chemoattractive for neutrophils, eosinophils, monocytes, and macrophages. The number of BLT1-expressing T cells is increased in asthma and BLT1 antagonists prevent airway inflammation and airway hyper-responsiveness in animal models.[42] However, the BLT1 antagonist LY293111 (Eli Lilly, USA) had no effect on allergen challenge in a small group of asthmatic patients,[43] although this has not been tested in patients with neutrophilic asthma.

Targeting chemokines and their receptors

Recruitment of inflammatory cells into the airway by chemokines is a crucial process in the development of asthma. Chemokines are classified into four families

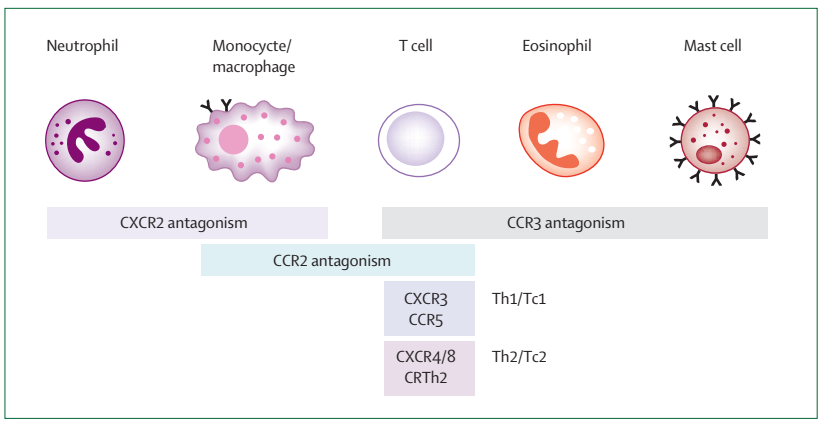

Figure 3: **Chemokine receptor modulation in asthma**
Prevention of inflammatory cell migration into the airways might be achieved by modulation of chemokine receptors. CCR3 antagonists would be predicted to prevent recruitment and activation of T cells, eosinophils, and mast cells in the airways, whereas CXCR2 antagonism would target monocytes/macrophage and neutrophil infiltration. Antagonism of the CCR2 receptor or biological agents against its ligands would prevent mast cell, monocytes/macrophage, and T-cell effects in asthma. Selective targeting of Th1/Tc1 or Th2/Tc2 cells could be achieved with CXCR3 and CCR4/8 or CRTh2 (chemoattractant receptor-homologous molecule expressed on Th2 lymphocytes) antagonism, respectively, or by activation of CCR5 in the case of Th1/Tc1 cells.

based on a conserved cysteine motif—C, CC, CXC, or CX3C—and act through specific receptors according to the ligand class.[42] The major focus of interest in asthma has been CCR3 and its ligands (CCL11, CCL24, and CCL26), which are increased in asthma[44] and mediate eosinophil recruitment[42] (figure 3). CCR3 is also expressed on mast cells and some Th2 cells. CCL11 is increased temporally before induction of CCL24 and CCL26 after allergen challenge.[45] Targeting of CCR3 would therefore be more reasonable than targeting its ligands, as is the case with most chemokine receptors. Antisense oligonucleotides that inhibit CCR3 and interleukin-5 receptor led to a reduction in sputum eosinophilia after allergen challenge associated with an inhibition of the early response, but only a trend for a reduced late response was noted.[46] Furthermore, inhibitors of CCR3 have been effective in inhibiting eosinophilic inflammation, airway hyper-responsiveness, and goblet cell hyperplasia in mouse models[47,48] and are currently undergoing clinical trials for asthma.

Other chemokines may be important in asthma. The expression of CCL17 and CCL22[49] and the number of CCR4-expressing Th2 cells is increased in asthmatic airways after allergen challenge,[50] and antibodies directed against CCL17 and CCL22 are effective in animal models of asthma.[42] Additionally, CXCL8, acting through CXCR1, is important for neutrophil chemotaxis and activation; the expression of CXCR1/2 and its ligands is increased in biopsy samples obtained from asthmatic patients during severe exacerbations that required mechanical ventilation.[51] CXCR1/2 antagonists or biological agents could therefore be particularly effective in the treatment of severe exacerbations or in patients with severe asthma with evidence of neutrophilia.[51,52] CCR2, which is expressed on monocytes and T cells, and CCR4 and CCR8, which are expressed on Th2 cells, are also targets for asthma therapy[42] (figure 3).

