

REVIEW

Molecular Mechanisms in Chronic Obstructive Pulmonary Disease

Potential Targets for Therapy

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Abstract

Chronic obstructive pulmonary disease (COPD) is a chronic inflammatory lung disease associated with progressive airflow obstruction. Tobacco smoking is the main risk factor worldwide. In contrast to asthma, anti-inflammatory therapies are rather ineffective in improving chronic symptoms and reducing inflammation, lung function decline, and airway remodeling. Specific drugs that are directed against the remodeling and chronic inflammation, thereby preventing lung tissue damage and progressive lung function decline, must be developed. Experimental models and expression studies suggest that anti-vascular endothelial growth factor (VEGF) receptor strategies may be of use in patients with emphysema, whereas anti-HER1-directed strategies may be more useful in patients with pulmonary mucus hypersecretion, as seen in chronic bronchitis and asthma.

Growth factors and cytokines including VEGF, fibroblast growth factors, transforming growth factor- β , tumor necrosis factor- α , CXCL1, CXCL8, and CCL2, and signal transduction proteins such as mitogen-activated protein kinase p38 and nuclear factor- κ B, seem to be important pathogenetic molecules in COPD. Specific antagonists for these proteins may be effective for different inflammatory diseases. However, their efficacy for COPD therapy has not yet been demonstrated. Finally, other drugs such as retinoic acids may provide restoration of lung tissue structure. Such approaches, however, must await the first results of growth factor or cytokine antagonist therapy in chronic lung diseases.

Index Entries: COPD, inflammation, airway remodelling, growth factors, cytokines, receptors, signal transduction, antagonists.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a chronic lung disease characterized by a progressive airflow limitation and associated with an abnormal inflammatory response of the lungs to noxious particles or gases (1,2). COPD ranks among the top five leading causes of death worldwide and it is estimated that it will be in the mortality top three by 2020. Annual healthcare costs are greatly affected by COPD, being estimated at 2.8×10^8 £ in The Netherlands (in 2000), 7.8×10^8 £ in the United Kingdom (in 1996), and more than 14.5×10^9 US\$ direct costs in the United States (in 1993) (2).

Tobacco smoking, the main risk factor, is associated with about 80% of COPD cases, and this includes passive smoking (3). Other risk factors include air pollution, the effects of indoor cooking, and asthma (2-4). However, only 15-20% of the smokers develop COPD, pointing to susceptibility factor(s) for COPD. Among these factors are genotype polymorphisms as exemplified by α 1-antitrypsin (5) and other gene polymorphisms in molecules such as tumor necrosis factor (TNF)- α , matrix metalloproteinase (MMP), or antioxidant genes (5-9) dependent on race and COPD phenotype (9).

PATHOGENESIS OF COPD

Airway Remodeling

COPD can be divided based on either clinical manifestations or pathological features into three different

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disorders: chronic bronchitis with airflow limitation, emphysema, and chronic bronchiolitis. COPD is characterized by a progressive lung function decline, measured as an increased annual decline in forced expiratory flow in 1 s (FEV₁) (1,2). Research in the past two decades has shown pathological features of COPD patients comprising lung tissue remodeling-like changes in mucosal tissue fiber types and/or fibrosis, pulmonary and systemic inflammation, lung vascular remodeling, and declines in skeletal muscle mass and function (10–17). In emphysema, alveolar septa are deteriorated, resulting in reduced gas exchange and loss of elastic recoil of lung tissue (14). In chronic bronchitis with airflow limitation, bronchial gland enlargement and goblet cell hyperplasia leads to overproduction of mucus, resulting in mucoid airways obstruction (14). Often, COPD patients show a combination of these disorders. The pulmonary inflammation consists of neutrophils, macrophages, CD8+ T-cells (less prominent in severe COPD), mast cells, and eosinophils during exacerbations or in COPD patients showing reversible lung function (16,18).

Pathophysiological features in addition to decreased lung function include chronic cough and dyspnoea. In smokers, epithelial cell metaplasia often occurs, whereas in chronic bronchitis, a goblet cell metaplasia is seen. Both types of metaplasia result in an impaired ciliary function. In chronic bronchitis, the hypersecreted mucus cannot be cleared sufficiently, resulting in chronic cough and dyspnoea. Although after cessation of smoking, the decline in lung function is reduced, lung function will not be restored and an increased airway resistance persists (19–20). This is probably due to pathological alterations in the airways like fibrosis and inflammation that reduce the diameter of airway lumen. In addition, after cessation of smoking an airway inflammation persists, hence contributing to the irreversibility of the decreased lung function (10,12,16,18).

