

Novel Strategies for the Treatment of Asthma

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Received: January 2, 2007; Accepted: January 9, 2007; Revised: January 11, 2007

Abstract: It is now clear that airway inflammatory processes characterized by eosinophils and Th2 lymphocytes are pivotal as the pathological features of asthma. Standard inhaled corticosteroids markedly suppress such inflammatory changes, resulting in clinical beneficial effects. However, it is also notified that airway wall remodeling including goblet cell hyperplasia, sub-epithelial collagen deposits, increased capillary networks and smooth muscle hypertrophy occur as a chronic consequence of this disorder even by the recommended strategies with steroid treatment. These pathologic changes play an important role in the increased airway obstruction and hyperresponsiveness, and eventually in the development of irreversible respiratory failure. Recent studies have elucidated that myofibroblasts and smooth muscle as well as mucosal epithelial cells play a vital role in these processes. Agents regulating proliferation, differentiation and activity of these cells, especially of low-molecular weight compounds, attract attention. Studies on molecular mechanisms of above processes, have led the development and patents of potential drugs including inhibitors of NF kappaB, statins, macrolides and phosphodiesterase-4 inhibitors.

Keywords: Asthma, mucosal epithelial cell, myofibroblast, smooth muscle cell, cytokines, chemokines, growth factor, signal transduction, transcription factor, statins.

INTRODUCTION

Asthma is characterized by allergic inflammatory responses with airway hyperresponsiveness, and its prevalence is increasing in many countries as one of the important socio-medical problems [1]. Both clinical and experimental studies suggest that eosinophils and Th2 type lymphocytes play a key role in the induction of airway inflammation and mucosal injury, which closely links to non-specific hyperresponsiveness in asthma [2]. In fact, inhaled corticosteroids markedly suppress the airway hyperresponsiveness and asthma symptoms along with decreased eosinophil infiltration in the airways. Clinical trials with anti-IL-5 antibody for the treatment of asthma, however, failed to show the clinical improvement despite apparent decrease in eosinophils of the peripheral blood, and hence, posed some doubt about the critical role of this inflammatory cell [3,4].

It is now proved that asthma is a heterogeneous and complex airway disease that involves both inflammatory and "non-inflammatory" processes [5,6]. It has been considered that asthma processes are affected by various interactions between airway epithelial cells and other mesenchymal cells such as myofibroblasts and smooth muscle cells. From viewpoints of the updated understanding, novel strategies are being proposed for better control of this disorder and drugs regulating not only inflammation, but also proliferation, differentiation and apoptosis of these cells attract attention.

UPDATED UNDERSTANDING OF ASTHMA PATHOGENESIS: ROLE OF AIRWAY REMODELING

Airway remodeling in asthma has been implicated as a significant pathological change that includes subepithelial

fibrosis, goblet cell hyperplasia, smooth muscle cells proliferation and microvascular changes (Fig. 1). Such structural alterations observed in asthmatic airways are believed to be related to the severity and therapeutic outcomes of asthma [7,8].

1. Subepithelial Fibrosis and its Molecular Pathogenesis

Bronchial subepithelial fibrosis is considered to be an important part of airway remodeling. A significant correlation was found between subepithelial layer thickness and degree of airway hyper-reactivity [9]. Increased deposition of collagens type I, III and V is thought to be induced by fibroblasts under the basement membrane. Fibroblasts, especially so-called myofibroblasts, are increased in the airways of asthmatic patients, and the number of myofibroblasts is correlated with the degrees of airway hyperresponsiveness [10,11]. Abnormal extracellular matrix (ECM) deposition is induced by imbalance between matrix metalloproteinases (MMPs) and tissue inhibitor of matrix metalloproteinases (TIMPs) [12]. Inflammatory cells such as neutrophils and eosinophils produce and release MMPs and TIMPs. Various ECM produced by myofibroblasts also act as growth and migration factors for fibroblasts. ADAM33 (A Disintegrin And Metalloproteinase33) has been identified as a gene that is linked to asthma in a Caucasian population [13]. ADAM33 is a membrane-anchored metalloproteinase [14]. The expression of this gene is abundant in airway fibroblasts and smooth muscles. Therefore, this protein may play an important role in airway remodeling.

