

Emerging Molecular Targets for the Treatment of Asthma

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Current therapeutic approaches for the treatment of asthma have limitations in their ability to target all the features of the disease. Indeed, existing pharmacological asthma therapies are based on decades old strategies that were developed prior to the rapid growth in knowledge stemming from cell and molecular biology in the past decade. Thus, there is an unmet need for developing new drugs to target these features along with improved efficacy and safety. In the present review, the limitations of prevalent pharmacological asthma therapy are discussed briefly, and some explanations are suggested as to why new therapeutic targets are required to treat asthma, and finally directions for novel asthma therapies are proposed.

Keywords: Asthma, Airway hyperresponsiveness, Inflammation, Airway remodeling, PDE4 inhibitors, Statins, Rho kinase, Cytokines, Bronchodilators, PI-3 kinase inhibitors, Antisense oligonucleotides, Transcription factors, Kinase inhibitors, CpG oligonucleotides, H4 receptors

Introduction

Asthma is now one of the most common chronic diseases in industrialized countries with increasing prevalence, leading to a substantial global economic burden. In later half of the 20th century, asthma was viewed as a disease of bronchoconstriction and treated predominantly with bronchodilators. However, at present, it is considered as a chronic inflammatory disease of the airways, and the mainstay of modern management is treatment with anti-inflammatory inhaled corticosteroids. Classically, asthma is characterized by reversible airway obstruction,

bronchial hyperresponsiveness and airway inflammation and more recent insight suggests the latter may underpin irreversible structural changes known as airway remodeling (Figure 1). Key pathological features include infiltration of the airways by inflammatory and immune cells, including eosinophils, lymphocytes, neutrophils and mast cells, structural damages to the bronchial epithelium, mast cell degranulation, mucous gland hyperplasia, increased mass of airway smooth muscle, and increased collagen deposition in the epithelial sub-basement membrane area associated with increased numbers of myofibroblasts.

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Abbreviations: AHR, airway hyperresponsiveness; ANP, atrial natriuretic peptide; AON, antisense oligonucleotide; CCR, chemokine receptor; IL, interleukin; ICAM-1, intercellular adhesion molecule 1; JNKs, Jun N-terminal kinases; 5LO, 5-lipoxygenase; LTRAs, leukotriene receptor antagonists; MAP, mitogen-activated protein; ODNs, CpG-oligonucleotides; PDE, phosphodiesterase; PG, prostaglandin; PI3K, phosphatidylinositol-3-kinase; TLR, toll like receptor; TNF- α , tumor necrosis factor- α ; VCAM-1, vascular cell adhesion molecule 1; VIP, vasoactive intestinal peptide.

Asthma pathology is associated with the release of a myriad of pro-inflammatory substances including lipid mediators, chemokines, cytokines, growth factors and other peptides. In addition to infiltrating leukocytes, structural cells in the airways that include smooth muscle cells, endothelial cells, fibroblasts and epithelial cells are all important sources of asthma-causing or -precipitating mediators¹. Existing therapies for asthma are aimed at controlling disease symptoms, chiefly airway responsiveness, reduced airflow resistance, cough and disease exacerbation. For the majority of asthmatics, inhaled corticosteroid and β_2 -adrenergic receptor agonist therapy is

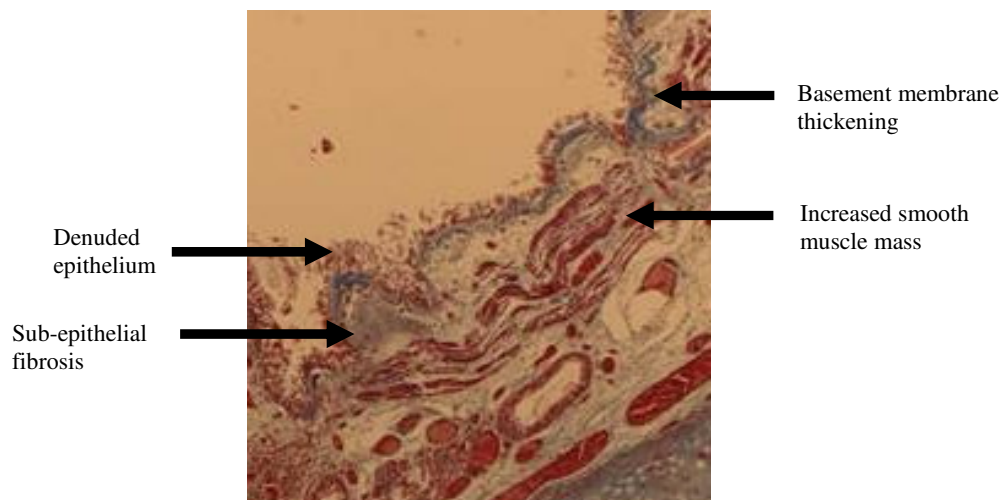


Figure 1—Picture of an asthmatic airway showing features of key structural changes in asthma (known as airway remodeling) which includes: damaged epithelium, thickening of the basement membrane, subepithelial fibrosis, increase in the mass of smooth muscle as a result of hyperplasia and hypertrophy, increased numbers of blood vessels

effective. However, this approach requires life-time therapy as its cessation leaves individuals subject to asthma exacerbation, which in some cases is worse than those experienced prior to the original initiation of therapy. Moreover, a subset of patients (~10-20%) remains symptomatic despite treatment and in fact, these patients account for at least 50% of the economic burden associated with asthma treatment. This situation creates a clear unmet medical issue. Recently, much interest has gone into developing more specific and effective treatments for asthma, but there is no therapy which can prevent all the features of this multi-factorial chronic disorder.