Other disturbed mechanisms are thought to include oxidative stress and protease–antiprotease balance (12,16,18). This may be due to genetic predisposition such as polymorphisms reported for glutathion-S-transferase, epoxide hydrolase, MMPs 1, 9, or 12, and tissue inhibitor of metalloproteinase (TIMP) 2 (5–9,21). Alternatively, an imbalance is due to chronic exposition to cigarette smoke and subsequent release of free radicals and proteases such as elastase and MMPs from activated inflammatory cells. In turn, this may cause a chronic state of tissue damage, repair, and remodeling (14). Frequent exacerbations in COPD patients may eventually result in increased airway inflammation with eosinophils and neutrophils and higher levels of inflammatory mediators such as cytokines (22,23).

Role of Growth Factors

Growth factors are small to large proteins that are divided in families based on their structural or functional homologies. The main families include the epidermal growth factor (EGF) family, transforming growth factor (TGF)- β family, fibroblast growth factors (FGFs), insulin-like growth factor (IGF) family, platelet-derived growth factor (PDGF) family, vascular endothelial growth factor (VEGF) family, and hepatocyte growth factor (HGF). This review will be confined to EGF-like growth factors, FGFs, VEGF, TGF- β , and specific cytokines. Growth factors are involved in a variety of processes including cellular proliferation, differentiation, migration, metabolism, and gene expression. Recently, localization of EGF-like growth factors in the human airways such as EGF, TGF- α , heregulins, beta-cellulin, and heparin-binding EGF (HB-EGF) has been demonstrated (24). One of the receptors for EGF-like growth factors, HER1 (EGFR or c-erbB1), has been shown to be involved in mucus production in goblet cells either directly via stimulation by TGF- α or HB-EGF, or indirectly via a variety of molecules including cytokines such as TNF- α , interleukin (IL)-4 or IL-13, cigarette smoke, or neutrophil-derived oxidants (25). In the airway epithelium of patients with asthma, expression of HER1, but not of the other HER or EGF family members, was enhanced as compared to healthy control subjects (26,27). Increased expression of HER1 has been advocated for its involvement in epithelial repair and airway wall remodeling in asthma (27). In the airway epithelium of patients with COPD, expression of HER1, heregulin, and EGF was higher as compared to control subjects (24). In contrast, Vignola et al. (28) could not find any increased bronchial expression of EGF in chronic bronchitis patients, perhaps as a result of differences in the study population. The literature fully supports the notion that HER1 is involved in hypersecretion of mucus in chronic bronchitis as well as in neutrophilic inflammation, and eventually contributes to the pulmonary tissue remodeling seen in COPD and severe asthma. Also, EGF-like growth factors may also be involved in pulmonary tissue remodeling in COPD.

FGF Family

The FGF family consists of 23 members in man and their functional receptors are designated FGFR1 to FGFR5 (29). These FGFs are mitogenic factors involved in development, tissue homeostasis, and repair processes; FGF-1 and FGF-2 are also angiogenic factors (30–32). Among other growth factors, FGF-1, FGF-2, and FGF-7, as well as their receptors FGFR1 and FGFR2, are widely distributed in the human lung. In a recent study from our lab, differences in pulmonary expression of FGF-1, FGF-2, and FGFR1 were found between non-

smokers and smokers with or without COPD (15,32). With regard to FGF-2, airway expression is higher in bronchial tissue from asthmatics as compared to healthy control subjects (33). In COPD, expression levels of FGF-1, FGF-2, and FGFR1 were observed to be increased in vascular and in epithelial compartments in lungs of patients with COPD as compared to subjects without COPD (15,32). FGF-1 can stimulate tissue remodeling by increasing collagenase expression and downregulation of collagen I expression in lung fibroblasts (30–32). It is yet unknown whether differences in FGF-7 expression patterns in lung tissue from patients with and without COPD or asthma exist. These data support a role for the FGF–FGFR axis in tissue remodeling in COPD.