Lastly, the glycosaminoglycan heparin is co-released with histamine from mast cells and can, in addition to its well-described anticoagulant properties, combine with chemokines to prevent their action and prevent exercise-induced bronchoconstriction.[53] A phase II study in patients with mild asthma using IVX 0142 (IVAX Research, USA), a novel heparin-derived oligosaccharide, has just been completed (ClinicalTrials.gov, number NCT00232999).

Targeting Th2-derived and inflammatory cytokines

Cytokines are major targets for new asthma therapies because of their key role in chronic inflammation and in remodelling of the airway.[27] The concept of asthma as a Th2-driven disease and the demonstration that inhibition of Th2-derived cytokines in many animal models of asthma prevented all aspects of disease drove the development of antagonists and antibodies directed against interleukins 4 and 5 (figure 1). However, these findings have not been replicated in asthmatic patients, illustrating the limitations of the animal models used.

Interleukin 5 is critical for terminal differentiation of eosinophils and for eosinophilic inflammation; however, a blocking antibody to interleukin 5 had no effect on airway hyper-responsiveness, lung function, or exacerbation frequency in asthmatic patients, despite depletion of blood and sputum eosinophilia.[54,55] In a subsequent study, an interleukin-5 antibody failed to prevent eosinophilia within the airway submucosa and resulted in some beneficial effects on aspects of airway remodelling.[56] It is possible that anti-interleukin-5 therapy may be more effective in patients with high levels of circulating and sputum eosinophils, as it has been shown to be effective in the treatment of hyper-eosinophilic syndrome.[57]

Both interleukin 4 and interleukin 13 are important in B-cell IgE isotype switching, and interleukin 4 is also important in maintaining the Th2 phenotype. Interleukin 13 is also involved in the modulation of eosinophilic inflammation and airway smooth muscle hyperplasia, the induction of goblet-cell hyperplasia, the recruitment of monocytes and T cells, and the induction of a corticosteroid-insensitive airway inflammation.[58] Early studies with a soluble recombinant human interleukin-4 receptor (altrakincept, Immunex [Amgen], USA) in patients with mild-to-moderate asthma showed some efficacy in maintaining asthma control when inhaled corticosteroids were being withdrawn,[59] but this effect was not subsequently confirmed and development stopped. Humanised interleukin-4-specific antibodies, antibodies that block the alpha subunit of the interleukin-4 receptor, and peptide-based vaccines against interleukin 4 are now being tested.[60] A recent study showed that interleukin-4 muteins (eg, pitrakinra) can inhibit the binding of interleukins 4 and 13 to the alpha subunit of

the interleukin-4 receptor, reducing the allergen-induced late-phase response in asthmatic patients.[61] Several monoclonal antibodies against interleukin 13, including CAT-354 and IMA-638, are undergoing clinical trials for asthma and these may have a better therapeutic index than small molecule inhibitors because the large size of the binding pocket could prevent selectivity.[60]

Suplatast tosilate selectively inhibits interleukin 4 and 5 production from T cells in vitro, attenuates allergen-induced goblet-cell metaplasia, and reduces blood and sputum eosinophilia and airway hyper-responsiveness in mild-to-moderate asthmatic patients.[60] In a small placebo-controlled clinical trial, suplatast was as effective as inhaled beclomethasone in improving the forced expiratory volume in one second (FEV_1) and mean morning peak expiratory flow and was corticosteroid-sparing in patients with moderate asthma.[60]

Thymic stromal lymphopoietin is highly expressed in airway epithelial cells of asthmatic patients and can either activate dendritic cells to orchestrate an allergic pattern of inflammation through the activation of Th2 cells or directly stimulate Th2 cytokine expression from T cells.[62,63] Additionally, mice in which the gene for thymic stromal lymphopoietin has been knocked out fail to develop an antigen-specific Th2-mediated inflammatory response in the airways. Clinical studies are now needed in patients with all types of asthma.

Some cytokines are intrinsically anti-inflammatory and are therefore potential therapeutic agents. The expression of interleukins 10 and 12 is reduced in patients with severe asthma;[64,65] restoration of levels of these cytokines has been proposed to restore asthma control. However, repeated injections of interleukin 12 have no effect on airway hyper-responsiveness despite marked effects on blood eosinophilia.[66] Administration of interleukin 10 has proved effective in animal models of asthma but no studies have been reported in asthmatic patients despite interleukin 10 being approved for psoriasis.[67] These cytokines have unacceptable side-effects and an alternative strategy to enhance endogenous expression of interleukin 10 through immunomodulatory pathways is preferable.