It is well appreciated that particularly in severe asthma, there are structural changes in the airway called airway remodeling that might reduce its ability to reverse airflow limitation and response to therapy. One of the major problems that new drug development is faced with is the fact that existing therapies for most compliant asthmatics, particularly combination inhalers are effective, inexpensive and reasonably safe². Another issue is that animal models of asthma are poorly predictive of efficacy of treatment in asthmatic patients; indeed, many drugs that have proved effective in pre-clinical models have failed in clinical trials. Some issues in developing new drugs for asthma are: (i) that they should be effective in severe, poorly controlled asthma; (ii) oral treatment without side effects is preferable to inhaled medications as compliance is better, particularly in children; and (iii) that they should modify the course of, or even reverse the disease.

To date, the approaches that have been most commonly taken are to improve existing treatments, such as β_2 -agonists or corticosteroids, or to find drugs against novel inflammatory targets, such as cysteinyl-leukotrienes or interleukin (IL)-5. Another consideration is growing appreciation that the phenotype of asthma is variable, making it difficult to treat with a single “magic bullet”, in particular when one considers interaction with other conditions such as obesity³ or current smoking⁴ on asthma therapies. In this review, we have included current knowledge on several new classes of drugs which are under development for the treatment of asthma.

Phosphodiesterase (PDE) inhibitors

Due to their ability to target multiple features of asthma, a therapeutic class that has shown excellent promise in the last decade is PDE inhibitors. Theophylline, a non-specific PDE inhibitor has been used clinically to treat airway diseases for over 50 years⁵; it has been demonstrated to have bronchodilating, anti-inflammatory and anti-fibrotic effects^{6,7}. Focus is shifting towards developing subtype specific PDE inhibitors to minimize some of the unwanted side effects associated with using this class of molecules. Therefore, apart from theophylline, first and second generation PDE-4 inhibitors like roflumilast, piclamilast, cilamifast, cilomilast and roflumilast have shown great promise in reducing features associated with airway remodeling both *in vitro* and *in vivo*^{8,9}. This effect is largely due to their ability to raise cAMP levels in

inflammatory, immune and airway smooth muscle cells by inhibiting degradation of the cyclic adenosine monophosphate moiety by PDE-4, which is the predominant subtype expressed in the airways^{10,11}.

There are a number of studies using selective PDE-4 inhibitors that suggest good promise for these compounds to treat asthma. In a chronic murine model of asthma, a new generation PDE-4 selective inhibitor, roflumilast, which is currently in phase 3 clinical trials, reduces sub-epithelial fibrosis and hypertrophy of the tracheal epithelium¹². Indeed, roflumilast inhibits TGF- β 1-induced production of extracellular matrix proteins by both asthmatic and non-asthmatic cultured airway smooth muscle cells and in tracheal rings *in vitro*; this further supports a potential role for PDE-4 inhibitors in preventing airway structural changes¹³. In addition, roflumilast can be protective in early and late allergic response and airway hyperresponsiveness (AHR) in *Aspergillus fumigatus*-sensitized mice¹⁴.

Another inhibitor ciclamilast dose-dependently attenuates ovalbumin-induced goblet cell hyperplasia, mucus secretion and tissue inflammation, thus too appears to have anti-remodeling effects¹⁵. Cilomilast, a PDE-4 inhibitor currently pending approval for use in the treatment of COPD¹⁶ has been shown to reduce both basal- and PDGF-stimulated migration of human airway smooth muscle cells *in vitro*¹⁷. In a mouse model of childhood allergic asthma, rolipram, another PDE-4 selective inhibitor is shown to reduce AHR, but does not prevent lung inflammation and atopy¹⁸. However, NIS-62949 has been found to be effective in suppressing effects of lipopolysaccharide-induced endotoxemia and pulmonary neutrophilia in mice, antigen-induced allergic airway inflammation, AHR and bronchoconstriction in guinea pig model of asthma and appears to be safer with a wider therapeutic window compared to second-generation PDE-4 inhibitors, such as roflumilast¹⁹. The randomized placebo-controlled studies of a highly selective PDE-4 inhibitor MK-0359 has been shown to improve lower airway function, symptoms and reduces rescue medication use in chronic asthma patients²⁰. Collectively, these findings provide evidence for the potential to develop PDE-4 selective inhibitors as a multi-faceted treatment for asthma.