Regulation of fibroblast proliferation is dependent on several growth factors via each specific as well as common signal transduction pathways. *In vitro* co-culture of bronchial epithelial cells and myofibroblasts as a model of airway remodeling of asthma has been reported, and when bronchial epithelial cells are damaged, growth factors [platelet-derived growth factor (PDGF), fibroblast growth

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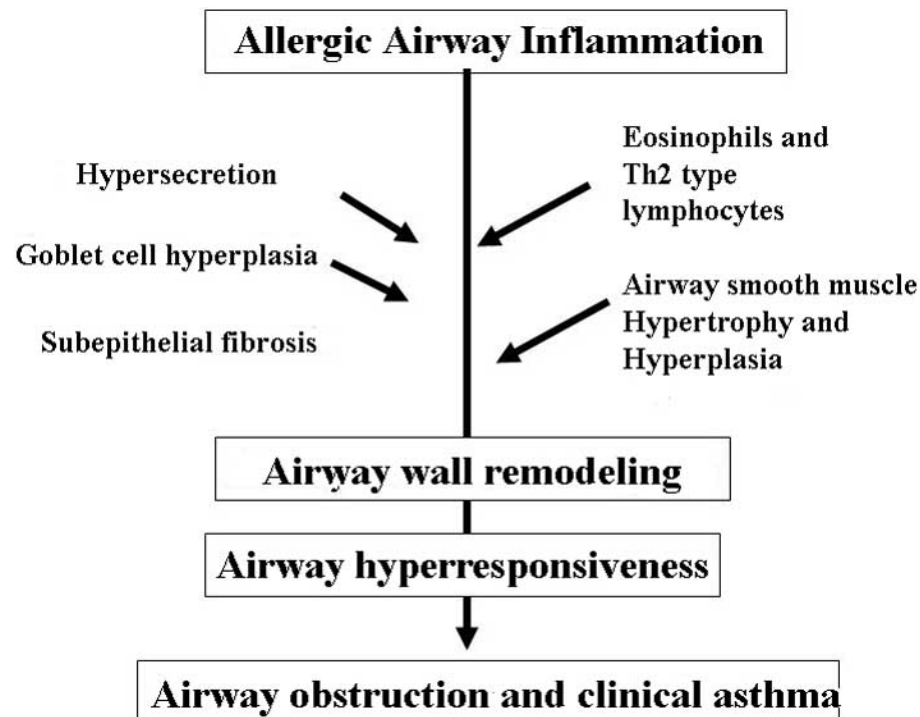


Fig. (1). Novel understanding of the pathogenesis of asthma.

Airway epithelial cells, fibroblasts and smooth muscle cells are capable of releasing various cytokines and growth factors which positively and negatively regulate mesenchymal cells, which are involved in airway remodeling.

factor-2 (FGF-2), insulin-like growth factor-1 (IGF-1), transforming growth factor (TGF)- β , endothelin-1 (ET-1)] are secreted by bronchial epithelial cells and these factors control myofibroblast proliferation and migration [15]. TGF- β 1 stimulates or inhibits proliferation depending on the condition of fibroblast cultures. In addition, prostanoids are involved in negative signals for fibroblast proliferation. We found that Th2 cytokines, IL-4 and IL-13 upregulated cell growth of normal lung fibroblasts, and a potent cyclooxygenase inhibitor indomethacin increased proliferation up to the level reached by the action of IL-4 or IL-13 [16].

Regulatory mechanisms of how fibroblasts differentiate into myofibroblasts have been studied. A potent fibrogenic growth factor TGF- β is known to induce myofibroblastic differentiation [17]. We found that IL-4 and IL-13 can directly induce differentiation of lung fibroblasts [16]. Importantly, such phenotypic differentiation was not attenuated by dexamethasone (DEX), a potent corticosteroid. As a Th1 type cytokine, interferon(IFN)- γ counteracted the effect of IL-4 and IL-13, and attenuated the expression of α -smooth muscle actin (SMA), a marker of myofibroblasts [16]. This result suggests that intervention to the Th1-Th2 imbalance in asthmatic airways can be beneficial for airway remodeling.