Tumour necrosis factor (TNF) α has been implicated as a pro-inflammatory cytokine in asthma and blood monocytes from patients with refractory asthma have increased expression of membrane-bound TNFα, TNFα receptor 1, and TNFα-converting enzyme.[68] In two small studies, patients with severe asthma on conventional therapy exhibited improved asthma control and bronchial hyper-responsiveness in response to TNFα blockade,[68,69] although a slightly larger study with etanercept, a soluble TNF receptor, showed no clinical efficacy despite effects on sputum cell numbers.[70]

Targeting adhesion molecules

Considerable effort has been made to exploit the importance of adhesion molecules in several facets of asthma—eg, leucocyte migration, exocytosis, cytokine production, and respiratory burst—by developing drugs that target these molecules[42] (figure 1). Much interest has been generated by the very late antigen-4 (VLA4, α4β integrin), which is involved in the recruitment of eosinophils and T cells.[42] However, the clinical outcome of trials of these agents in patients with asthma have been disappointing[71–73] and indeed the development of some classes of these drugs (eg, α4β integrin antagonists) was placed on hold by the US Food and Drug Administration because of reports of progressive multifocal leucoencephalopathy in patients taking natalizumab. Although now lifted, the ban delayed approval causing widespread implications for the development of adhesion molecule antagonists for the treatment of asthma.[42]

Other therapeutic areas

Infections

Respiratory viral and bacterial infections are a major cause of asthma exacerbations.[1] Therefore, antibiotics and antivirals may be beneficial for asthma exacerbations. Telithromycin, a macrolide antibiotic, caused a small but significant reduction in asthma symptoms without changes in lung function compared with placebo when administered to patients with acute exacerbations of asthma.[74] Additionally, clarithromycin can reduce levels of interleukin 8 and sputum neutrophilia and can improve asthma-related quality of life in patients with refractory non-eosinophilic asthma.[75] Whether these antibiotics act by directly inhibiting infections or by inhibiting neutrophil-based inflammation is unclear. Therapeutic interventions aimed at treating viral or bacterial infections by inhibition of Toll-like receptors (TLRs) are also under investigation, although evidence in animal models is inconclusive.[76]

Rhinovirus infection can reduce nuclear translocation of the glucocorticoid receptor and corticosteroid function;[77] conversely, inhibition of the glucocorticoid receptor-associated heat shock protein 90 (hsp90) by geldanomycin or its analogues can attenuate rhinovirus replication without the formation of drug-resistant strains,[78] implying mutual antagonism between viral infection and corticosteroids.

Intracellular signalling pathways

Phosphodiesterase 4 (PDE4) inhibitors such as roflumilast and cilomilast prevent eosinophilic inflammation in ovalbumin-sensitised Brown Norway rats with an efficacy similar to that seen with budesonide.[79] Moreover, PDE4 inhibitors are able to suppress neutrophilic inflammation and exert distinct anti-inflammatory effects compared with those seen with corticosteroids,[80] suggesting that PDE4 inhibitors could be useful in the treatment of severe asthma. The suppressive activity of PDE4 inhibitors on inflammatory cells in asthma involves the inhibition of the generation of cytokines, oxidants, pro-inflammatory mediators, the reduction or inhibition

of eosinophilic migration, and the mitigation of degranulation; the compounds can also modify airway hyper-responsiveness and remodelling by reducing smooth muscle cell proliferation and migration, the pro-inflammatory activity of epithelial cells, and plasma exudation that can lead to oedema.[80] Both cilomilast and roflumilast show dose-dependent inhibition of early and late phase responses to allergen and exercise challenges.[80] In a parallel-group trial, cilomilast caused small improvements in FEV_1 at 6 weeks, although this was lost by 12 months.[80] By contrast, roflumilast (500 µg daily) produced greater effects and caused improvements in FEV_1, morning and evening peak flow, and asthma symptoms over 12 weeks similar to those seen with beclomethasone 400 µg.[81] The problem of nausea and vomiting that occurs at the top of the dose–effect curve has not been overcome despite improved isoform selectivity, and administration of these compounds by inhalation is being considered.

Kinase inhibitors

Kinases have a critical role in the expression and activation of inflammatory mediators in the airway, in both resident and infiltrating cell function and airway remodelling[82,83] (figure 4). Although different kinase pathways can activate specific downstream transcription factors, there is considerable cross-talk between pathways.[82,83] Changes in kinase activation status have been reported in all asthmatic patients, but particularly in those with severe asthma where an association with reduced glucocorticoid responsiveness has been proposed.[84] Thus, enhanced activation of extracellular signal-related kinase (ERK), c-Jun N-terminal kinase (JNK), p38 mitogen-activated protein kinase (MAPK), and the janus kinase/signal transducers and activators of transcription (JAK/STAT) signalling pathway have all been proposed to have a role in steroid-insensitive asthma in a stimulus-dependent manner.[7,85,86]