In addition to PDE-4 inhibitors a number of inhibitors that target multiple PDE isozymes have been developed. LASSBio596, a PDE-4/5 inhibitor in a murine model of chronic asthma prevents collagen

deposition, when administered to sensitized animals before they are challenged with allergen²¹. A PDE-4 and -1 inhibitor KF19514 significantly prevents allergen-induced changes in bronchial responsiveness, inflammatory cells and eosinophils infiltration to the lamina propria, thickening of the epithelial and subepithelial collagen layers, and induction of lung hydroxyproline content in mice²². Recently, NT-702, a PDE-3 inhibitor has also been found to suppress ovalbumin-induced late asthmatic responses, AHR and the accumulation of inflammatory cells in bronchoalveolar lavage fluid of guinea pig²³. Thus, there is compelling data to suggest that PDE inhibitors that target the type-4 isoform and those that have a broader selectivity can prevent various aspects of asthma, such as AHR, airway remodeling and inflammation both *in vitro* and *in vivo*. However, more thorough and systematic clinical studies are warranted to assess their safety and efficacy, before they can become future routine therapies for asthma.

Rho kinase inhibitors

Rho kinases are serine/threonine kinase effectors of RhoA, a monomeric GTP-binding protein that cause Ca²⁺ sensitization of airway smooth muscle by inactivating myosin light chain phosphatase, the enzyme chiefly responsible for tempering contraction and causing relaxation. Two isoforms of Rho kinase ROCK-1 and -2 that show overlapping expression profiles have been identified, though the latter has been implicated to be relatively more important in biological responses associated with neural tissues and cells²⁴. As to date, there are no isoform selective inhibitors available and both ROCK-1 and -2 are expressed in lung and inflammatory cells, we use the term Rho kinase without discriminating the relative importance of either isoform. The major roles of Rho kinase in cellular physiology include contraction, cell attachment and migration, and proliferation and cell survival. These cellular activities are all thought to be of importance in the pathogenesis of and patho-physiological manifestation of asthma.

There is accumulating evidence that activation of the Rho kinase pathway is substantially involved in the pathogenesis of asthma and that Rho kinase inhibitors may be useful for the treatment of asthma. Indeed, in animal model of asthma, Rho kinase inhibitors appear to be effective in suppressing both acute airway hyperresponsiveness and airway remodeling arising from chronic allergic

inflammation²⁵⁻²⁹. One the best known and most widely studied Rho kinase inhibitors at present is Y-27632 ([4-(1-aminoethyl)-N-(4-pyridyl)cyclohexanecarboxamide dihydrochloride). It is a cell permeable, potent competitive inhibitor that can induce bronchodilatory effects, when delivered as an aerosol to guinea pigs³⁰. Furthermore, it appears to have minimal side effects on systemic blood pressure.

Several analogues of Y-27632 have been synthesized; these have similarly high inhibitory constants for Rho kinase, promote smooth muscle relaxation, but also inhibit cAMP-dependent protein kinase at relatively low concentrations^{31,32}. Insulin-like growth factor-1 and angiotensin II induced contraction of human bronchial smooth muscle is almost completely inhibited by Y-27632³³. Intra-nasal administration of Y-27632 inhibits the number of eosinophils recovered from bronchoalveolar lavage fluid in ovalbumin sensitized and challenged mice and this is accompanied by a significant inhibition in the development of AHR²⁵. Also of interest, Rho kinase appears to be involved with "activation" of fibroblasts to a myofibroblasts that contribute to local inflammation and collagen deposition in the airways of asthmatics²⁹.

Another well-described Rho kinase inhibitor is fasudil or HA-1077 (1-(5-isoquinolinesulfonyl)-homopiperazine), which has a similar affinity for Rho kinase as Y-27632, as judged by its Rho kinase activity inhibition constant (0.33 μM)³⁴. However, it exhibits less selectivity for Rho kinase, as it has binding affinity in the mid-to-high nanomolar range for a number of other kinases, such as protein kinase A. But, despite its lower selectivity compared to Y-27632, fasudil has been investigated rigorously in animal models and is in fact the only currently available Rho kinase inhibitor for clinical use; in Japan, fasudil is approved for the prevention of vasospasm in patients with subarachnoid hemorrhage³⁵. It serves as an effective intervention for this purpose and has been used in a large number of patients and notably to date, evidences for adverse reactions or side effects are extremely low. Collectively, these results suggest that Rho kinase possibly contributes significantly to asthma pathogenesis and morbidity, in particular bronchoconstriction and inflammatory cell recruitment to the airways. And there is a growing body of evidence that Rho kinase inhibitors can effectively attenuate AHR, suppress smooth muscle growth and also

prevent the phenotypic change of lung fibroblasts into myofibroblasts. Thus, this class of inhibitors holds excellent promise to target acute (bronchoconstriction) and chronic (inflammation and airway remodeling) aspects of asthma.