2. Airway Smooth Muscle Cells: Mechanisms of Proliferation and Hypertrophy

Airway narrowing in asthma is the result of spasms of airway smooth muscles (ASM). Hypertrophied ASM lead to the severe airway narrowing. ASM cell size is greater in

patients with severe asthma as compared with that in control subjects [18]. Bronchial biopsies revealed that numbers and sizes of ASM cells were negatively associated with pre-bronchodilator and post-bronchodilator FEV1 values in asthma [18].

Regulatory mechanisms of proliferation of airway smooth muscle cells have been investigated. Nitric oxide and prostaglandin (PGE)₂, which are released by bronchial epithelial cells, inhibit proliferation of smooth muscle cells [19,20]. PDGF, TGF- β , IGF-I have growth stimulating activity [21], which are also released by bronchial epithelial cells. ASM cells of bronchial asthma themselves produce connective tissue growth factor (CTGF) in response to stimulation with TGF- β [22]. CTGF release by smooth muscle cells may contribute to the increased production of fibronectin and collagen deposition in the remodeled airway wall. ASM in asthma release less endogenous PGE₂ than normal smooth muscle cells [23].

Infiltrated inflammatory cells including eosinophils affect proliferation of smooth muscle cells via TGF- β , PDGF, and tumor necrosis factor (TNF) [24]. Mast cell infiltration in airway smooth muscle layer is a unique feature in asthma [25]. Mast cell-derived histamine and tryptase induce proliferation of smooth muscle cells [26] whereas, mast cell chymase modifies cell matrix interactions and inhibits mitogen-induced proliferation of human ASM cells [27].

3. Goblet Cell Hyperplasia

In asthma, goblet cell hyperplasia and increased mucus production occur specially in the small airways. Goblet cell

hyperplasia is thought to be the result from the airway epithelial injury. Th2 cytokines interact with the repairing epithelium to promote goblet cell hyperplasia [28]. IL-13 induces the goblet cell hyperplasia by the production of heparin-binding-epidermal growth factor (EGF) [29]. Calcium-activated chloride channel-1 (CLCA1) gene may also be related to induce goblet cell hyperplasia in the airways of bronchial asthma [30].

4. Vascular Components

Airway remodeling also include changes of vascular components in the airway. Increased vascularity, vasodilation, and microvascular leakage occur, which are related to the increased airway wall thickness. Even among patients with mild asthma, increased number of small vessels in submucosal layer was reported, and this fact suggests that angiogenesis is a component of the chronic airway remodeling in asthma [31]. These changes of vascular components are thought to be induced by vascular endothelial growth factor (VEGF), which is induced by PDGF and platelet activating factor (PAF) [32].

AIRWAY REMODELING AS A RESULT OF DYSREGULATED EPITHELIAL MESENCHYMAL TROPIC UNITS

Above mentioned data strongly suggest that airway epithelial cell, fibroblast and smooth muscle cell closely interact with each other and exquisitely regulate the repair process and/or remodeling. In the asthmatic airways, dysregulated repair process might result in structural and functional changes known as remodeling. In the regeneration of epithelial cells, EGF plays an important role, and it is reported that the expression of EGFR (epidermal growth factor receptor) is upregulated in asthmatic bronchial epithelium, while proliferation of the epithelium does not take place appropriately [33]. This observation suggests that bronchial epithelial cells in asthma lack in functional response to the binding of EGF.

In contrast, negative growth factors such as TGF- β seem to play an important role in epithelial repair of asthma. While intrinsic fragility and impaired proliferation of epithelial cells might be a cause of epithelial damage and subsequent profibrogenic growth factor production, much has yet to be clarified to explain the precise mechanism of remodeling.

Above mentioned progress in the understanding of the molecular mechanisms of airway inflammation and remodeling in asthma leads us to re-evaluate the present and future drugs for asthma treatment. Although complex and heterogeneous processes are involved, one can understand that certain signal transduction pathways play pivotal roles as final common processes, and thus, it is crucial to determine the target molecule(s) and route of administration for proper drug design (Fig. 2).