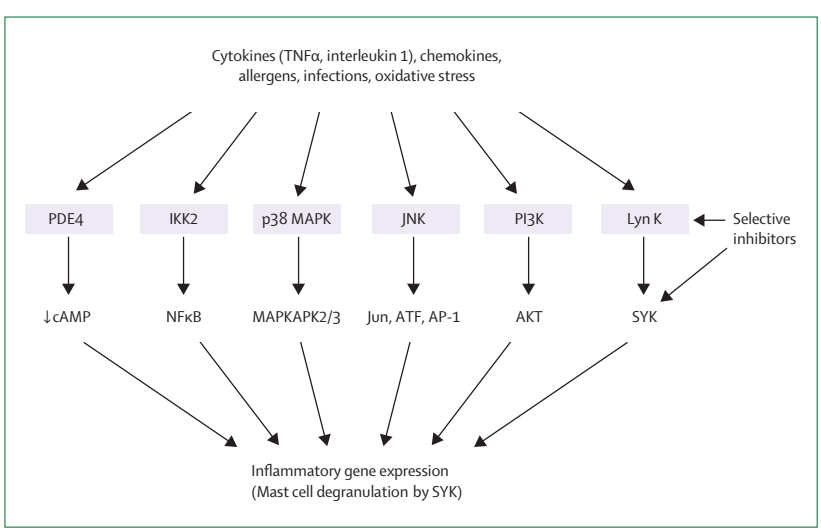

Figure 4: **Major pro-inflammatory kinases in asthma**
Many stimuli, including cytokines, allergens, chemokines, and infectious agents known to be important in asthma and asthma exacerbations, can activate key intracellular signalling pathways in a cell and stimulus dependent manner. Activation of these pathways results in enhanced recruitment and activation of infiltrating and resident cell types and increases the expression of inflammatory mediators, including other cytokines and chemokines that can act, in turn, in an either an autocrine or paracrine manner to further drive the inflammatory response in asthma. Selective inhibitors of these pathways are under development with the intention of dampening the inflammatory response. Some of these pathways (eg, IKK2 and MAPK) are also implicated in corticosteroid function and suppression of these may be corticosteroid-sparing. Additionally, inhibitors of SYK are able to prevent mast cell degranulation. AP-1=activator protein-1. ATF=activating transcription factor. cAMP=cyclic adenosine monophosphate. IKK=inhibitor of κB kinase. JNK=c-Jun N-terminal kinase. NFκB=nuclear factor κB; MAPK=mitogen-activated protein kinase. MAPKAPK=MAPK-activated protein kinase. PDE=phosphodiesterase. PI3K=phosphoinositide-3 kinase. SYK=spleen tyrosine kinase.

p38 MAPK is involved in many inflammatory processes in the lower airways and in tissue remodelling. Selective second generation p38 MAPK inhibitors such as SB2439063 (GlaxoSmithKline, UK) and the p38 MAPK antisense ISIS 101757 (ISIS Pharmaceuticals, USA) reduce the release of inflammatory mediators and some characteristics of allergic inflammation in animal models with no hepatic or neurological toxicity.[82] Interestingly, the corticosteroid insensitivity seen in peripheral blood cells and macrophages obtained from bronchoalveolar lavage fluid in patients with severe asthma can be overcome by the combination of a p38 MAPK inhibitor and dexamethasone.[7,87] Safety issues remain a concern for long-term use, although delivery to the airways by aerosol may reduce side-effects. An alternative approach is to target downstream substrates such as MAPKAPK2 since, by contrast with p38 MAPK knockout mice, MAPKAPK2 knockouts are viable and exhibit an anti-inflammatory phenotype.[88]

JNK activity is increased in corticosteroid-resistant asthma[89] and SP600125 (Celgene, USA), a JNK inhibitor, reduces accumulation of eosinophils and lymphocytes in bronchoalveolar lavage fluid, cytokine release, serum IgE production, and smooth muscle proliferation after repeated allergen exposure in acute and chronic animal models of asthma.[90,91]

Spleen tyrosine kinase (SYK, p72Syk) has a pivotal role in triggering mast cell degranulation through the high affinity IgE receptor (FcεRI).[92] SYK is also involved in B and T lymphocyte antigen receptor signalling and in eosinophil survival, suggesting that this might be an important potential target for the development of new anti-asthma drugs.[93] BAY 61-3606 (Bayer, Japan), a potent and selective inhibitor of SYK inhibited lipid mediator release, cytokine synthesis, and mast cell degranulation, had inhibitory effects on human basophils, eosinophils, and monocytes,[94] and attenuated ovalbumin-induced airway inflammation in rats.[94] The SYK inhibitor R112 (Rigel Pharmaceuticals, USA), given topically, rapidly reduced symptoms of allergic rhinitis[95] and its follow-up R343 (Rigel-Pfizer, USA) began phase I studies in asthma in late 2007.

