Biological targets for therapeutic interventions in COPD: clinical potential

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Abstract: COPD is a widespread inflammatory respiratory disorder characterized by a progressive, poorly reversible airflow limitation. Currently available therapies are mostly based on those used to treat asthma. However, such compounds are not able to effectively reduce the gradual functional deterioration, as well as the ongoing airway and lung inflammation occurring in COPD patients. Therefore, there is an urgent need to improve the efficacy of the existing drug classes and to develop new treatments, targeting the main cellular and molecular mechanisms underlying disease pathogenesis. These therapeutic strategies will be highlighted in the present review.

Keywords: COPD, bronchodilators, anti-inflammatory drugs, cytokine and chemokine antagonists, signal transduction inhibitors

Introduction

COPD is a chronic respiratory disease characterized by a progressive, not fully reversible airflow limitation, which is associated with an abnormal inflammatory response of the lungs to noxious particles or gases (Celli et al 2004). In particular, the main risk factor for COPD is tobacco smoking, whose pathogenic action may be potentiated by other harmful agents such as air pollution. However, because about 20% of COPD patients are not smokers and only 15%–20% of smokers develop COPD, a genetically based individual susceptibility also plays an important role in disease pathogenesis. Indeed, in addition to the well known defects occurring in the α1-antitrypsin gene, which are responsible for a very small percentage of COPD cases, several different polymorphisms involving other genes encoding tumor necrosis factor-α (TNF-α), matrix metalloproteinases (MMPs), antioxidants, and other molecules have also been detected (Molfino 2004). These complex interactions between environmental and genetic factors make COPD one of the leading causes of death worldwide, and the current epidemiological projections predict further increases in its prevalence and mortality during the next decades.

The main cellular elements of the inflammatory process that characterizes COPD include neutrophils, macrophages, and predominantly cytotoxic (Tc) CD8+ T lymphocytes (Lacoste et al 1993; Saetta et al 1999; Shapiro 1999). Migration and activation of these cells are mediated by a complex network of cytokines and chemokines synthesized by both inflammatory and resident cells, the latter including fibroblasts, and epithelial, endothelial, and airway smooth muscle cells. In COPD, airway and lung inflammation is associated with pulmonary structural changes, arising from a relative imbalance between elastolytic proteinases and their inhibitors (Shapiro and Ingenito 2005). Therefore, the resulting altered turnover of extracellular matrix leads to the parenchymal destruction and loss of elastic recoil typical of emphysema. Conversely, collagen deposition and fibrosis characterize the small airways, thereby thickening their wall and further contributing to airflow limitation in COPD. On the other hand, the same proteinases that cause elastolysis such as MMP-9, may also

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activate fibrogenic growth factors like transforming growth factor-β (TGF-β), which is in turn involved in MMP-9 activation thus triggering a positive, feed-forward pathogenic circuit (Barnes et al 2003). Moreover, oxidative stress is heavily implicated in both the inflammatory and tissue-damaging processes detectable in COPD patients, who are subjected to a remarkable oxidant burden due to cigarette smoking, air pollution, and intense metabolic activity of inflammatory and structural cells (Maselli and Pelaia 2005).

Currently available therapies for COPD are mostly based on those used to treat asthma, mainly including bronchodilators and anti-inflammatory drugs such as corticosteroids. In COPD, however, these compounds are less effective than in asthma. With regard to bronchodilators such as long-acting β_2-adrenergic agonists and anticholinergics, though airflow limitation in COPD is characterized by poor reversibility, when given to COPD patients these drugs seem to be quite effective in improving lung function, exercise tolerance, and health status, as well as in reducing symptoms and frequency of exacerbations (Cazzola and Donner 2000; Casaburi et al 2002). Much more evident is the relatively low sensitivity of COPD patients to inhaled corticosteroids, possibly for several reasons including corticosteroid-dependent reduction of neutrophil apoptosis, putative resistance of CD8+ T cells to corticosteroids and, most importantly, oxidative stress-induced inhibition of expression and activity of histone deacetylases (HDACs) in alveolar macrophages (Ito et al 2005); HDACs are indeed essential for corticosteroid action in that these enzymes play a key role in remodeling chromatin structure and switching off pro-inflammatory genes.

Therefore, there is an urgent need to achieve a much better control of COPD, and ongoing pharmacological research is at least in part focused on improving the efficacy of currently available drug classes, but it especially aims to develop novel therapeutic strategies, specifically targeting the main cellular and molecular mechanisms underlying disease pathogenesis. This review will outline the most promising approaches under development for COPD treatment, including improved bronchodilators and corticosteroids, as well as new compounds such as selective phosphodiesterase inhibitors, leukotriene modifiers, adhesion molecule blockers, tachykinin antagonists, inhibitors of cytokines and growth factors, chemokine inhibitors, anti-inflammatory cytokines, inhibitors of transcription factors and intracellular signal transduction, proteinase inhibitors, and antioxidants (Table 1).

Improvements in existing classes of drugs

Improved long-acting β_2-adrenoceptor agonists

Currently used long-acting β_2-adrenoceptor agonists such as salmeterol and formoterol are, like all other marketed compounds of this drug class, racemic mixtures of both R(right)- and S(sinister)-isomers. However, only R-enantiomers are pharmacologically active, whereas S-isomers are essentially inert (Handley et al 2000). Therefore, the newly developed pure R-form of formoterol (arformoterol) appears to be quite promising for a better control of COPD patients (Molfino 2005). Moreover, the pharmacological industry has tried to combine the therapeutic advantages of R-enantiomers with a once-daily dosing approach, thus developing a non-catechol, pure (*R,R*)-isomer and 24-hour-lasting β_2-agonist named carmoterol (TA-2005) (Kikkawa et al 1994; Cazzola et al 2005). This drug has been shown to have a high binding affinity for β_2-adrenergic receptors, and to be more potent than formoterol, salmeterol, salbutamol, procaterol, and isoprenaline. The carmoterol-derivative indacaterol (QAB-149) produces a very long bronchodilation, which lasts more than 24 hours, and preliminary clinical data suggest that such a compound is strongly efficacious in COPD (Cazzola et al 2005). It can be thus argued that COPD patients will probably benefit from these further advances in β_2-agonist pharmacology.