POTENTIAL CHOICES FOR THE TREATMENT OF ASTHMA: A FUTURE PERSPECTIVE

1. Inhibitors of Transcription Factors

As mentioned above, increased expression of cytokines, chemokines, adhesion molecules and growth factors are the

essential features of persistent, chronic asthma with inflammation and airway wall remodeling. Several transcription factors have been implicated in the pathogenesis of asthma, including the glucocorticoid receptor (GR), nuclear factor kappa B (NF kappaB), Activator Protein-1 (AP-1), Nuclear Factor of Activated T-cells (NF-AT), cyclic AMP Response Element Binding Protein (CREB) as well as signal transducer and activator of transcription (STAT)1 [34] and more recently, the CCAAT/Enhancer Binding Protein (C/EBP), STAT6, Peroxisome Proliferator-activated Receptor (PPAR), STAT6, Peroxisome Proliferator-activated Receptor (PPAR) and the bZIP transcription factor, nuclear factor E2-related factor 2 (Nrf2) [35]. In clinical practice, inhaled corticosteroids and beta agonists are commonly used for the treatment in asthma and are often used together. Recent evidence suggests that many of the anti-inflammatory actions of corticosteroids are mediated by cross-talk between the activated GR and other transcription factors such as the pro-inflammatory NF kappaB. In a randomized, placebo controlled, crossover study of six weeks' treatment with inhaled budesonide (400 microg twice daily), terbutaline (1 mg four times daily), and combined treatment were recruited [36]. Biopsy samples of the bronchial mucosa were obtained after each treatment and analysed for the DNA binding activity of GR, CREB, and NF kappaB. Budesonide increased GR activity and decreased NF kappaB activity. No treatment combination altered CREB activity and terbutaline had no significant effects on any transcription factor. Thus, effects of inhaled corticosteroids might be due to, at least in part, the dual effects on GR and NF kappaB activity in bronchial mucosa.

NF kappaB Inhibitors

NF kappaB is an inducible transcription factor that plays a central role in the regulation of many immune and inflammatory responses. While NF kappaB is required for cell survival and immunity, abnormal expression and/or activation of NF kappaB leads to the development of many pathological states, especially those involved in chronic and acute inflammation. A variety of signal transduction pathways, originating from various cellular stresses and stimuli, lead to the downstream molecular targets: the NF kappaB/IkappaB complex and its activating kinase (inhibitor of kappaB kinase, IKK). Several inhibitors of these processes have been patented. For examples, new pyrazoloisoquinoline derivatives are inhibitors of NF-kappa-B inducing kinase, especially IKK2 (also known as IKK β) [37], and can be useful in the treatment of disorders associated with inappropriate activity, such as rheumatoid arthritis, asthma, and COPD (chronic obstructive pulmonary disease). New Indazole carboxamide derivative or its salt is another inhibitor of IKK2 activity [38]. New crystalline monopotassium salt form of 2-((2-(2-methylamino-pyrimidin-4-yl)-1H-indole-5-carbonyl)-amino)-3-(phenylpyridin-2-ylamino)-propionic acid is IKK inhibitor [39]. New anilinopyrimidine derivatives are selective inhibitors of IKK, particularly IKK-2 [40].

Another invention is related to the co-administration of a dehydroepiandrosterone (DHEA) congener in combination with a parthenolide, a naturally occurring NkappaB inhibitor, to reduce inflammation [41].