Phosphoinositide 3-kinases (PI3Ks) catalyse the phosphorylation of phosphoinositides and regulate a number of distinct cellular responses including cell growth and division, cell apoptosis and survival, and activation in response to cytokines, antigens, and co-stimulatory molecules.[83,96] PI3Ks may also contribute

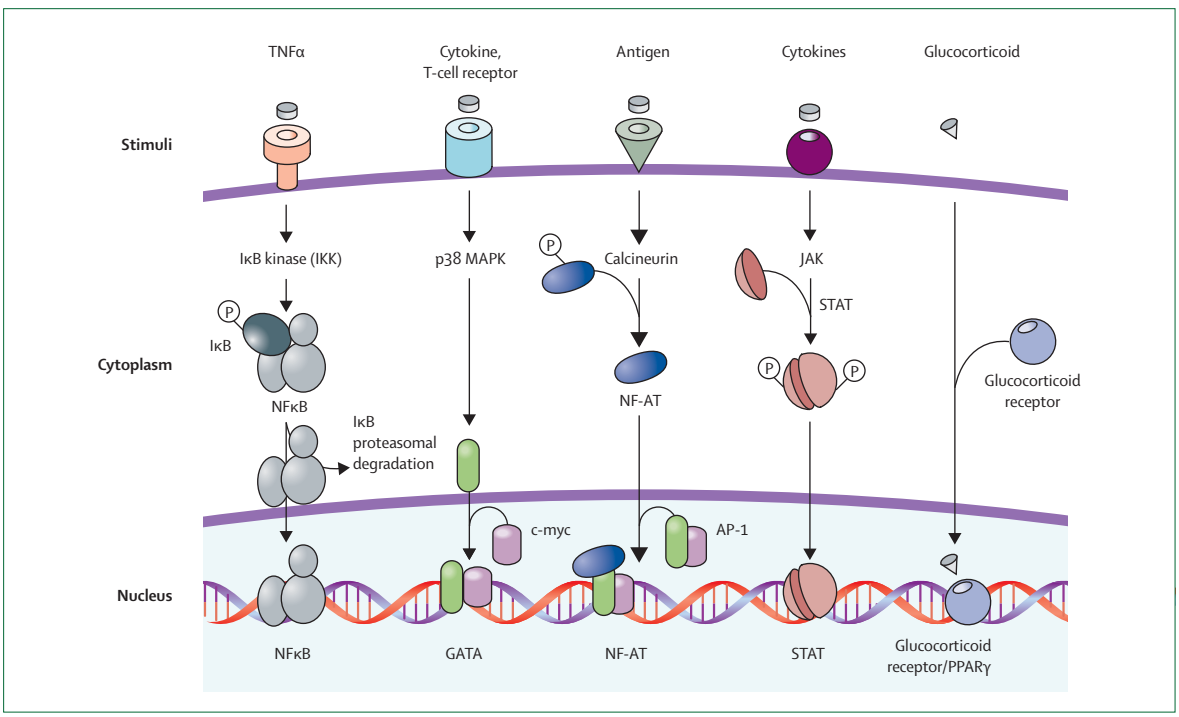

Figure 5: **Stimulation of cell surface or intracellular receptors can activate a number of signalling cascades that alter the DNA binding or transcriptional activation status of key pro-inflammatory transcription factors**

The activity of the transcription factors can be modulated by crosstalk between factors at the level of DNA binding or transactivation—eg, AP-1 and c-myc can associate with GATA-3 or NF-AT to enhance transcription. By contrast, activation of nuclear hormone (steroid) receptors in the cytoplasm leads to nuclear import and either gene induction after DNA binding or gene repression by crosstalk between other factors such as NFκB and AP-1. IKK=IκB kinase. GATA-3=GATA binding protein 3. IκB=inhibitor of κB. JAK=Janus kinase. NF-AT=nuclear factor of activated T cells. NFκB=nuclear factor κB. p38 MAPK=p38 mitogen-activated protein kinase. PPARγ=peroxisome proliferator-activated receptor γ. STAT=signal transducers and activators of transcription.

to the pathogenesis of asthma by affecting airway smooth muscle proliferation and eosinophil recruitment.[83,96] The need for isoform specificity may limit the usefulness of current PI3K inhibitors in asthma.