Improved anticholinergics

In addition to the long-acting tiotropium bromide, which dissociates slowly from M_3 and M_1 muscarinic receptors with respect to the inhibitory, pre-junctional M_2 autoreceptors, more selective M_1/M_3 antagonists are in clinical development. For instance, the M_3 anti-muscarinic compound LAS34273 is a long-acting anticholinergic

Table 1 New therapeutic interventions for COPD

Selective phosphodiesterase inhibitors
Leukotriene modifiers
Adhesion molecule blockers
Tachykinin antagonists
Inhibitors of cytokines and growth factors
Chemokine inhibitors
Anti-inflammatory cytokines
Inhibitors of transcription factors
Signal transduction inhibitors
Protease inhibitors
Antioxidants

characterized by a rapid and significant bronchodilatory action, coupled with a good tolerability pattern in both healthy subjects and COPD patients (Schelfhout et al 2003). Revatropate is an M_1/M_3 antagonist, with no effect on M_2 autoreceptors and available for inhalation, which has been shown to be an effective and well-tolerated bronchodilator in COPD patients (Alabaster 1997).

Improved corticosteroids

As already mentioned, corticosteroids are quite unable to suppress airway and lung inflammation in COPD, mainly because of the inhibitory effect exerted by cigarette smoke and oxidative stress on histone deacetylation, which is required for the repressive action of corticosteroids on pro-inflammatory gene expression. However, the new, non-halogenated, inhaled glucocorticoid pro-drug ciclesonide is under development for both asthma and COPD (Dent 2002). This compound, which is converted into a ciclesonide-active principle (CIC-AP) in the lung, seems to be more potent than the currently available corticosteroid agents.

Improved theophyllines

The current renewed interest for theophylline, whose clinical use has been quite neglected during recent years, arises from its suggested anti-inflammatory action, detectable even at low plasma concentrations (Barnes 2003). These newly recognized pharmacological properties are likely independent from either phosphodiesterase (PDE) inhibition or adenosine antagonism, and appear to be due to the ability of theophyllyne to activate HDACs (Barnes et al 2005). The latter mechanism may be very useful to restore HDAC activity impaired by oxidative stress, thus resensitizing COPD patients to the anti-inflammatory effects of corticosteroids. Therefore, there is a rational basis for a future design of novel theophylline-like molecules with improved HDAC-activating properties and devoid of the side-effects mainly caused by adenosine receptor antagonism. Such types of drugs could be indeed usefully utilized alone or in combination with corticosteroids, thereby increasing significantly the therapeutic potential of these anti-inflammatory agents, rather limited in COPD at present.

New therapeutic strategies

Selective phosphodiesterase inhibitors

At least 11 PDE families are currently known (Soderling and Beavo 2000), among which PDE4 is the predominant isoenzyme expressed in neutrophils, CD4+/CD8+ T cells, and monocytes/macrophages, as well as in airway epithelial and smooth muscle cells. Therefore, PDE4 is a suitable therapeutic target for treatment of COPD (Sturton and Fitzgerald 2002). Specific PDE4 inhibitors such as cilomilast and roflumilast are active in some animal models of neutrophil inflammation and have been tested in COPD patients (Barnes and Hansel 2004). A 6-week study performed in patients with moderate to severe COPD showed that cilomilast improved lung function (Compton et al 2001), and this drug was also able to reduce airway inflammation in biopsy samples (Gamble et al 2003). Furthermore, TNF-α release from peripheral blood monocytes was significantly inhibited by roflumilast (Reid 2002), which in a placebo-controlled trial also increased FEV_1 in COPD patients (Molfino 2005). This latter compound is better tolerated than cilomilast, especially with regard to gastrointestinal side-effects like vomiting, due to inhibition of the PDE4D gene preferentially expressed in the chemosensitive trigger zone in the brain stem (Lamontagne et al 2001). Cilomilast is indeed a selective PDE4D inhibitor whereas roflumilast does not target any specific member of the PDE4 family, thus exhibiting a more favorable therapeutic index that could be further improved by the future availability of selective inhibitors of the PDE4B isoform, which appears to play a prominent role in inflammatory cells (Barnes and Stockley 2005).

Other PDE4 inhibitors in clinical development for COPD and asthma include ONO-6126, arofylline, and AWD-12-281. Oral administration of ONO-6126 has been reported to inhibit both airway inflammation and bronchoconstriction (Molfino 2005). Inhaled arofylline, tested for 3 months in a double-blind, placebo-controlled Phase III trial involving 141 subjects with moderate to severe COPD, improved FEV_1 and reduced the incidence of disease exacerbations (Molfino 2005). AWD-12-281 is a new compound, already available as oral and inhaled formulations, which has the potential for inhibiting the release of pro-inflammatory mediators such as histamine, TNF-α, and granulocyte-macrophage colony stimulating factor (GM-CSF), and appears to be effective also in in vivo models of airway diseases (Kuss et al 2003). More recently, a selective PDE7 inhibitor named BRL 50481 has been developed (Smith et al 2004) that targets the PDE7 isoform expressed by monocytes/macrophages, CD8+ T-cells, and neutrophils, thus potentiating the anti-inflammatory actions of PDE4 inhibitors.

Leukotriene modifiers

Differently from the pathogenic mechanisms underlying asthma, which are characterized by an important role played by cysteinyl leukotrienes (LTC_4, D_4, E_4), LTB_4 is the leukotriene mostly involved in the inflammatory process associated with COPD. In fact, LTB_4 concentrations are markedly increased in both sputum and exhaled breath of patients with COPD (Hill et al 1999; Montuschi et al 2003), especially during exacerbations. LTB_4 interacts with the high-affinity BLT_1 receptor expressed by immune/inflammatory cells, thus exerting a powerful chemoattractant effect particularly on neutrophils, but also on CD8+ T-cells (Martin et al 1989; Goodarzi et al 2003).