Statins

Evidences indicate that statins have a greater health benefit than reducing serum cholesterol and there are a growing number of clinical trials testing their impact on lung health, including the treatment of allergic asthma³⁶⁻³⁹. Statins reduce various allergic responses via regulation of small GTPase proteins (eg. Ras, Rac, Rho), mitogen-activated protein (MAP) kinases and nuclear factor-kappa B in airway inflammatory cells and lung tissues from allergen challenged mice⁴⁰. Interestingly, these effects occur concomitantly with a reduction of serum IgE levels, inflammatory cell number in the airways (including macrophages, neutrophils and eosinophils), as well as cytokine and cell adhesion molecules expression (CD40, CD40L, VCAM-1, IL-4, IL-13, TNF- α , etc.)⁴⁰. These positive results have been verified in other studies, for example, fluvastatin has a suppressive effect on cytokine and chemokine production by peripheral blood mononuclear cells harvested, following allergen-specific and non-allergen-specific stimulation in patients with asthma⁴¹. However, there are some conflicting reports as in another mouse model of asthma, though lovastatin ameliorates airway eosinophilia, it has no significant effect on the levels of these inflammatory mediators or IgE⁴². Thus, at present the positive effects of statins are equivocal, as there are additional reports that question the efficacy of statins for the treatment of human asthma⁴³. A caveat to these studies showing a lack of effect of statins is that only mild asthmatics were studied and statin use was of relatively short duration. Due to these issues, at least two large randomized control clinical trials for lovastatin in asthma control are underway.

Simvastatin, a lipophilic statin that is closely related in structure to other statins such as the widely used lovastatin has been studied extensively *in vitro* and in animal models of asthma. Very recently, it has been shown that simvastatin attenuates airway inflammation and improves lung physiology in a mouse model of allergic asthma⁴⁴. Also, in ovalbumin-induced allergic asthma in mice, simvastatin reduces ovalbumin-specific IgE levels,

the number of inflammatory cells in bronchoalveolar lavage fluid, expression of CD40, CD40L and VCAM-1, mRNA and protein levels of interleukins IL-4, and IL-13 and tumor necrosis factor (TNF)- α , goblet cell number, activity of matrix metalloproteinases, small G proteins, MAP kinases and NF-kappa B in bronchoalveolar lavage cells and lung tissues⁴⁵. Pravastatin is shown to suppress systemic sensitization to allergen with concomitant downregulation of IL-17 production and also an ongoing immune response in the airway, partly by suppressing antigen presentation⁴⁶. Addition of atorvastatin to inhaled corticosteroids though does not cause short-term improvement of asthma control, but reduces sputum macrophage counts in mild to moderate atopic asthma⁴⁷. Though investigations into the use of statins to treat asthma are in infancy, studies to date suggest this class of compound could be a novel therapeutic option, as statins are well-tolerated and lack side effects in long-time users for hyperlipidemia.

Anti-cytokine therapy

Cytokines are important for maintaining normal physiology and mediating the appropriate response to injury and insult; however, when inflammation is excessive, these proteins also play a principal role in orchestrating deleterious chronic inflammation in many diseases, including asthma. Multiple cytokines and chemokines have been implicated in the pathophysiology of asthma^{1,48}. There is now an intensive search for cytokine-based specific therapies in asthma. Recent studies suggest multiple Th2 cytokines, such as IL-4, IL-5 and IL-13 play an important role in acute and chronic pathobiology, including the development of airway remodeling⁴⁹⁻⁵².

IL-5 plays an essential role in orchestrating the eosinophilic inflammation of asthma⁵³. In IL-5 gene knock-out mice, the eosinophilic response to allergen and the subsequent AHR are markedly suppressed, validating the strategy to inhibit IL-5. Blocking antibodies to IL-5 inhibit eosinophilic inflammation and AHR in animal models of asthma, including primates⁵⁴. Humanized monoclonal antibody mepolizumab markedly reduces blood eosinophils for several weeks and prevents eosinophil recruitment in to the airways after allergen challenge in patients with mild asthma⁵⁵. In mice, a blocking antibody against IL-5 prevents increased collagen deposition in airways in response to repeated allergen exposure⁵⁶.

Though there is no substantial clinical data showing the protective effect of anti-cytokine therapy in airway wall remodeling in asthmatics, it has been demonstrated in an allergic mouse model that anti-IL-5 neutralizing antibodies inhibit mucous gland hyperplasia and the development of subepithelial fibrosis^{50,56,57}. However, the lack of clinical benefit of anti-IL-5 antibodies has made this a less attractive approach.

Accumulating evidence suggests that IL-13 in mice contributes to many of the hallmark features of human asthma, including AHR, mucus hypersecretion and airway fibrosis, independent of eosinophilic inflammation⁵⁸. Knocking out the IL-13, but not the IL-4 gene in mice prevents the development of AHR after allergen exposure despite a vigorous eosinophilic response⁵⁹. Anti-IL-13 treatment effectively inhibits goblet cell hyperplasia and mucous production and reduces reticular basement membrane thickening and collagen deposition in the airways of a mouse model of allergic asthma^{50,57}. IL-13 signals through the IL-4R α , but may also activate different intracellular pathways via activation of IL-13R α 1⁶⁰, thus may be an important target for the development of new therapies. A second specific IL-13 receptor IL-13R α 2 exists in soluble form and has a high affinity for IL-13. Soluble IL-13R α 2 is effective in blocking the actions of IL-13, including IgE generation, pulmonary eosinophilia and AHR in mice⁶¹. Humanized IL-13R α 2 is now in clinical development as a therapeutic approach for asthma. These findings are in agreement with the absence of sub-epithelial fibrosis in IL-13 knock-out mice^{62,63}. A recent study also indicates that IL-13 peptide-based vaccines may be an effective therapeutic approach to inhibit airway inflammation, remodeling and AHR⁶⁴.