Inhibition of transcription factors

Nuclear factor κB (NFκB) is induced by many factors involved in asthmatic inflammation including allergen challenge, cytokines, chemokines, and bacterial and viral infection, and induces the expression of many mediators, growth factors, receptors, and enzymes important in the inflammatory cascade, leading to a feed-forward enhancement of inflammation.[82] Activation of NFκB is enhanced in mild asthma and further increased in severe asthma;[97,98] genetic studies also implicate the NFκB pathway in corticosteroid-resistant asthma.[99] Small molecule inhibitors that target the stimulating kinase (IKK2) completely suppressed inflammatory responses in animal models of asthma and in macrophages from bronchoalveolar lavage of asthmatic patients[100,101] (figure 5). Targeting IKK2 may also have additional benefits since it seems that current IKK2 inhibitors can also modulate the corticosteroid-insensitive release of CXCL10 that is induced by interferon γ.[102,103] There are concerns that inhibition of NFκB may cause side-effects such as increased susceptibility to infections.[82] Delivery by inhalation could decrease the risk of serious side-effects and these drugs may be particularly effective for exacerbations.

NFκB is a major target for corticosteroids and downstream co-activators or co-repressors may also be potential therapeutic targets as steroid-sparing agents. Recruitment of histone deacetylase 2 (HDAC2) is involved in the glucocorticoid receptor-mediated suppression of NFκB and its expression and activity is reduced in some corticosteroid-insensitive diseases.[104,105] Importantly, over-expression of HDAC2 in corticosteroid-insensitive cells can restore corticosteroid responsiveness.[106] The suppression of HDAC2 activity may be due to tyrosine nitration[106] further implicating a potential therapeutic role for anti-oxidants or inhibitors of nitric oxide synthase 2 in restoring corticosteroid responsiveness. Theophylline and curcumin both enhance HDAC2 activity under conditions of oxidative stress, leading to a restoration of corticosteroid responsiveness,[107,108] which might explain why adding a low, sub-bronchodilator dose of theophylline is more effective than increasing the dose of inhaled corticosteroids in patients with poorly controlled disease and why theophylline withdrawal worsens control of patients with severe asthma.[109]

Expression of the transcription factor GATA-3 is markedly increased in T cells and bronchial biopsies from asthmatic patients[110,111] and in cells from bronchoalveolar lavage after allergen challenge.[112] GATA-3 is also involved in the regulation of early T-cell development in the thymus, the differentiation of invariant natural killer T cells,[113] and the control of regulatory T (Treg) cells.[114] GATA-3 has a critical role in Th2 differentiation from naive CD4 T cells[113] and knockdown of GATA-3 expression using small interfering RNA in human T cells results in the loss of anti-CD3/CD28-mediated Th2 cytokine expression.[115] These findings suggest that antisense oligonucleotides may be an excellent novel anti-asthma therapy.[116]

The nuclear factor of activated T cells (NF-AT) regulates the release of cytokines from Th2 cells by forming complexes with GATA-3 and AP-1, another transcription factor (figure 5). The immunosuppressive drugs ciclosporin A, tacrolimus, and pimecrolimus block calcineurin-dependent dephosphorylation of NF-AT, thereby preventing its activation.[116] Systemic administration of ciclosporin A to patients with severe asthma has provided some improvement in lung function but has unacceptable side-effects.[116] It is hoped that the development of the locally active T-cell modulator MLD987 (Novartis, Switzerland), an inhaled derivative, may reduce the side-effects.[116] Alternative strategies for inhibiting NF-AT include the use of peptides known as inhibitors of NF-AT-calcineurin association (INCA) that block docking of calcineurin to NF-AT.[116]

STATs are the primary signal-specific mediators of cytokine-regulated gene expression activated by receptor-associated JAKs. For example, interleukins 4 and 12 drive the differentiation of Th2 and Th1 cells, respectively, through activation of STAT6 and STAT4.[117] Thus, targeting JAK/STAT pathways could be an effective therapeutic strategy for asthma. Indeed, a STAT1 decoy oligonucleotide (AVT-01, Avontec, Germany) has proved successful in an animal model of asthma[118] and is now in phase IIa clinical trials.[60] Of the many STAT proteins, only STAT6 is unique to the asthma-related cytokines interleukin 4 and interleukin 13.[60]

The characteristics of Treg cells have made these cells attractive candidates for immunotherapy.[119] The activity of Treg cells is under the control of the transcription factor forkhead box P3 (FOXP3). Stable over-expression or induction of FOXP3 by retinoic acid drives the conversion of naive T cells to Treg cells and this has been proposed to modulate asthmatic inflammation.[119]