VEGF Family

The VEGF family consists of seven members (VEGF-A–F and placenta growth factor [PiGF]), which can bind to their respective cellular receptors VEGF receptor (VEGFR)-1 (also referred as Flt-1), VEGFR-2 (or KDR or Flk-1), and VEGFR-3 (or Flt-4). VEGF is predominantly known as a paracrine-acting, angiogenic factor stimulating mitogenesis, migration, and permeabilization of the vascular endothelium. Several studies support the idea that VEGF and its receptors contribute to tissue remodeling and disease severity in chronic lung diseases including asthma (34,35). In patients with COPD, higher pulmonary VEGF expression was found in bronchial and alveolar epithelial in vascular smooth muscle as well as alveolar macrophages, whereas higher VEGFR-1 and VEGFR-2 expression was found in the endothelium as compared to patients without COPD (35). In this study, patients were not further stratified into emphysema severity classes. In patients with severe emphysema, VEGF expression in total lung extracts has been reported to be lower as compared to patients without COPD (36). Furthermore, VEGF levels in induced sputum were higher in patients with chronic bronchitis and airflow limitation, whereas they were lower in patients with emphysema as compared to healthy controls. In agreement with immunohistochemical data from our lab (35), VEGF sputum levels in patients with chronic bronchitis and airflow limitation were inversely correlated with FEV₁ values (36). These data point at a COPD stage- and/or subtype-dependent expression of VEGF. This idea is supported by another study (37) demonstrating higher VEGF expression in lung tissue from patients with moderate COPD, but a lower expression in lung tissue from patients with severe emphysema as compared to subjects without COPD. Overexpression of VEGF in mice caused pulmonary emphysema, aberrant structure of capillary endothelium, and pulmonary inflammation with macrophages (38). Also, transgenic overexpression of

PiGF caused apoptosis of pneumocytes, emphysema, and a reduced endothelial cell number and VEGF expression (39). In contrast, adult mice in whom pulmonary VEGF expression was impaired by genetic targeting showed up to 86% lower VEGF levels as well as VEGFR-2 expression concomitant with alveolar and bronchial epithelial apoptosis, loss of elastic recoil, and airspace enlargement but no inflammation (40). Although inconclusive, these data from animal studies provided further support for a role of VEGFs and their receptors in tissue and vascular remodeling seen in COPD.

Assigning functional differences of VEGFRs in the pathogenesis of COPD can further support this. Activation of VEGFR2 is involved in angiogenesis by stimulating mitogenesis of endothelial cells during vascular damage-repair processes (41). In contrast, VEGFR1 is rather involved in stimulating vascular smooth muscle MMP activities and endothelial plasminogen activator and its inhibitor, activities needed for blood vessel maturation (41,42). VEGFR-1 is also involved in inflammation, e.g., by stimulating migration of monocytes and CD34+ cells (43). Expression of VEGFR2 during repair is followed by VEGFR1 expression. As VEGFR1 has a higher affinity for VEGF than VEGFR2, it is thought that VEGFR1 is involved in the bronchial angiogenic process (35,37,44). In COPD, pulmonary vascular expression of VEGF and VEGFR2 were reported to be increased in smokers with COPD or chronic bronchitis, whereas VEGFR1 expression did not differ between smokers with or without COPD (35). In addition, subjects with COPD showed an increased pulmonary vascular remodeling and thicker vessel walls (44). In contrast, in subjects with emphysema, pulmonary VEGF and VEGFR2 expression is lower as compared to subjects without COPD (45). Concomitant with the decreased expression, endothelial cell death and decreased endothelial proliferation occurred (45). Supporting evidence for the role of VEGFR2 in emphysema was provided in an animal model in which VEGFR2 was blocked, resulting in pulmonary endothelial cell apoptosis and emphysema (45).

Based on these data, we hypothesize that in COPD/chronic bronchitis, the higher VEGF and VEGFR2 expression leads to an increased angiogenesis and vascular remodeling, which is inefficiently compensated by the low expression of VEGFR1. In subjects with COPD/emphysema, VEGFR2 and VEGF expression are lower. Because VEGFR1 has a higher affinity for VEGF than VEGFR2, VEGFR1 scavenges VEGF from VEGFR2, resulting in specific VEGFR1 activation and in endothelial apoptosis, higher MMP activity, and vascular and alveolar destruction. Hence, the balance among VEGF,

VEGFR1, and VEGFR2 is critical in the pathogenesis of COPD subtypes.