IL-4 is involved in eosinophil recruitment to the airways and is also critical for the synthesis of IgE by B-lymphocytes⁶⁵. One of the important functions of IL-4 is to promote differentiation of Th2 cells and, therefore, acts at a proximal site in the allergic response, making IL-4 an attractive target for inhibition in asthma pathogenesis. Indeed, IL-4 knockout mice exhibit reduced subepithelial airway collagen deposition and reduced goblet cell number after brief or chronic allergen exposure⁶³. IL-4 blocking antibodies inhibit allergen-induced AHR, goblet cell metaplasia and pulmonary eosinophilia in a murine model⁶⁶. A single nebulized dose of soluble humanized IL-4 receptors (sIL-4r) prevents the fall in

lung function induced by withdrawal of inhaled corticosteroids in patients with moderately severe asthma⁶⁷. Studies have shown that weekly nebulization of sIL-4r improves asthma control over a 12 week period⁶⁸. Also, administration of novel IL-4 based vaccine leads to an overall decrease in the development of airway allergic inflammatory responses in mouse model of allergic asthma⁶⁹.

There are a number of other novel cytokine-based therapies for asthma that are being considered for development. An endogenous inhibitor of STATs, suppressor of cytokine signaling (SOCS-1) is a potent inhibitor of IL-4 signaling pathways and offers a new therapeutic target⁶⁰. Another possible anti-cytokine therapy includes strategies to block IL-9 and these are now in development⁷⁰. IL-25, which is released from mast cells via an IgE-dependent mechanism, is also a possible target for inhibition in the treatment of asthma⁷¹. Pro-inflammatory cytokines, in particular IL-1 β and TNF- α that amplify the inflammatory response in asthma and are linked to disease severity suggest that blocking IL-1 β or TNF- α may have beneficial effects, particularly in severe airway disease. Evidences suggest that anti-cytokine therapy is useful in reducing the inflammatory cell load in the airways of asthmatic patients and in preventing some aspects of airway remodeling *in vivo*. However, more thorough and systematic clinical studies are warranted to more clearly identify their potential as therapeutical interventions for treating asthma.

PI-3 kinase inhibitors

The phosphatidylinositol-3-kinase (PI3K) family of proteins catalyzes the phosphorylation of phosphorinositides at the 3-hydroxyl position and generates lipids that control a wide variety of intracellular signaling pathways. PI3K activity has been found in all eukaryotic cell types and is linked to an incredibly diverse set of key cellular functions, including cell growth, proliferation, motility, differentiation, survival and intracellular trafficking⁷²⁻⁷⁴. Therefore, it is conceivable that PI3K signaling plays a critical role in the pathogenesis of cancer, chronic inflammatory disease, allergy, metabolic disease, diabetes and cardiovascular problems.

For airway inflammatory diseases, numerous components of the PI3K pathway participate in regulation of the expression and activation of inflammatory mediators, inflammatory cell recruitment, immune cell function, airway remodeling

and corticosteroid insensitivity. Levels of IL-4 and IL-5 in bronchoalveolar lavage fluids induced by allergen challenge are significantly reduced by the intra-tracheal administration of the PI3K inhibitors wortmannin or LY294002^{75,76}. These inhibitors also reduce the allergen-induced airway inflammation, AHR, increased numbers of inflammatory cells, including eosinophils in the airways and increased eosinophilic cationic protein levels in bronchoalveolar lavage. Increased levels of adhesion molecules (ICAM-1 and VCAM-1), chemokines (including RANTES and eotaxin), TNF- α and IL-1 β after allergen inhalation are also significantly decreased in the lungs by treatment with LY294002 or wortmannin⁷⁷. Intra-tracheal administration of LY294002 also dramatically inhibits allergen-induced tissue eosinophilia and airway mucus production in mice⁷⁸. In addition, *in vitro* tests have shown that PI3K plays a role in regulating the contraction and migration of airway smooth muscle cells^{79,80}, as well as in the transformation of airway smooth muscle cells into the contractile phenotype observed in asthma^{76,81}. This implies that inhibition of PI3K could provide bronchodilatory impact and may prevent or even reverse the increase in airway smooth muscle mass that characterizes asthma.

Recently, the biological significance and molecular mechanisms of PI3K δ (one of the PI3K isoforms) in allergic airway inflammation and hyperresponsiveness using IC87114, a selective PI3K δ inhibitor in a murine asthma model has been shown^{82,83}. Both p110 δ knock-out mice and mice expressing an inactive form of the p110 δ catalytic subunit display impaired B-cell and T-cell antigen receptor signaling^{84,85}. The 50% inhibitory concentration (IC₅₀) of IC87114 for PI3K δ inhibition is 0.5 mmol/L, whereas the IC₅₀ values for PI3K α , PI3K β and PI3K γ are >100, 75 and 29 mmol/L, respectively^{86,87}. Although there is no optimal concentration of LY294002 that will selectively antagonize any single class of PI3K, IC87114 selectively antagonizes PI3K δ at concentrations of 0.3-10 mM⁸⁷. As expected, IC87114 treatment markedly attenuates ovalbumin-induced serine phosphorylation of Akt1, a downstream effector of PI3K signaling⁸².