Several selective BLT_1 antagonists are currently under investigation for their potential therapeutic use in COPD. LY293111 and SB225002 are able to inhibit the neutrophil chemotactic activity of sputum from COPD patients (Crooks et al 2000; Beeh et al 2003). A powerful and long-lasting activity characterizes the pharmacological profile of the LTB_4 receptor antagonist amelubant (BIIL-284), which is in clinical development for treatment of COPD, asthma, and cystic fibrosis (Birke et al 2001). BIIL-284 is an orally active pro-drug that is converted by ubiquitously expressed esterases into two active metabolites (BIIL-260 and BIIL-315). A more limited therapeutic potential seems to characterize LTB 019, another LTB_4 receptor antagonist that was tested in COPD patients, but did not elicit significant changes in FEV_1 as well as in induced sputum levels of neutrophils, TNF-α, and interleukin-8 (IL-8) (Gronke and Beeh 2002). Since LTB_4 is synthesized by 5′-lipoxygenase (5-LO), some 5-LO inhibitors such as CJ-13610 and BAYx1005 have been evaluated in COPD patients without showing marked activity in reducing sputum concentrations of LTB_4 and neutrophil expression of activation markers (Gompertz and Stockley 2002). It is thus likely that more potent 5-LO inhibitors are still needed.

Adhesion molecule blockers

Airway and lung infiltration by inflammatory cells depends on their adhesion to vascular endothelium and the subsequent migration into the respiratory system. In this regard a key role is played by the expression on neutrophils, monocytes, CD8+ T-lymphocytes, and endothelial cells, of several different families of adhesion molecules, which represent another potential target for anti-COPD therapies (Matera and Cazzola 2004). For instance, rolling of inflammatory cells along vascular endothelium is mediated by P-selectin, whose function may be inhibited by an antagonist (TBC1269) of its sialyl-LewisX ligand, which preferentially blocks neutrophil adhesion (Davenpeck et al 2000). Another selectin inhibitor, the imidazole compound OC 229648, is under evaluation for treatment of COPD and asthma (Romano and Slee 2001). The Mac-1 (CD11b/CD18) integrin might be a further interesting target because this adhesion molecule is overexpressed on neutrophils from COPD patients (Noguera et al 1998), and it is also present on the surface of monocytes and macrophages. However, these therapeutic approaches raise some concerns due to the possible risk of infections caused by a defective neutrophil migration.

Tachykinin antagonists

Antagonists of NK-1, NK-2, and NK-3 tachykinin receptors, stimulated by substance P (SP) and neurokinins A (NKA) and B (NKB), respectively, have been suggested to be potentially beneficial in both asthma and COPD (Joos and Pauwels 2001). Therefore, some single (NK-3), dual (NK-1/NK-2), or even triple (NK-1/NK-2/NK-3) orally active, NK receptor antagonists such as CS-003, DNK-333, and SB-223412 (talnetant), are in clinical development for COPD (Molfino 2005).

Inhibitors of cytokines and growth factors

TNF-α inhibitors

TNF-α is a pro-inflammatory cytokine involved in activation and migration of neutrophils, monocytes/macrophages, and T lymphocytes. In mice models, TNF-α is implicated in the pathogenesis of airway inflammation sustained by neutrophils and macrophages, as well as in cigarette smoke-induced emphysema (Churg et al 2002). In COPD patients, high concentrations of this protein have been detected in induced sputum and plasma, and increased amounts of TNF-α-mRNA have also been found in skeletal muscles (Keatings et al 1996; de Boer 2005). On the other hand, the severe wasting occurring in many patients with advanced COPD might be caused by TNF-α through the induction of skeletal muscle cell apoptosis. In support of this hypothesis, some TNF-α gene polymorphisms appear to be associated with a poorer disease prognosis (Keatings et al 2000).

Therefore, TNF-α seems to be a suitable target for the development of anti-COPD drugs, and different approaches have been tried to inhibit either the production or functions of this cytokine. Many of these compounds are in clinical trial for COPD and other chronic disorders such as asthma,

Crohn's disease, rheumatoid arthritis, and psoriasis. TNF-α inhibitors include monoclonal, non-human or chimeric anti-TNF-α antibodies (infliximab, afelimomab, and CytoTab), humanized antibodies (adalimumab, CDP-571, CDP-870), human soluble TNF receptors (onercept) or TNF receptor fusion proteins (etanercept), and antisense oligonucleotides (ISIS-104838) that inhibit TNF-α mRNA translation into pre-TNF-α proteins (de Boer 2005). The aim of humanization is to reduce the immunogenicity of therapeutic antibodies and the consequent side-effects. The latter cannot, however, be completely avoided, in that local reactions around the site of injection and, less frequently, a delayed hypersensitivity-like reaction and a new onset of autoimmunity have also been reported (de Boer 2005). Very recently, the results of the first Phase II clinical trial evaluating the effects of infliximab in COPD patients have been published (van der Vaart et al 2005). According to this double-blind, placebo-controlled study involving 22 current smokers with mild to moderate COPD, 14 of which received 3 infusions of infliximab (5 mg/kg) at weeks 0, 2, and 6, respectively, such a treatment did not induce any change in respiratory symptoms, FEV_1, quality of life, and percentage of inflammatory cells in induced sputum. Anyway, larger and longer-term trials are still needed to better understand whether infliximab may have a place in COPD management. Another anti-TNF-α strategy can be achieved by inhibiting TNF-α converting enzyme (TACE), which cleaves pre-TNF-α into the mature cytokine. Furthermore, TACE inhibitors (marimastat, BMS-561392, or DPC-33) may also reduce MMP activity and mucin 5AC production (Shao et al 2004), thereby providing potential additional benefits for COPD patients. However, anti-TNF-α therapies are only beginning to be evaluated for COPD treatment and, therefore, it is too early to predict the future impact of the above mentioned experimental approaches.

Interleukin-1 inhibitors

Cigarette smoke and lipopolysaccharide (LPS) stimulate macrophages and epithelial cells to produce interleukin-1 (IL-1), which in turn induces TNF-α expression in macrophages (de Boer 2005). When compared with healthy subjects, IL-1 concentrations have been reported to be increased in induced sputum from patients with mild COPD (Dignetti et al 2002). Anti-IL-1 strategies include the use of endogenous, soluble IL-1 receptor antagonists (sIL-1Ra), anti-IL-1 receptor antibodies, IL-1 binding proteins such as IL-1 Trap, anti-IL-1β antibodies (CDP 484), and inhibitors of IL-1β converting enzyme (ICE). A recombinant IL-1 receptor antagonist (anakinra) has been already tested in subjects with rheumatoid arthritis, improving both symptoms and tissue damage typical of this chronic disease (Olsen and Stein 2004). Preliminary data suggest that administration of anakinra to patients with COPD may increase the risk of bacterial lung infections (de Boer 2005). However, despite the apparently disappointing results of these early clinical studies, the various approaches aimed at inhibiting IL-1 functions and/or synthesis might be potentially beneficial in COPD, also because of the consequent reduction of TNF-α expression.