Peroxisome proliferator-activated receptors (PPARs) are nuclear receptors activated by polyunsaturated fatty acid derivatives, oxidised fatty acids, and phospholipids[120] (figure 5). PPARγ ligands decrease antigen-induced airway hyper-responsiveness, lung inflammation and eosinophilia, cytokine production, and GATA-3 expression, as well as serum levels of antigen-specific IgE in different animal models of asthma.[120] Activation of PPARγ also alters the maturation of dendritic cells and rosiglitazone reduces the proliferation of antigen-specific T cells while increasing the production of interleukin 10 by these cells.[120] Interestingly, the panel of inflammatory genes regulated by PPARγ agonists is distinct from that regulated by corticosteroids and the combination of both a PPARγ agonist and a corticosteroid might have a greater anti-inflammatory effect than either drug alone,[121] particularly since corticosteroids can induce PPARγ expression.[122]

Oxidative stress

Oxidative stress has been implicated as a driving force behind the inflammatory response and lack of corticosteroid sensitivity in severe asthma.[123] Moreover, oxidative stress and its byproducts may drive a Th2-dependent immune response.[124] Anti-oxidants including N-acetylcysteine, nacystelyn, and the superoxide dismutase mimetic AEOL 10150 (Aeolus Pharmaceuticals, USA) are able to restore corticosteroid functions that were reduced in response to cigarette smoke or other oxidative stresses in both primary human cells and in animal models,[123] but current anti-oxidants do not seem to affect the redox balance in the airways of human beings. Therefore, smarter and more potent drugs are required to target the correct cellular compartment. Oxidative stress in combination with the high levels of nitric oxide seen in asthma[125] will result in the formation of peroxynitrite, tyrosine nitration, and lipid peroxidation products,[126] which have been linked to corticosteroid insensitivity. Inhibitors of nitric oxide synthase 2 have been shown to be safe, but ineffective, in mild asthma[127] but they may have an important role in treatment of patients with severe asthma and in asthmatic patients who smoke, who have increased levels of oxidative or nitrosative stress.[123]

Resveratrol (3,5,4'-trihydroxystilbene), a component of red wine, has anti-inflammatory and antioxidant properties.[123] Although resveratrol inhibits cytokine release by alveolar macrophages from patients with chronic obstructive pulmonary disease, it is not clear if this is due to its anti-oxidant properties. Nevertheless, this compound may also be beneficial for patients with severe asthma.[128] Curcumin has many therapeutic properties as a result of its anti-oxidant, anti-inflammatory, and anti-cancer effects.[108,129] Despite the low bioavailability of curcumin after oral application, it is now in phase I clinical studies for several respiratory diseases.[130]

Immunomodulation and anti-allergy treatments

Since asthmatic patients are often atopic, much effort has been directed at modulating the allergic response (figure 1). IgE is the immunoglobulin that mediates the acute allergic response in mast cells and basophils through cross-linking of high-affinity IgE receptors, and may increase allergen uptake by dendritic cells. A

humanised monoclonal antibody that binds to IgE (omalizumab) has been introduced for the treatment of severe allergic asthma. Omalizumab is a useful add-on therapy in some patients who are affected by frequent exacerbations since it reduces the rate of exacerbations,[131] but it is expensive and its cost-effectiveness is debated.[132] Other more potent anti-IgE antibodies are in development and other strategies also being considered include the development of peptide-based vaccines or immunotherapy to induce IgE-specific antibodies. An antibody directed against the low-affinity IgE receptor (FcεRII or CD23), lumiliximab (Biogen Idec, UK), reduces IgE concentrations in atopic patients.[133] A similar effect has been reported in mild asthma from the same group but the results have not been published in full.[134] Development of therapies directed at T-cell co-stimulatory molecules such as CD23, ICOS, and OX40 is also progressing.[60]

Dendritic cells mount and maintain immune responses to inhaled allergen and modulation of their function represents a new approach to asthma treatment. The sphingosine-1 phosphate receptor antagonist, FTY720 (fingolimod, Novartis, Switzerland), strongly attenuates established lung inflammation in mice through inhibition of activation of dendritic cells,[135] and is currently in clinical trials for the treatment of multiple sclerosis and transplant rejection.[135] Selective prostaglandin D receptor agonists such as BW245C (Wellcome Research Laboratories, UK) also suppresses dendritic cell function, leading to decreased airway inflammation and bronchial hyper-reactivity in a mouse model of asthma through the induction of FOXP3-expressing Treg cells.[136] A similar mechanism may account for the actions of the prostacyclin analogue, iloprost.[62]