Selective inhibition of PI3K δ with IC87114 appears to hold significant potential as a therapy for asthma. The compound has been used to explore the importance of PI3K δ in neutrophil migration⁸⁶⁻⁸⁸.

Intra-tracheal administration of IC87114 in a mouse asthma model attenuates ovalbumin-induced lung influx of leukocytes, eosinophils, neutrophils and lymphocytes, and reduces the production of Th2 cytokines (IL-4, IL-5 and IL-13), adhesion molecules (ICAM-1 and VCAM-1) and chemokines (RANTES and eotaxin) in a dose-dependent manner. IC87114 also reduces serum levels of total and ovalbumin-specific IgE, as well as leukotriene C4 release into the airspace. Furthermore, it inhibits allergen-induced lung tissue eosinophilia, airway mucus production and inflammation score and suppresses AHR. These findings have been supported by other studies in which genetic inactivation of the haemopoietic cell-restricted PI3K isoform p110 δ shows that Th2 cytokine responses (IL-4, IL-5 and IL-13) are significantly decreased in mutant mice⁸⁹. Furthermore, p110 δ mutant mice have reduced Th2 cytokine-independent airway inflammation elicited by intranasal allergen challenge and this is linked with reduced pulmonary levels of eosinophils, mucus production and AHR. Of significant interest, an in contrast to the otherwise beneficial effects of p110 δ blockade, a recent study shows that genetic or pharmaceutical inhibition of p110 δ enhances allergen-induced IgE production, although T-cell receptor function is also suppressed⁹⁰. This raises an important issue that needs to be clarified experimentally before proceeding with large clinical trials using PI3K inhibitors for asthma. Collectively, emerging data demonstrate a novel biological role of p110 δ signaling pathway in allergic airway inflammation and highlight the importance of p110 δ isoforms as a new target for therapeutic intervention in asthma.

Novel bronchodilators

Although several novel classes of bronchodilator have been explored, it is difficult to find a drug class of comparable efficacy and safety to β 2-adrenoceptor agonists, which counteract all known bronchoconstrictors. Some newer bronchodilators that may hold promise for asthma are: (i) vasoactive intestinal peptide (VIP), a potent relaxant of constricted human airways *in vitro*. However, its degradation in airway epithelium indicates that it is ineffective in asthmatic patients⁹¹. A more stable cyclic analogue of VIP (Ro-25-1553) has a more prolonged effect *in vitro* and *in vivo* and is effective in asthmatic patients by inhalation⁹²; (ii) intravenous infusion of atrial natriuretic peptide (ANP) produces a significant bronchodilator response and protects

against bronchoconstriction induced by inhaled spasmogens, such as methacholine⁹³; thus, it is possible that useful non-peptide agonists of ANP receptors could be developed in the future; and, (iii) K⁺ channel openers have been studied in phase I/II trials. However, recently the use of current forms of these drugs to treat asthma has been halted, because of dose-limiting vasodilator side effects (headaches and postural hypotension)⁹⁴.

Transcription factors blockade

Many transcription factors are involved in the expression of inflammatory genes in asthmatic airways and are, therefore, possible targets for anti-inflammatory drugs. Selective inhibitors of IKK2 or the κ B proteasome (the multi-functional enzyme that degrades I κ B) and, therefore, of NF- κ B are currently in development⁹⁵. However, one concern about long term NF- κ B inhibition is that it could result in immune suppression and impair host defenses. NF- κ B is also an important pro-survival signal in most cells, so chronic inhibition could pose issues related to unregulated tissue changes due to disequilibrium between physiological proliferation and cell death. Inhaled formulations of the immunosuppressors cyclosporin and tacrolimus are being tested for efficacy in asthma, but it remains to be determined whether this would provide a favourable therapeutic ratio. Blocking GATA3 with an antisense oligonucleotide or a dominant-negative mutant prevents the differentiation of TH2 cells and the development of eosinophilic inflammation in mice and thus could also be a possible target for asthma⁹⁶.

Kinase inhibitors

There has been particular interest in inhibitors of the p38 mitogen-activated protein (MAP) kinase pathway, which is involved in expression of several inflammatory proteins that are relevant to asthma⁹⁷. p38 MAP kinase inhibitors decrease eosinophil survival by activating apoptotic pathways⁹⁸ and several inhibitors of p38 MAP kinase are now in phase II development. Whether this new class of anti-inflammatory drugs will be safe in long-term studies remains to be established; it is likely that such a broad-spectrum anti-inflammatory drug will have some toxicity, but inhalation might be a feasible therapeutic approach to limit exposure to afflicted tissues, the airways.

Jun N-terminal kinases (JNKs) are involved in activation of the transcription factor AP-1, which has been shown to be induced in asthmatic airways and small-molecule JNK inhibitors have now been developed that have anti-inflammatory effects in allergen-exposed sensitized animals⁹⁹. Steroid resistance in asthma can be associated with increased activation of JNKs, indicating that JNK inhibitors could be useful in severe asthmatic patients with reduced steroid responsiveness. Syk (p72Syk) is a cytoplasmic tyrosine kinase that is pivotal in signaling through the high-affinity IgE receptor (FcεRI) in mast cells. In Syk-deficient mice, mast-cell degranulation is inhibited, indicating that the kinase could be a useful target for the development of mast-cell-stabilizing drugs¹⁰⁰. Indeed, aerosolized Syk antisense oligodeoxynucleotide inhibits allergen-induced inflammation in a rat model, suggesting that this could be a target for asthma drug development¹⁰¹.