Interleukin-6 inhibitors

In the exhaled breath condensate, as well as in serum during disease exacerbations, COPD patients exhibit elevated levels of interleukin-6 (IL-6) (Wedzicha et al 2000; Bucchioni et al 2003), a pleiotropic cytokine released by several different cells and capable of stimulating the generation of cytotoxic T lymphocytes. Anti-IL-6 antibodies are currently in clinical development for many inflammatory disorders (Nishimoto and Kishimoto 2004), and they should thus be experimented also for COPD treatment.

TGF-β inhibitors

In patients with COPD, high expression levels of TGF-β are detectable in airway epithelium and macrophages of small airways (Takizawa et al 2001). TGF-β1 is a potent fibrogenic agent which plays a pivotal role in inducing the fibrosis and narrowing of peripheral airways that characterize obstructive bronchiolitis in COPD (Hogg et al 2004). As already mentioned, TGF-β also contributes to proteinase imbalance by activating MMP-9. Furthermore, TGF-β has been shown to promote apoptosis of both bronchial and alveolar epithelial cells (Hagimoto et al 2002; Pelaia et al 2003), which may represent a very initial event in COPD pathogenesis (Shapiro and Ingenito 2005). Therefore, the currently available low-molecular-weight inhibitors of TGF-β receptor kinase (Yakymovych et al 2002) could be used to evaluate their effects in COPD patients.

Chemokine inhibitors

Among the four known families of chemokines, CC and CXC chemokines, synthesized by both inflammatory and structural cells, are those mostly involved in regulating the traffic of macrophages, neutrophils, and CD8+ T

lymphocytes, thus significantly contributing to COPD pathogenesis.

Inhibitors of monocyte/macrophage migration

Migration of monocytes/macrophages is mainly mediated by CC chemokines including monocyte chemoattractant protein 1 (MCP-1 or CCL2), acting on its CCR2 receptor, and macrophage inflammatory proteins 1α (MIP-1α or CCL3) and 1β (MIP-1β or CCL4), interacting with their CCR5 receptor. Both CCL2 and CCR2 proteins are highly expressed on macrophages from COPD patients (de Boer et al 2000; Traves et al 2002), thereby representing attractive targets for the development of small molecule antagonists (Figure 1). The humanized mouse monoclonal anti-CCL2 antibody MLN1202 and the selective CCR2 antagonist INCB3284 have been taken into consideration for treatment of rheumatoid arthritis (de Boer 2005). Bindarit is another CCL2 antagonist that was shown to inhibit monocyte production of CCL2 and TNF-α, being also capable of reducing monocyte number in animal models of arthritis (Sironi et al 1999; Guglielmotti et al 2002). Binding of CCL2 to its CCR2 receptor can also be blocked by RS-504393, which is able to inhibit macrophage infiltration and activation (Kitagawa et al 2004). Therefore, the potential utility of these compounds for COPD therapy should be explored.

With regard to the interactions between CCR5 receptor and its CCL3/CCL4 ligands, whose expression is increased in COPD, some CCR5 antagonists such as TAK220 and AK602 are now available (Figure 1) (de Boer 2005). However, these drugs are unable to significantly reduce macrophage chemoattraction because they inhibit only CCL3, but not CCL4 binding to CCR5, thus probably not being of particular interest for COPD treatment.

Inhibitors of neutrophil chemotaxis

The CXC chemokines IL-8 (CXCL8) and growth-related oncoprotein-α (GRO-α or CXCL1) play a key role in neutrophil chemotaxis via stimulation of their CXCR1 and CXCR2 receptors (Figure 2). IL-8 is overexpressed in COPD, thus being considered as an important target for therapeutic intervention (de Boer 2003; Fuke 2004). In a recent study, COPD patients received 3 injections (800 mg at baseline and 400 mg in the 2 subsequent months) of a monoclonal antibody (ABX-IL-8) against IL-8, which attenuated the severity of dyspnea without, however, improving lung function, quality of life, and performance of the 6-minute walking test (Mahler et al

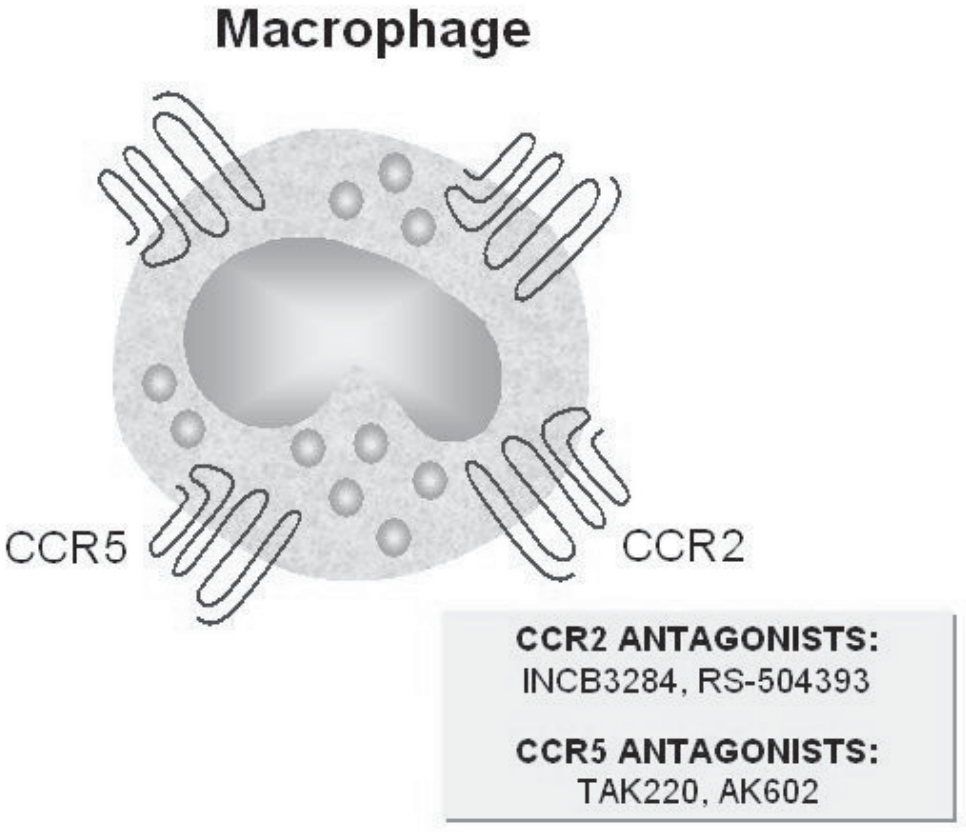

Figure 1 Main chemokine receptors expressed by macrophages.