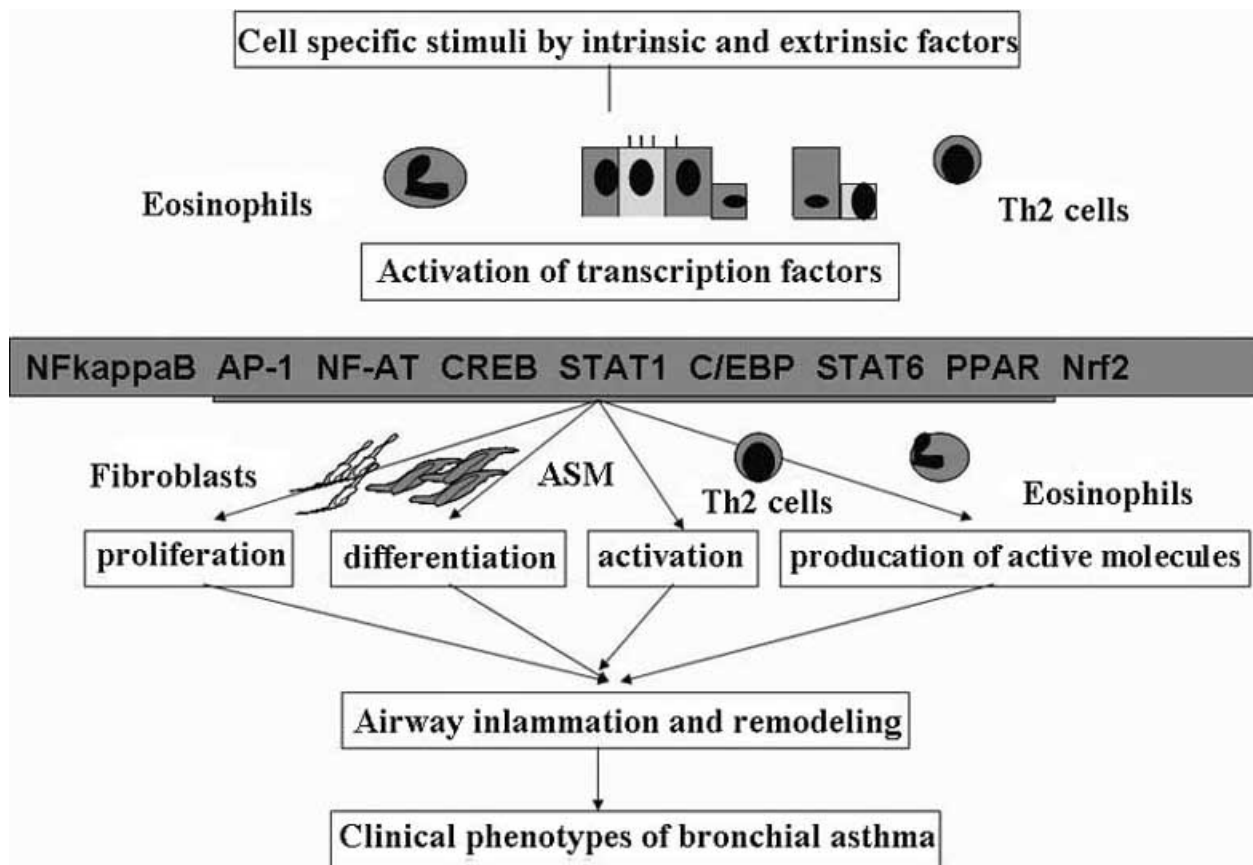


Fig. (2). Cell specific signal transduction in the molecular mechanisms of airway inflammation and remodeling in asthma.

Certain signal transduction pathways resulting in several transcription factors activation play pivotal roles as final common processes, and thus, it is crucial to determine the target molecule(s) and route of administration for proper drug design.

Although these low-molecular products seem to have a potential to be a novel choice for the treatment of intractable asthma and COPD, it is worrisome to suppress these pivotal transcription processes, since many transcription factors play a central role in tissue and organ homeostasis. Cell type specific application of decoy or antisense oligonucleotides or inhaled formulations to antagonize against NF kappaB, may help to control the inflammatory responses in the affected airways, with little adverse effects.

2. Statins

Statins reduce cholesterol levels by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and have an established role in the treatment of atherosclerotic disease. Recent research has identified anti-inflammatory properties of statins. Statins appear to reduce the stability of lipid raft formation with subsequent effects on immune activation and regulation, and also inhibit signaling molecules with subsequent downregulation of gene expression. Both these effects result in reduced cytokine, chemokine, and adhesion molecule expression, with effects on cell apoptosis or proliferation. In allergic asthmatic models of mice, simvastatin reduced ovalbumin-specific IgE level, the number of total inflammatory cells, including macrophages, neutrophils, and eosinophils into bronchoalveolar lavage fluid, the expressions of CD40, CD40L or VCAM-1, the mRNA and protein levels of interleukin (IL)-