TGF Family

The TGF- β subfamily has five members, of which TGF- β 1–3 have been shown to be present in mammals. TGF- β shows a plethora of effects. It is known to act in a biphasic way on fibroblast proliferation, whereas it inhibits proliferation of other cells such as epithelial, endothelial, and smooth muscle cells. In addition, it stimulates (terminal) differentiation of several cell types, and enhances synthesis and secretion of extracellular matrix (ECM) proteins such as collagens. TGF- β 1 can drive wound repair by cellular migration and cell flattening toward wound closure, as well as restoration of tissue differentiation. A recent study pointed out that overexpression of TGF- β 1 in mice leads to Smad3-dependent pulmonary expression of procollagens and antiproteinases as well as fibrosis (46). On the other hand, TGF- β is chemotactic for monocytes/ macrophages and mast cells. Several studies provided evidence for an aberrant pulmonary expression of TGF- β 1 in patients with COPD. Pulmonary TGF- β 1 protein and mRNA expression was higher in lung tissue including airway epithelial cells of patients with mild to moderate COPD (47–49). The higher expression correlated with the increased number of macrophages in the airways (47), supporting a role for TGF- β 1 in the pathogenesis of COPD. Baraldo et al. (50) demonstrated that bronchial glands of patients with chronic bronchitis with airway obstruction have fewer cells showing a positive TGF- β RII staining as compared with smokers without COPD. No differences between the patients groups were found with regard to the number TGF- β 1-positive cells. Thus, the literature supports the idea that the role of TGF- β 1 in COPD is dependent on the COPD subtype.

AIRWAY SMOOTH MUSCLE AND ECM

Focus is now on the bi-directional relationship between airway smooth muscle (ASM) cells and the ECM. Evidence is mounting that ASM cells can modulate ECM homeostasis as they express and release a variety of ECM proteins (51). Altered ECM deposition contributing to the airway wall remodeling is an important feature of chronic airway diseases such as asthma and COPD. We have demonstrated the increased bronchial deposition of ECM proteins, such as collagen I and III, fibronectin, and laminin as part of the airway remodeling process in patients with COPD (52). Staining for fibronectin was increased in bronchial blood vessels and for laminin in ASM as well as the microvasculature in patients with COPD as compared to controls (52). The synthetic capabilities of ASM cells

in response to various mediators such as serum, TGF- β , and LTD4 are documented by the fact that they can produce fibronectin, perlecan, elastin, laminin ($\alpha_1, \beta_1, \beta_2, \gamma_2$), and various collagen subtypes (51–55). Under chronic inflammatory conditions, a number of cytokines and leukotrienes are produced in airways, which could directly act on ASM cells. In addition to their role in ECM deposition, ASM cells have been shown to have the capacity to produce MMPs (54,55), which include the collagenases, gelatinases, stromelysins, elastases, and membrane-bound forms. MMPs are secreted in inactive form and are tightly regulated by TIMPs (56), thus eventually contributing to the alteration in ECM. ECM remains in a state of dynamic equilibrium of new synthesis and degradation controlled by the actions of MMPs and their tissue inhibitors (TIMPs). ASM contributes to this equilibrium by producing both ECM proteins (51) and MMPs and TIMPs (56), as well as growth factors such as TGF- β 1 (57) and connective tissue growth factor (CTGF) (58).

In turn, ECM proteins promote the survival, proliferation, cytokine synthesis, migration, and contraction of human ASM cells. There is accumulating evidence that ECM, in turn, alters a number of aspects of ASM biology through integrin signaling (52,55). Thus, the intertwined relationship of ASM and ECM and their response to stimuli such as chronic inflammation in diseases like asthma and COPD contribute to the remodeling seen in airways of patients with these diseases.