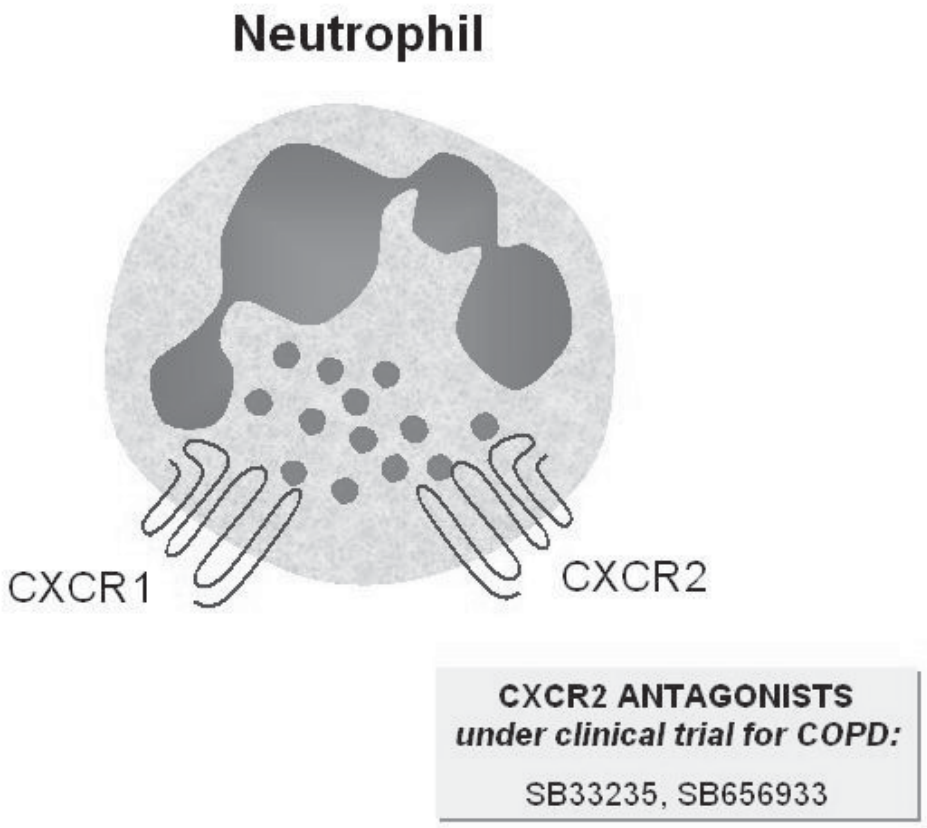

Figure 2 Main chemokine receptors expressed by neutrophils.

2004). CXCL1- and IL-8-mediated neutrophil chemotaxis can be inhibited by the non-peptide CXCR2 antagonist SB33235, whose safety profile and pharmacokinetic characteristics are not yet known (de Boer 2005). SB656933 is another CXCR2 antagonist, currently under clinical investigation for COPD treatment, that was shown to impair neutrophil activation and recruitment into the bronchoalveolar lavage fluid (BALF) from rats exposed to aerosolized LPS (Carpenter et al 2004; Salmon et al 2004).

Inhibitors of CD8+ T cell recruitment

It has been recently suggested that COPD can be considered as an immunological disease featured by a predominant Tc1/Th1-mediated response (Di Stefano et al 2004). In fact, a significant up-regulation of both interferon-inducible protein-10 (IP-10 or CXCL10) and its CXCR3 receptor, which is preferentially expressed by CD8+ Tc1 and CD4+ Th1 cells, was found in peripheral airways of COPD patients (Saetta et al 2002). Specific inhibitors of CXCL10/CXCR3 interaction might therefore be useful for COPD treatment. In this regard, an anti-CXCL10 antibody has been recently developed and shown to inhibit CXCR3+ T cell recruitment in an experimental model of lung inflammation (Hildebrandt et al 2004).

Anti-inflammatory cytokines

In contrast to the pro-inflammatory cytokines implicated in COPD pathophysiology, interleukin-10 (IL-10) may exert some anti-inflammatory actions that could be exploited for therapeutic purposes. In particular, a recombinant human IL-10 (ilodecakin) is currently under evaluation for treatment of Crohn's disease, rheumatoid arthritis, psoriasis, and hepatitis C (Asadullah et al 2003). Administered to healthy volunteers before being exposed to endotoxin, IL-10 reduced pulmonary neutrophilia and was also capable of inhibiting the expression of TNF-α, IL-6, IL-8, CCL2, CCL3, and CCL4 (Paikrt et al 1997). Repression of these pro-inflammatory genes is probably due to the inhibitory effect of IL-10 on activation of the transcription factor nuclear factor-κB (NF-kB) (Driessler et al 2004). Clinical studies performed in patients with psoriasis showed that subcutaneous injections of IL-10 improved skin lesions and reduced the expression of Th1 cytokines (Asadullah et al 2003), thus suggesting an IL-10-induced shift from Th1 to Th2 immune response. This hypothesis appears to be

confirmed by trials involving patients with chronic hepatitis C infection, treated for up to 1 year with IL-10, who exhibited an improvement in liver histology associated with a shift from interferon (IFN)γ+CD8+ T cells towards a Th2 secretory pattern (Nelson et al 2003). However, subjects with either rheumatoid arthritis or Crohn's disease did not experience significant clinical improvements after treatment with IL-10, that surprisingly stimulated IFNγ expression and IFNγ-dependent production of CXCL10 (Asadullah et al 2003), which may thus result in increased inflammation associated with CXCR3+ T lymphocytes, as occurring in COPD. On the other hand, it has been recently reported that inhaled corticosteroid therapy may promote in COPD patients the production of IL-10 from alveolar macrophages, though this effect was not accompanied by significant FEV_1 increases (de Boer 2005).