CpG oligonucleotides

CpG-oligonucleotides (ODNs) are bound by CpG-binding proteins at the cell surface, and the ODNs then undergo endocytosis, where they become bound to TLR9 in the endosomes¹⁰². TLR9 interacts with MyD88 and the IKK complex, leading to nuclear activation of NF-κB, which initiates the induction of cytokines and chemokine transcription. Administration of CpG-ODNs to mice increases the ratio of TH1 to TH2 inflammation and decreases the formation of specific IgE and eosinophilic response to allergen¹⁰³. CpG-ODN treatment also reverses established allergen-driven eosinophilic inflammation in mice¹⁰⁴ and AHR in mice sensitized to ragweed pollen antigen¹⁰⁵. These promising animal studies show that CpG-ODN and DNA vaccines might prevent or cure atopic diseases in the future, and clinical trials of these compounds are currently underway^{102,106}.

Antisense oligonucleotides

Antisense oligonucleotide (AON) molecules modulate expression of a target gene by binding to its mRNA and preventing translation¹⁰⁷. An inhaled antisense oligonucleotide directed against the adenosine A1 receptor reduces AHR in a rabbit model of asthma, thereby demonstrating the potential of this delivery route¹⁰⁸. Respirable antisense oligonucleotides (RASONS) represent a truly novel approach to asthma therapy, and clinical trials with the A1 receptor oligonucleotide EPI-2010 (EpiGenesis)

have shown that this therapy is well tolerated¹⁰⁹. Decoy double-stranded oligonucleotides containing the DNA-binding motif of transcription factors look promising as blockers of specific transcription factors, such as NF-κB and STATs¹¹⁰.

TPI ASM8 and TPI 1100 (Topigen) are two products containing modified phosphorothioate AONs, which are undergoing development for the treatment of asthma and COPD, respectively. TPI ASM8 is comprised of two AONs, one targeting the human chemokine receptor 3 (CCR3) and the other targeting the common beta-chain of the IL-3/IL-5/GM-CSF receptors. TPI 1100 is also a dual-AON compound targeting the PDE 4 and 7 isotypes. Both products are administered by inhalation to patients and TPI ASM8 is currently undergoing phase 2 clinical trials¹¹¹. Thus, the application of oligonucleotide technology, such as antisense to regulate the transcription of disease-related genes *in vivo* has important therapeutic potential for asthma therapy.

Other possible therapies

The recent discovery of H4 receptors expressed on mast cells, T cells and eosinophils has raised the possibility that H4 receptor antagonists could be of use in asthma¹¹². Classical antagonists of the H1 receptor are of little clinical value in asthma, despite the clear association of mast cell released histamine with allergen-induced asthma exacerbation. The selective H4 receptor antagonist JNJ 7777120 potently inhibits mast-cell activation and chemotaxis and might therefore be of potential benefit in asthma¹¹³. This is supported by growing evidence that the accumulation of mast cells in the airway smooth muscle layer may be a pathological feature that predicts steroid refractory asthma^{114,115}. Several classes of prostaglandin antagonists have been evaluated as a treatment for asthma¹¹⁶, however, blocking the production of PGD2 with cyclooxygenase inhibitors has not proven to be of significant benefit. Endothelin antagonists¹¹⁷, nitric oxide inhibitors¹¹⁸ and adenosine antagonists¹¹⁹ have also been developed recently, but their potential use in asthma has still to be fully evaluated.

Concluding remarks

Though current asthma therapy is safe and effective in at least 70% of patients, efforts are being made to improve existing therapy, in particular for those patients in whom current therapies are ineffective. In most patients, inhaled corticosteroids

Table 1—Pipeline of anti-asthma drugs at different stages of clinical development