CYTOKINES AND CHEMOKINES

Inflammatory cells of both the innate and the acquired immune system seem to play an important role in the pathophysiology of COPD (14,16,18). In patients with COPD, higher numbers of macrophages and CD8+ T-cells were found in the airway walls and in vascular smooth muscle, whereas more neutrophils were observed in bronchial glands and the airway lumen (16,18). Both cytokines and chemokines provide a chemotactic gradient and can activate these cells. Classical cytokines include ILs, interferons, and TNF- α . Based on structural homology around four cysteine residues, chemokines can be subdivided into four subclasses, -C-, -CC-, -CXC-, and -CXXXC-, in which X substitutes for any amino acid. Two subclasses account for most of the chemokines: CXC (α) and CC (β) chemokines (59). Cytokines and chemokines are synthesized by both structural cells (such as fibroblasts and epithelial, endothelial, and muscle cells) and inflammatory cells of the innate and the adaptive immune system. They act via specific membrane-bound receptors resulting in cell-specific reactions. With regard to COPD, protein and/or mRNA levels of

Table 1
Expression and Polymorphisms of Growth Factors, Cytokines,
and Chemokines in Chronic Obstructive Pulmonary Disease (COPD)

Molecule	Product ^a	Compartment ^b	Data ^c
EGF	Protein	Lung tissue	↑ (CB)
EGFR	Protein	Lung tissue	↑ (COPD) ^d
HER3	Protein	Lung tissue	↑ (COPD)
FGF-1	Protein	Lung tissue	↑ (COPD)
FGF-2	Protein	Lung tissue	↑ (COPD)
FGFR1	Protein	Lung tissue	↑ (COPD)
TGF-β1	Protein	Lung tissue	↑ (COPD)
	mRNA	Lung tissue	↑ (COPD)
VEGF	Protein	Lung tissue	↓ (emphys)
		Lung tissue	↑ (COPD)
	Protein	sp	↓ (emphys)
		sp	↑ (COPD)
VEGFR1	Protein	Lung tissue	↑ (COPD)
VEGFR2	Protein	Lung tissue	↓ (emphys)
			↑ (COPD)
TNF-α	Protein	ser, sp, leg muscle	↑ (COPD)
	mRNA	ser, sp	↑ (COPD)
	Polymorph	Gene	yes (COPD)
sTNFR p55	Protein	sp ^e	↑ (COPD)
sTNFR p75	Protein	ser	↑ (COPD)
CCL2	mRNA	Lung tissue	↑ (COPD)
	Protein	sp, BAL	↑ (COPD)
CCR2	mRNA	Lung tissue	↑ (COPD)
	Protein	Lung tissue	↑ (COPD)
CXCL1	Protein	sp, Lung tissue	↑ (COPD)
CXCL8	mRNA	Lung tissue	Higher
	Protein	ser, sp, BAL	
		Lung tissue	Higher
CXCR2	Protein	PBL	Higher
	mRNA	BAL cells	Higher

^aPolymorph, polymorphism; no., number; epi, bronchial epithelial.

^bser, serum; sp, induced sputum; BAL, bronchoalveolar lavage; PBL, peripheral blood lymphocytes.

^cExpression or levels in COPD as compared to controls; yes, polymorphisms reported; CB, chronic bronchitis; emphys, severe emphysema.

^dEx-smokers with COPD.

^eInverse correlation with FEV₁. Data were obtained from references (15,16,24,28,35–37,47,48,59,60,72).

different cytokines and chemokines have been found to be altered compared with subjects without COPD (Table 1). Among these, TNF-α or TNF-α receptor (TNFR) levels, soluble IL-1 receptor antagonist (sIL-1Ra), CCL2 (or monocyte chemoattractant protein [MCP]-1) and its receptor CCR2, CCL3 (or macrophage inflammatory protein [MIP]-1α), and CCL4 (or MIP-1β)

and their receptor CCR5, CXCL8, (or IL-8), and CXCL10 (or interferon-inducible protein [IP]-10) can be discerned as pro-inflammatory factors. In Fig. 1, a brief outline of the proposed remodeling and inflammatory mechanism in COPD is provided (18,59). Based on this, specific anti-inflammatory therapies are being developed for COPD (60). In addition to the inflammatory