In vitro, in addition to decreasing the synthesis of TNF-α and IL-8 by macrophages, IL-10 also induced the expression of tissue inhibitor of metalloproteinase-1 (TIMP-1), without affecting MMP-9 levels (Lim et al 2000). Such actions might be particularly beneficial for patients with COPD, who are characterized by an unbalanced proteinase/antiproteinase ratio, shifted towards lung tissue destruction. These patients also have, when compared with normal subjects, lower IL-10 concentrations in induced sputum (Takanashi et al 1999). However, in order to better delineate the potential therapeutic use of IL-10, its pathogenetic role in COPD needs to be further elucidated. Moreover, recombinant IL-10 can cause adverse side-effects including anemia, headache, and immunological reactions (de Boer 2005). Therefore, all these considerations at present preclude COPD patients from entering trials aimed at evaluating the clinical effects of IL-10.

Inhibitors of transcription factors

Among the transcription factors that regulate the expression of pro-inflammatory cytokines and chemokines, a prominent role is played by NF-κB, which is activated in macrophages and epithelial cells of patients with COPD, especially during disease exacerbations (Di Stefano et al 2002; Caramori et al 2003). NF-κB is a dimeric transcription factor which in its inactive form is bound in the cytoplasm to an inhibitory protein (inhibitor of NF-κB: IκB), that upon IκB kinase (IKK)-dependent phosphorylation undergoes ubiquitin-mediated proteolysis thus resulting in the release of active NF-κB, able to translocate into the nucleus and to interact with the NF-κB binding sites of target genes (Baldwin 1996). Translation of NF-κB p65 subunit has been experimentally blocked in airway epithelial cells by small interfering (si)RNAs, which in vitro reduced the expression of both IL-6 and IL-8 (de Boer 2005). Furthermore, an antisense antagonist for p65 is now in clinical trial for Crohn's disease (Hibi et al 2003).

A more promising approach is based on the use of orally active, small-molecule IKK inhibitors including SPC600839 and BMS345541. In a mouse model of collagen-induced arthritis, BMS345541 was shown to suppress NF-κB nuclear translocation as well as TNF-α and IL-1β expression, thereby improving clinical symptoms and joint lesions (McIntyre et al 2003). Therefore, such compounds might be beneficial in COPD by inhibiting the production of cytokines and chemokines by both inflammatory and structural cells. However, these therapeutic strategies should be considered with extreme caution in that a prolonged inhibition of NF-κB, which is crucially involved in the control of innate and adaptive immune responses, could expose recipient patients to a high risk of infections.

Inhibitors of signal transduction pathways

Inhibitors of mitogen-activated protein kinases (MAPK)

Among the various MAPK families (Figure 3), p38 is mostly implicated in cytokine biosynthesis as well as in recruitment of inflammatory cells (Pelaia et al, 2005). In particular, p38 MAPK significantly contributes to neutrophil recruitment by up-regulating the vascular expression of intercellular adhesion molecule-1 (ICAM-1) and the release of TNF-α into airspaces (Tamura et al 1998; Nick et al 2000). Moreover, monocyte differentiation and chemotaxis are regulated by p38, which also stimulates the release of TNF-α and macrophage inflammatory protein-2 (MIP-2) from human macrophages (Ayala et al 2000; Nick et al 2000). Several pyridinylimidazole inhibitors of p38 are now available, and the combination of mutagenesis studies, X-ray crystallography and mechanistic enzymology has enabled elucidation of the molecular basis of inhibitor specificity, as well as development of second-generation compounds with increased potency and selectivity (Wang et al 1998; Lee et al 2000; English and Cobb 2002). The first p38 inhibitors able to block the biosynthesis of pro-inflammatory cytokines included the bicyclic pyridinylimidazole SKF 86002 and the 2,4,5-triaryl imidazole SB 203580 (Lee et al 1993; Gallagher et al 1995). Subsequently, other compounds such as SB 220025, HEP