Compound	Stage	Proposed mechanism of action	Company	Intended route
Roflumilast	Phase-III	PDE-4 inhibitor	Nycomed/ Forest	PO
Mepolizumab	Phase-III	Anti-IL-5R antibody	GSK	IV, SC
Darapladib	Phase-III	Phospholipase A2 inhibitor	GSK/Human Genome Sciences	PO
GS 256066	Phase II	PDE-4 inhibitor	GSK	
ATL 1102	Phase-II	Integrin α 4 inhibitor	Isis Pharmaceuticals	Inhalation, PO, SC
AZD 1981	Phase-II	PG receptor antagonist	AstraZeneca	PO
Anrukinzumab	Phase-II	IL-13 inhibitor	Wyeth	IV, SC
MEDI528	Phase-II	Anti-IL-9 antibody	AstraZeneca	IV, SC
CAT354	Phase-II	Anti-IL-13 antibody	AstraZeneca	IV, SC
MEDI 563	Phase-II	Anti-IL-5R antibody	AstraZeneca	IV, SC
CAT 354	Phase-II	IL-13 inhibitor	Cambridge Ab Tech/MedImmune	IV, IV, SC
MK 0633	Phase-II	5-LO inhibitor	Merck & Co	PO
Oglemilast	Phase-II	PDE-4 inhibitor	Glenmark/Forest Labs	PO
OX 914	Phase-II	PDE-4 inhibitor	Orexo	PO
QAV 680	Phase-II	PGD2 receptor antagonist	Novartis	PO
TG 100115	Phase-I/II	PI3K inhibitor	TargeGen	Inhalation, IV
ABN 912	Phase-I	Chemokine receptor antagonist	Novartis	IV-infusion
AIR 645	Phase-I	IL-4 receptor antagonist	Isis Pharmaceuticals	Inhalation
AM 211	Phase-I	PGD2 receptor antagonist	Amira Pharmaceuticals	PO
AMG 761	Phase-I	CCR4 receptor antagonist	Amgen	
AMG 853	Phase-I	PGD2 receptor antagonist	Amgen	
AP 768	Phase-I	PGD2 receptor antagonist	Bayer HealthCare	
AVE 0675	Phase-I	TLR-9 agonist	Coley Pharmaceutical Group	Inhalation
AZD 5985	Phase-I	PGD2 receptor antagonist	AstraZeneca	PO
AZD 8075	Phase-I	PGD2 receptor antagonist	AstraZeneca	PO
BT 061	Phase-I	CD4 antigen inhibitor	Biotest	IV
CVT 6883	Phase-I	Adenosine A2 receptor antagonist	CV Therapeutics/Gilead Sciences	PO
DSP 3025	Phase-I	TLR-7 agonist	Dainippon Sumitomo Pharma	Intranasal
JNJ 18054478	Phase-I	Chymase inhibitor	Johnson & Johnson	PO
N 30 201	Phase-I	Nitric oxide donor	NitroMed	Inhalation
QAX 935	Phase-I	TLR-9 agonist	Idera Pharmaceuticals	
R 343	Phase-I	Syk kinase inhibitor	Rigel Pharmaceuticals	Inhalation
R 4930	Phase-I	OX40 ligand inhibitor	Genentech / Genmab / Roche	Parenteral
SAR 21609	Phase-I	TLR-9 agonist	Coley Pharma / Sanofi-aventis	
SAR 389644	Phase-I	PGD2 receptor antagonist	Sanofi-aventis	
TBC 4746	Phase-I	Integrin α 4 β 1 / VCAM antagonist	Encysive Pharmaceuticals	PO
PF 4191834	Phase-I	Dual-acting β 2 agonist/ M3 antagonist	Pfizer	Inhalation
PF 3526299	Phase-I	Dual-acting β 2 agonist/ M3 antagonist	Pfizer	Inhalation
PF 3893787	Phase-I	Dual-acting β 2 agonist/ M3 antagonist	Pfizer	Inhalation

* Source: Adis R&D Insight

are effective as a chronic treatment that is capable of suppressing underlying inflammation that predisposes to asthma exacerbation. It is most likely that combination inhalers that include a corticosteroid and long-acting β 2 agonist will remain the principal approach to asthma therapy, while leukotriene receptor antagonists (LTRAs) will remain a validated first line add-on treatment for all age groups. LTRAs also offer a protective effect, as they can be used in allergic rhinitis which is a risk factor for asthma.

The real issue today lies in the fact that 30% of patients are refractory to inhaled corticosteroids and ~10% of asthmatics represent a severe disease

sub-phenotype that is linked with very poor quality of life, increased hospital admission and death rate and accounts for at least 50% of all health care costs associated with asthma. On this basis, the search for new therapies that meet the needs of this void is essential. In addition, it cannot be overstated that current asthma therapy is somewhat of a “bandage approach”, because when used appropriately it does control disease symptoms, but they do not reverse the disease and upon removal asthma reappears, oftentimes in a more severe form. This situation also creates a void, albeit a “silent” one that necessitates efforts to develop new therapies can fill this gap.

Targeting pathways that determine Th2 function appears to be promising while treatments based on targeting IgE, though expensive appear to be effective in a subset of patients with difficult-to-treat asthma. The potential for non-allergic mechanisms to contribute to the pathogenesis of asthma might mean that novel treatments targeted at the allergy arm of the asthma phenotype may not be as efficacious as hoped for. It seems that the strategy of targeting single mediators, cytokines or chemokines may ultimately lead to less than optimal effect at relatively high cost, therefore approaches that have multiple effects and indirect targets may have greater impact in the long run.

Over the last two decades, there has been a great effort to develop new therapies for asthma as can be seen in Table 1, which lists the current drugs in clinical development. There is some hope for anti-inflammatory therapies, such as PDE-4, p38 MAP kinase, PI3K, Rho kinase, and IKK2 inhibitors, although these drugs are likely to have dose-limiting side effects if taken orally, thus will likely require inhaled administration. Emerging work on statins, which are very well tolerated and taken orally hold some promise in particular, since current basic research focus is on identifying the mechanisms for their seemingly pleiotropic effects. Through understanding the mechanism of action of statins, which is not related to serum cholesterol lowering in relation to a disease such as asthma, new classes of proteins for therapeutic targeting may be identified. Ultimately, the challenge for the next decade is to develop drugs that can fill the voids that current first line asthma therapy hold.

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