4, IL-13 and TNF-alpha, the numbers of goblet cells, activities of MMPs, and further small G proteins, mitogen-activated protein kinases and NF-kappaB activities in bronchoalveolar lavage cells and lung tissues [42]. In clinical studies, lung transplant recipients with statin therapy had a better survival rate than those without it [43]. This result was probably reflected by down regulation of myofibroblast function with statin [44]. The important key cell signaling molecule affected by statins appears to be Ras, which is a small guanosine triphosphate (GTP) - binding protein and is a key signaling molecule acting downstream of growth factors. Lovastatin can inhibit the activation of Ras through a modification of Ras localization to the inner plasma membrane of fibroblast [44]. Studies have demonstrated that lovastatin potently induces apoptosis in fibroblasts constitutively expressing Myc, and that lung fibroblasts isolated from fibrotic lesions constitutively express growth-promoting genes [44]. Clinically achievable concentrations of lovastatin induce apoptosis in normal and fibrotic lung fibroblasts *in vitro*, as evidenced by acridine orange staining, terminal transferase nick end translation (TUNEL), and DNA laddering. Apoptosis of human lung fibroblasts was dose- and time-dependent, and blocked by exogenous mevalonic acid. Furthermore, apoptosis was associated with decreased levels of mature Ras, a molecule directly implicated in fibroblast rescue from apoptosis. The ability of lovastatin to induce fibroblast apoptosis *in vivo*

was ascertained using a guinea pig wound chamber model [44]. These findings support further study of statins as potential therapy for patients with fibroproliferative disorders.

It is also considered that mevalonate metabolites play an essential role in transducing EGF receptor (EGFR)-mediated signaling cascades. Targeting HMG-CoA reductase using lovastatin induces a potent apoptotic response in a variety of tumor types at therapeutically achievable levels of this drug [45]. As mentioned above, there exists a persistent activation of EGFR signaling in mesenchymal cells of asthmatic airways, and therefore, the effects of lovastatin on EGFR function might be applied to the prophylaxis and/or treatment of airway remodeling in chronic asthma [46].

A patented invention provides medicaments comprising combinations of bronchodilators, glucocorticosteroids and HMG-CoA reductase inhibitors in the treatment of respiratory disorders such as COPD as well as asthma [47].

3. Macro-Steroids

Erythromycin and its 14-member macrolide analogues have attracted attention for their effectiveness in a variety of airway diseases including diffuse panbronchiolitis (DPB), sinobronchial syndrome, chronic sinusitis and bronchial asthma. *in vitro* As well as *in vivo* studies strongly suggested that macrolides have potentials to inhibit expression of inflammatory cytokines and chemokines. In chronic airway inflammation, there is a prominent increase in a variety of cytokines such as IL-1, TNF, and IL-8. Treatment with 14-ring member macrolide antibiotics resulted in decreased cytokine levels in the airway lining fluids, suggesting that they have potentials to inhibit cytokine production in the local milieu. *in vitro* Studies demonstrated that they have, indeed, inhibitory effects on cytokine /chemokine production by several kinds of cells [48]. Recent progress for the elucidation of molecular mechanisms of their unique and novel anti-inflammatory actions indicated that these agents inhibit activation of several transcription factors including NF kappaB and AP-1 [49]. They have also been shown to inhibit fibroblast proliferation, suggesting anti-remodeling activity. Recent reports [50,51] showing beneficial effects of anti-TNFalpha receptor antagonist on severe intractable asthma, further suggested therapeutic possibilities of this group of drugs. In this regards, macrolide conjugates with anti-inflammatory activity is interesting [52]. New macro-lide derivatives are glucocorticoid receptor antagonists which are expected to be useful to treat inflammatory diseases, disorders and conditions, and immune disorders associated with e.g. COPD, asthma and bronchitis. This invention might improve therapeutic action and the use in the treatment of inflammatory diseases and conditions in humans and animals. New decladinoyl macrolide derivatives are cytokine inhibitors used for treating inflammatory disorders e.g. asthma and adult respiratory distress syndrome [53]. The present invention relates to novel semi-synthetic macrolides with anti-inflammatory activity. More particularly, the invention relates to 14- and 15-membered macrolides lacking cladinose sugar substituted at the C-3 position, to their pharmaceutically acceptable derivatives, to processes and intermediates for their preparation, to pharmaceutical compositions containing them and to their

activity and use in the treatment of inflammatory diseases and conditions in humans and animals, especially those diseases associated with excessive secretion of TNF-alpha, IL-1, IL-6, IL-8, IL-2 or IL-5 [53].