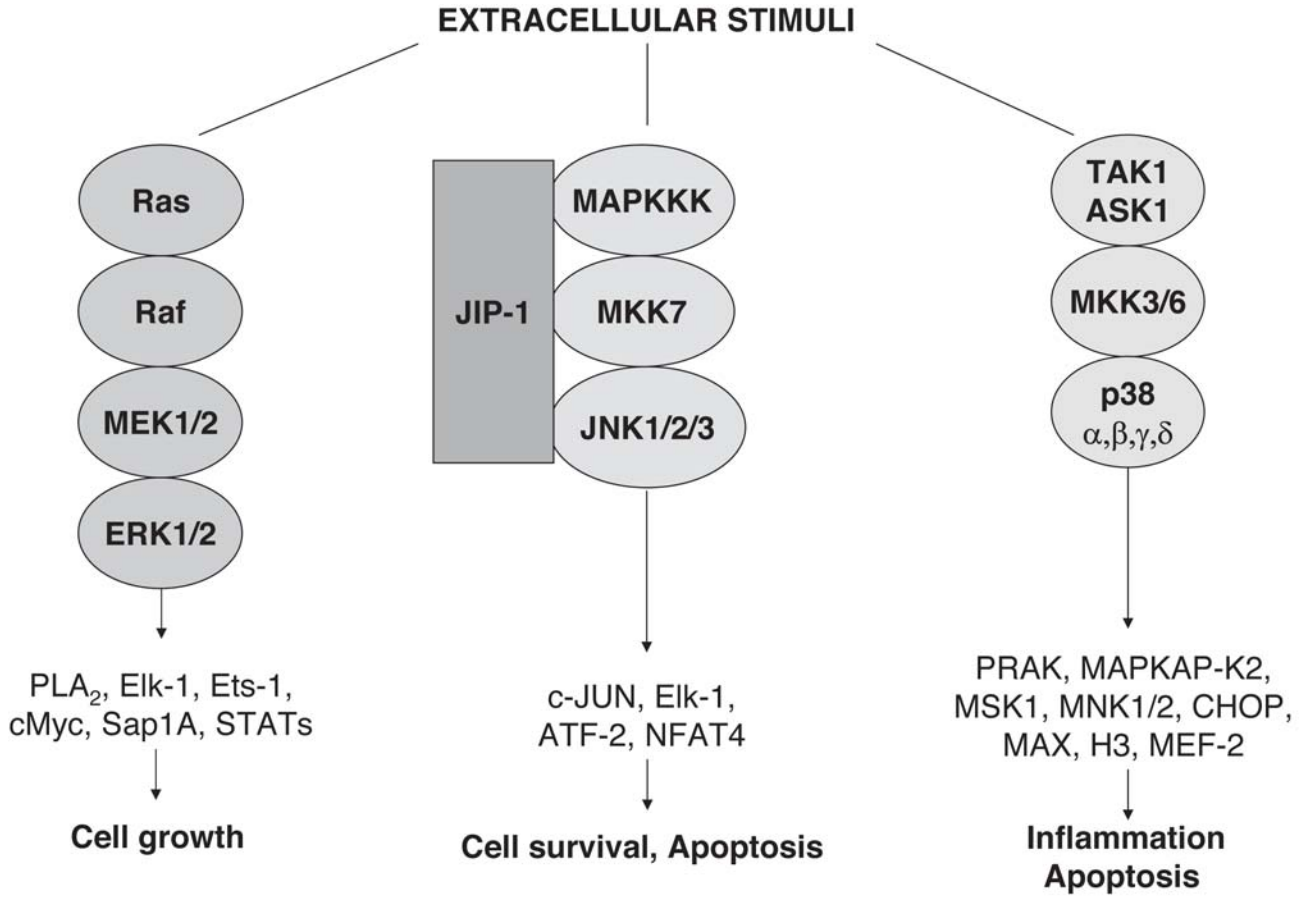

Figure 3 Mitogen-activated protein kinases (MAPK) signalling pathways.

689, and VX-745 have been designed; more recently, combinatorial chemistry strategies have led to the identification of a new class of highly potent p38 inhibitors, characterized by the replacement of the pyrazole ring with either an isoxazole or a thiophene (Dumas et al 2000). These latter molecules are very promising, as shown by their ability to inhibit, within a submicromolar range of concentrations, IL-6 production induced by TNF-α and IL-1.

Over the past few years, the potential therapeutic role of p38 inhibitors has been tested in several different models of lung inflammation. SB 239063, a small-molecule p38 inhibitor, attenuated neutrophil infiltration induced by inhaled LPS and decreased IL-6 and MMP-9 levels in rat BALF (Underwood et al 2000), thus being possibly useful for COPD treatment. Another p38 inhibitor, SD-282, reduced LPS-induced production of TNF-α by human lung macrophages, without affecting the expression of IL-8 (de Boer 2005). IL-8 release into cell-free supernatants of primary cultures of human bronchial epithelial cells, exposed to oxidative stress, was significantly inhibited by SB 203580 (Pelaia et al 2004). In these same cell cultures, SB 203580 completely prevented apoptosis elicited by TGF-β (Pelaia et al 2003). On the basis of all such in vitro findings, a p38 inhibitor named GSK-681323 has been developed for treatment of COPD, atherosclerosis, and rheumatoid arthritis, but clinical data are not yet available (Molfino 2005). However, the pharmacological strategy of inhibiting p38 MAPK should be considered with caution as a treatment option in mammalian diseases, in that p38α knockout mice are characterized by a defective placental development (Adams et al 2000; Mudgett et al 2000). An alternative therapeutic approach might thus consist of targeting the p38 substrate mitogen-activated protein kinase-activated protein kinase 2 (MAPKAP-K2) (Figure 3), which mediates the stabilizing function exerted by p38 MAPK on many mRNAs encoding pro-inflammatory cytokines (Winzen et al 1999). This strategy, however, also raises some concern because MAPKAP-K2 is probably required for the optimal effectiveness of immune responses; indeed, mice carrying a mutation in the gene encoding MAPKAP-K2 are highly susceptible

to infections by intracellular pathogens such as *Listeria monocytogenes* (Lehner et al 2002). A more suitable approach could thereby be represented by the development of inhalant formulations of either p38 or MAPKAP-K2.

Inhibitors of phosphoinositide 3-kinase (PI-3K)

The PI-3K enzymatic pathway, leading to the generation of lipid second messengers, provides pro-inflammatory signals involved in recruitment and activation of neutrophils, monocytes, and T lymphocytes. Knockout of the PI-3Kγ is indeed responsible for inhibition of neutrophil migration and activation, as well as for impairment of T cell and macrophage functions (Sasaki et al 2000). Therefore, the selective small-molecule inhibitors of PI-3Kγ which are now in development may exert anti-inflammatory actions potentially useful for COPD therapy (Ward et al 2003; Barnes and Stockley 2005).

Proteinase inhibitors

COPD is characterized by a relevant imbalance between proteinases (serine elastases, cysteine proteinases, MMPs), which degrade elastin and other structural proteins of lung parenchyma, and the protective array of antiproteinases (α1-antitrypsin, elafin, secretory leukoprotease inhibitor, and tissue inhibitors of MMPs) (Barnes et al 2003). In fact, smokers with a rapid decline in pulmonary function exhibit an increased urinary excretion of desmosine, a compound derived from elastin cross-links (Gottlieb et al 1996), which is a marker of connective tissue destruction. Neutrophil elastase is a powerful proteolytic enzyme that is predominantly inhibited by α1-antitrypsin (α1-AT). The latter is currently administered as an extracted protein to patients with genetically determined, low serum levels of α1-AT and concomitant lung disease (Sandhaus 2004). In future, α1-AT could be provided in recombinant form or delivered by viral vector-driven gene strategies (Luisetti and Travis 1996; Stecenko and Brigham 2003). Furthermore, synthetic inhibitors of neutrophil elastase have been developed, including ONO-5046, FR901277, DX-890, and midesteine (Luisetti et al 1996; Fujie et al 1999; Barnes and Stockley 2005; Molfino 2005). In animal models, FR901277 neutralized the action of elastase and other neutrophil serine proteinases such as cathepsin and proteinase 3, thus inhibiting acute inflammation and pulmonary emphysema (Fujie et al 1999). DX-890 is a recombinant protein derived from the human inter-α-trypsin inhibitor (Wark 2002), which after inhalation was well tolerated and detectable in its active form in BALF from healthy volunteers (Molfino 2005). Midesteine is an orally active elastase inhibitor that, given for 4 weeks to patients with COPD, in a subgroup of treated subjects induced a post-treatment decrease of urine desmosine levels (Luisetti et al 1996). Non-selective MMP inhibitors such as marimastat seem to induce relevant muscular-skeletal side-effects (Belvisi and Bottomley 2003). This problem could perhaps be overcome by the development of specific inhibitors of MMP-9 (Matter and Shudok 2004), which is the major elastolytic enzyme released by alveolar macrophages from COPD patients (Russell et al 2002).