Immunomodulatory therapies offer the opportunity to reverse the abnormal immune function observed in asthma by enhancing Treg expression or function or altering T-cell class switching away from the Th2 response.[137] Sublingual immunotherapy has been used in asthma, although questions remain about effective doses, treatment schedules, and treatment duration.[138]

Specific subcutaneous immunotherapy increases the production of interleukin 10 from Treg cells[137,139] and allergen-specific T-cell peptides that enhance Treg function and increase the release of interleukin 10 are now under development.[27] Treating Treg cells from corticosteroid-resistant patients with vitamin D3 in combination with dexamethasone restores the ability of these cells to release interleukin 10.[140] This, in turn, allowed interleukin 10 to upregulate the expression of the glucocorticoid receptor and reverse the dexamethasone-induced reduction in expression of this receptor. Impressively, oral administration of vitamin D3 (0·5 μg daily) for 7 days to corticosteroid-resistant asthmatic patients enhanced ex vivo Treg responses to dexamethasone.[140] This observation suggests that vitamin D3 could potentially increase the therapeutic response to glucocorticoids in corticosteroid-resistant patients.

The potential of non-selective skewing of the T-cell response in asthma using non-pathogenic bacterial products, such as immunostimulatory oligodeoxynucleotides, including CpG oligodeoxynucleotides, which target TLR9, is under active investigation.[141] However, early indications in asthma are not encouraging despite evidence of a robust increase in interferon γ and genes that are induced by interferon γ.[142] The long-term consequences of this approach need to be carefully assessed, especially since they would probably need to be used in young children before the onset of asthma.

Conclusions

Several new treatments are now under development for mild or moderate asthma but many of them are highly specific, targeting a single receptor, enzyme, or mediator, and are unlikely to have a major clinical impact. New treatments also have a high barrier to overcome in that combination therapy is efficacious and it is likely that once-a-day, fast-onset combinations of long-acting β agonists and steroids will be available soon. Because of the overexpression of interleukin 13 in asthma and its role in many immuno-inflammatory processes in asthma, it is possible that biological agents targeted against the interleukin-13 receptor may buck this trend. Compliance is an issue with current therapy and the development of an effective oral therapy for mild or moderate asthma may be an advantage, but it is likely that such treatments will have major side-effects. Although we have not discussed these issues here, drug delivery and comorbidities, particularly in patients with severe asthma, will affect the pharmacopoeia used to treat all asthmatic patients.

Prospects for prevention or cure are currently remote but might arise from the development of vaccines and immune therapies directed against Treg and Th17 cells, such as combinations of vitamin D3 and steroids. It is important to recognise that distinct subgroups of patients may respond better to particular therapies, such as those targeting the leucotriene pathway, although it is unlikely that these will be successful as monotherapies.

There are several approaches that can be taken to treat those patients with severe and treatment-refractory asthma. The type of inflammation in these patients may be distinct and targeting this inflammation with selective therapeutic agents such as inhaled p38 MAPK and IKK2 inhibitors may be beneficial. More selective targeting of drugs to patients with particular subtypes of asthma might be possible in the future with the development of discriminatory handprints of clinical phenotypes, biomarkers, and genetic profiles. Rapid tests that distinguish these subtypes may make the advent of selective therapy closer and more effective.

An alternative approach is to try to restore steroid sensitivity rather than prevent inflammation per se. Understanding the multiple mechanisms underlying

corticosteroid-resistant asthma might indicate patient-specific abnormalities in signalling pathways that could be targeted to restore asthma control—eg, inhaled p38 MAPK and JAK inhibitors—which could result in a reduced need for inhaled or systemic corticosteroids.

Finally, the fact that combination therapies are more effective than monotherapy, as has been previously observed in the treatment of rheumatoid arthritis, emphasises the need to examine multidrug approaches to asthma that are tailored to the genotype or phenotype of the particular asthma subtype.

Contributors

IMA prepared the initial draft; KFC and GC revised and corrected the subsequent versions and helped to prepare the figures. All authors saw and approved the final version.

Conflict of interest statement

IMA has received consultancy fees from Chiesi, GSK, and Novartis, lecture fees from Altana, GSK, Novartis, Pfizer, and Sanofi-Aventis and contributions towards attending ATS and ERS meetings from Boehringer-Ingelheim and GSK over the past 3 years. KFC has been remunerated for attending advisory board meetings and for delivering lectures at meetings sponsored by pharmaceutical companies marketing respiratory drugs. GC has received lecture fees from GSK (Italy) and MSD (Italy), and contributions towards attending ATS and ERS meetings from Chiesi and Novartis (Italy) over the past 3 years.

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