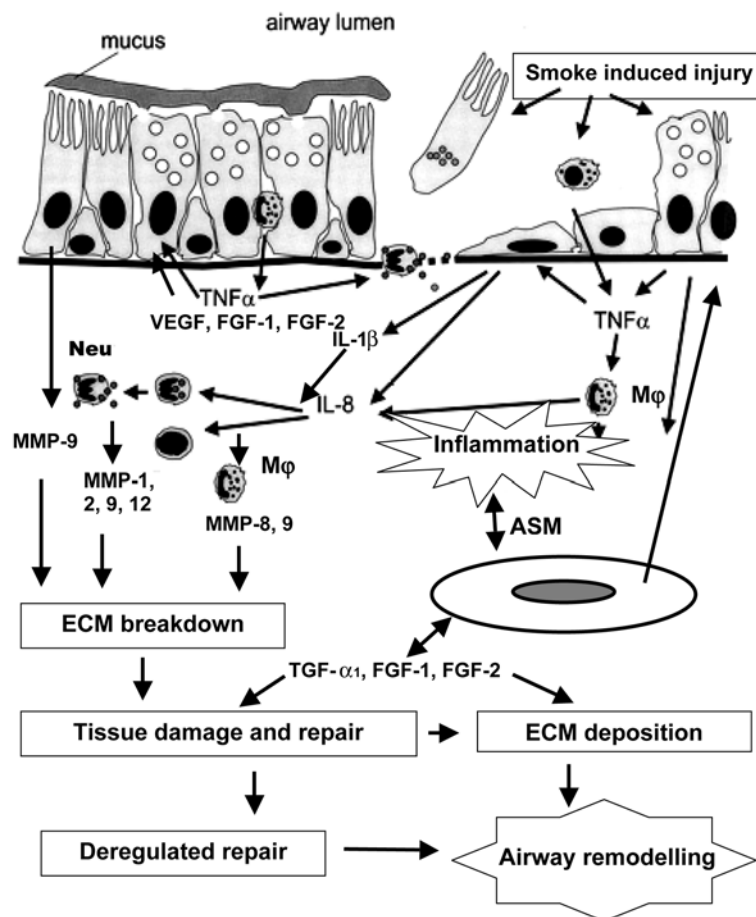


Fig. 1. Simplified scheme of cytokine and growth factor actions in human airways. On triggering, e.g., with tobacco smoke, epithelial cells are damaged, epithelial cells and resident macrophages produce inflammatory mediators such as tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , and IL-8. In turn, inflammatory mediators stimulate migration of monocytes/macrophages, neutrophils, and CD8+ T-lymphocytes to the airway. Both TNF- α and IL-8 can cause degranulation of neutrophils with production and release of serine-proteinases and matrix metalloproteinases (MMPs) as well as free radicals that can cause matrix and epithelial damage. In turn, TNF- α and released growth factors such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF)-1 and -2 orchestrate epithelial repair. Ongoing inflammation and tissue breakdown trigger the release of growth factors such as transforming growth factor (TGF)- β 1, inducing extracellular matrix (ECM) production by myofibroblasts. Repetitive tissue damage and repair can lead to excessive ECM deposition and subepithelial fibrosis. Neu, neutrophil; M ϕ , macrophage; T, CD8+ T-lymphocytes.

effects, recent studies provided more evidence that cytokines and chemokines are also involved in tissue remodeling.

Several studies have shown that epithelial cells express the CXCL8 receptor CXCR2 suggesting a role for CXCL8/CXCR2 in wound repair (18). In vivo, impaired CXCR2 expression in mice resulted in delayed skin wound repair as compared to wild-type mice, which could not be restored by exogenous CXCL8 application (61). Alternatively, topical application of CXCL1 or CXCL8 stimulated human skin wound repair in chimeric mice (62). The role of CXCL8 in wound repair was further substantiated by in vitro experiments. These data support a direct role for CXCL8 in

repair of epithelial injury often seen in airway epithelium in COPD (18).

CCL2 and CCL3 are produced by a variety of cells including macrophages and endothelial and epithelial cells. Wound healing studies in mice and man demonstrated that CCL2 and CCL3 expression are enhanced during repair of injured skin (63) or skeletal muscle in a time-dependent manner and coinciding with macrophage influx. TNF- α seems to be involved in the regulation of CCL2 expression, as TNF- α scavenging with a TNF- α antibody reduced CCL2 levels during wound repair (63).