Antioxidants

Oxidative stress plays a key role in the development of COPD, in that the major cause of this disease is cigarette smoking, which represents a rich source of oxidant agents. Furthermore, other factors involved in COPD pathogenesis and progression, such as air pollutants, occupational dusts, and respiratory infections, also have the ability to produce oxidative stress. Indeed, smokers and patients with COPD are characterized by high concentrations of exhaled hydrogen peroxide (H_2O_2), which become even higher during disease exacerbations (MacNee 2001). Moreover, increased levels of lipid peroxides, including 8-isoprostane and hydrocarbons such as ethane and pentane, are also detectable in the exhaled air condensate of patients with COPD (Habib et al 1995). Lipid peroxidation products positively correlate with airway obstruction, thus suggesting that oxidative stress is closely associated with the progressive decline in lung function occurring in COPD (Boots et al 2003). In addition, oxidative inactivation of the antiproteinase α1-AT favors the increase in elastase burden which is responsible for the development of pulmonary emphysema. Oxidants largely contribute to the inflammatory and structural changes underlying COPD by inducing the production of several mediators and cytokines such as TNF-α and IL-8. In this regard, we have reported that H_2O_2 elicited a concentration-dependent increase in the amount of IL-8 released from bronchial epithelial cells, whose apoptotic death was also stimulated by oxidative stress (Pelaia et al 2004). H_2O_2 is also able to induce, in a time-dependent manner, the acetylation of histone H4 and the closely related synthesis of IL-8 by both bronchial and alveolar epithelial cells (Gilmour et al 2003; Tomita et al 2003). In other cell types such as alveolar macrophages, it has been shown that H_2O_2 and cigarette smoke can stimulate

IL-8 secretion by inhibiting the activity of HDAC enzymes (Ito et al 2001). HDACs indeed repress gene transcription by deacetylating core histones, thus enhancing chromatin condensation and DNA supercoiling (Ayer 1999). In particular, oxidative stress might impair HDAC activity by enhancing, in the presence of high NO levels, the production of peroxynitrite and the subsequent nitration of tyrosine residues on HDAC or associated proteins. This mechanism could also help explain the low therapeutic efficacy, observed in COPD patients when compared with asthmatics, of inhaled glucocorticoids, whose anti-inflammatory actions largely depend on their ability to recruit and activate HDACs (Barnes et al 2004).

Therefore, antioxidants may exert potentially beneficial effects in COPD. In fact, in rats exposed to tobacco smoke, the intra-tracheal administration of a catalytic antioxidant (AEOL 10150) elicited a significant decrease in BAL neutrophils and macrophages, detected at 2 days and 8 weeks, respectively (Crapo 2003). Anti-oxidant defences can be potentiated by the cysteine-donor N-acetyl cysteine (NAC), able to stimulate the production of the endogenous tripeptide glutathione (GSH), which provides an effective intra- and extracellular shield against oxidative stress. However, a large trial carried out on 523 patients with COPD, randomly receiving oral NAC (600 mg daily) or placebo for 3 years, has very recently demonstrated that NAC did not produce any change neither in the annual decline in FEV_1 nor in the number of exacerbations per year (Decramer et al 2005). Nacystelyn is a NAC-related thiol antioxidant that has been shown to be effective in vitro, as well as in in vivo animal models (Antonicelli et al 2004); it also has the advantage of being suitable for aerosolized administration. Furthermore, the development of more powerful antioxidants including stable GSH compounds, analogs of superoxide dismutase, and selenium-based drugs is underway (Barnes and Stockley 2005). In this regard, the potential therapeutic utility for COPD of BXT-51072, the lead compound in a series of small-molecule GSH peroxidase mimics, is interesting. BXT-51072 is capable of increasing the rate of peroxide metabolism, as well as of inhibiting both inflammation and oxidative damage (Molfino 2005).

Conclusions

Because COPD is not currently well controlled by available drugs, which are unable to resolve inflammation and to prevent lung tissue destruction and the associated progressive decline in pulmonary function, novel and more effective therapies are badly required. This need is made even more urgent by the known difficulties, due to both addiction and psychological reasons, encountered by patients and healthy subjects trying to quit smoking. Therefore, the common goal of the several different experimental approaches in development for COPD treatment should be a more successful targeting of disease causative mechanisms. In this regard, there is no doubt that during the past few years significant progress has been made towards a better understanding of COPD pathophysiology. As a result, such new advances have led to a significant expansion of the pharmacological options now under evaluation. Within this renewed therapeutic context, many strategies appear to be quite promising for the near future, including selective PDE inhibition, interferences with the cytokine/chemokine network, and blockade of MAPK signal transduction pathways. However, knowledge of the basic cellular and molecular events underlying COPD pathogenesis still needs to be further improved, possibly through the identification of the genetic factors involved, and also via the application of proteomic techniques aimed at characterizing the specific patterns of protein expression related to the various COPD phenotypes. The potential future achievements will probably open the way for exciting perspectives, thus theoretically leading to the design of compounds capable of either directly modulating relevant genes or, alternatively, targeting their protein products.

Finally, a further contribution to the progress of COPD treatment may arise from the so-called regenerative medicine, aimed at restoring the integrity of lung tissue structure, destroyed in patients with emphysema. In particular, interesting data have been provided by experiments showing the activation of growth and repair processes in rat alveoli, induced by retinoic acid (Belloni et al 2000). In this regard, more beneficial results may perhaps be pursued by biological strategies based on the use of stem cells (Otto 2002). Therefore, our hope is that the combination of all these new approaches will eventually enable, in the next decades, a definitive cure for such a widespread and severe respiratory disease.

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