4. PDE4 Inhibitors

Phosphodiesterase-4 (PDE4) is an important cyclic adenosine monophosphate (cAMP)-metabolising enzyme in immune and inflammatory cells, airway smooth muscle and pulmonary nerves. PDE4 plays a significant role in modulating the activity of cAMP, an important second messenger that mediates the relaxation of airway smooth muscle and suppresses inflammatory cell function, thereby attenuating the inflammatory response. Selective inhibitors of this enzyme show a broad spectrum of activity in animal models of COPD and asthma [54]. These drugs block the hydrolysis of cAMP via inhibition of PDE4 and are attractive candidates for novel anti-inflammatory drugs. At present, two second-generation PDE4 inhibitors for the treatment of COPD and asthma patients are being tested in clinical Phase III trials. The first compound is the orally active, selective PDE4 inhibitor cilomilast (cis-4-cyano-4-[3-cyclopentyloxy-4-methoxyphenyl]-cyclohexanecarboxylic acid) [54]. Cilomilast shows high selectivity for cAMP-specific PDE4, an isoenzyme that predominates in pro-inflammatory and immune cells and that is 10-fold more selective for PDE4D than for PDE4A, -B or -C. *in vitro*, Cilomilast suppresses the activity of several pro-inflammatory and immune cells important in asthma and COPD. Moreover, it is highly active in animal models of these diseases [54]. Another PDE4 inhibitor, roflumilast was suggested to regulate the ECM and therefore processes of airway remodeling in asthma [55].

New pyrazolo-naphthyridinone compounds are PDE4 inhibitors useful for treating many diseases, including respiratory disease especially asthma [56]. New fluorene compounds are other group of PDE4 inhibitors [57], which down regulate or inhibit the production of TNF-alpha and therefore might be useful in the treatment of variety of allergic and inflammatory diseases including asthma and COPD.

5. Modulation of Th1/Th2 Balance

It has been documented that Th1/Th2 imbalance toward the predominance of Th2 cells play an important role in the airway inflammatory changes of asthma. As mentioned above, modulation of Th1/Th2 balance might also be a useful tool for the prophylactic treatment of airway remodeling. In fact, there is increasing body of literature suggesting that interferon may have a beneficial effect especially in severe persistent asthma [58-60]. A group of Th1 cytokines including interferon, IL-12 and other related substances, therefore, are worthy to be evaluated.

6. Other Anti-Inflammatory and Anti-Remodeling Drugs

Other groups of anti-inflammatory and anti-remodeling agents have been patented as potential promising compounds in the treatment of asthma and related inflammatory airway diseases.

MONOCYCLIC AROYLPYRIDINONES AS ANTI-INFLAMMATORY AGENTS

New monocyclic aroylpyridinones [61] useful for treating acute and chronic inflammatory processes e.g. asthma, chronic obstructive pulmonary disease.

PYRIMIDYL SULPHONE

New sulfonamidopyrimidine derivatives [62] are chemokine receptor modulators useful to treat asthma, rheumatoid arthritis, psoriasis and osteoporosis.

METALLOPROTEINASE INHIBITORS

New pyrrolidinone derivatives are metalloproteinase inhibitors [63] to be used for treating cancer, Alzheimer's disease, asthma, rhinitis and rheumatoid arthritis. These compounds are potent inhibitors of MMP12.

CURRENT AND FUTURE DEVELOPMENTS

Most of the drugs for the treatment of asthma have been developed based on the inhibitory activity on allergic airway inflammation and bronchial smooth muscle contraction. Recent progress in the understanding of molecular events in chronic asthma has proved that airway remodeling is a new potential target of asthma treatment. A variety of anti-inflammatory and anti-remodeling compounds have been attracting attention and patented. It would be vital to determine the target molecule(s) for re-evaluating each compound for novel drug design in this field [64].

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