In vitro, CCL2 and its receptor, CCR2, were demonstrated to be directly involved in endothelial and lung

epithelial cell proliferation and migration and wound closure (18,64,65). In addition, CCL2 was found to stimulate collagen synthesis in rat lung fibroblasts via a TGF- β 1-dependent pathway, hence potentially contributing to a fibrogenetic remodeling as seen in COPD (66). In turn, TGF- β 1 was reported to induce CCL2 protein levels via downstream intracellular mechanisms including reactive oxygen species, mitogen-activated protein kinase (MAPK) p38, and p42/44 in mesangial cells (67). Hence the CCL2-TGF- β axis may potentially contribute to fibrogenetic remodeling as seen in COPD.

CURRENT THERAPY FOR COPD

Current therapies for COPD are predominantly based on anti-inflammatory drugs for treating asthma. These include corticosteroids or theophylline with or without bronchodilators, including β_2 -agonists (1,68). Upon treatment with corticosteroids, some studies reported reduction of the number of exacerbations and improved quality of life assessed with FEV₁ values after short- or long-term treatment (69), or no effect on lung function. However, steroid treatment in COPD is rather ineffective in reducing airway inflammation and the decline in lung function, in contrast to steroid treatment in patients with asthma and COPD with bronchial hyperresponsiveness and eosinophilia. Furthermore, side effects of steroids include increased risk of hip fractures, skin bruising, and candidiasis, and the airway remodeling is not positively affected by the current treatment.

Novel possible broad-acting inhibitors of inflammation include specific inhibitors of phosphodiesterase 4 (PDE4), leukotriene receptor antagonists, inhibitors of 5-lipoxygenase (leukotriene synthesis inhibitor), and cyclo-oxygenase (COX) inhibitors (which inhibit prostaglandin synthesis) (70,71). However, more specific therapies directed against inflammation and remodeling without adverse effects are needed. Anti-inflammatory cytokine antagonists seem useful in several Th1- cytokine-driven diseases, such as rheumatoid arthritis (RA), psoriasis, and inflammatory bowel disease or Crohn's disease. Inhibitors of remodeling include antagonists for growth factors such as TGF- β and VEGF, and inhibitors of MMPs. This part of the review focuses on the recent developments in this field.

POTENTIAL DRUGS: GROWTH FACTOR OR CYTOKINE ANTAGONIST DRUGS

Different intervention levels can be explored. Currently examined levels of intervention include (1) scavenging and neutralization of proteins by binding to soluble receptors or neutralizing antibodies; (2) inhibition of protein binding to its receptor by small com-

pounds or incomplete and nonactivating cytokines; (3) inhibition of protein activation (processing of preprotein into mature protein); and (4) inhibition of signal transduction and transcription via inhibition of receptor-dependent tyrosine kinases, MAPKs, or nuclear factor (NF)- κ B and its inhibitors IKK and I κ B and via antisense mRNA or small interfering (si)RNA molecules for growth factors or cytokines. Many potential drugs are still in preclinical development or in clinical trial phases for diseases other than COPD (Tables 2 and 3). A few are, however, in clinical trials, with little information on them having been published for COPD in peer-reviewed journals. General adverse effects of injected drugs include reactions around the site of administration and, less frequently, a delayed hypersensitivity-like reaction and new onset of autoimmunity; on rare occasions, drug-induced systemic lupus erythematosus and demyelination, serious infections, vasculitis, or malignancies can occur (59,60). This may lead to a loss of efficacy. In the following sections, some potential drugs are highlighted.

EGFR/HER

Little has been published on the expression of EGF-like growth factors and HER1-family members in COPD. A few studies reported a higher expression of HER1, EGF or heregulin in airway epithelium in patients with chronic bronchitis or COPD (24,28,72). A recent study also demonstrated a higher bronchial epithelial expression of HER3 in patients with COPD as compared with smokers without COPD and healthy subjects (72). Several studies pointed to the involvement of HER1 in mucus overproduction and goblet cell metaplasia. This supports a potential targeting of these molecules for treatment of tissue remodeling in COPD. Among the antagonists of HER1 or HER2-4 as well as inhibitors of their ligands are antibodies directed against the ligand-binding site such as cetuximab and h-R3, or small molecules directed against the tyrosine kinase domain such as gefitinib or trastuzumab. Several of these antagonists are already in clinical phase studies or are already approved by the Food and Drug Administration (FDA) for treatment of specific cancers (cetuximab, erlotinib, trastuzumab, gefitinib) (Table 2).

Cetuximab is a chimeric immunoglobulin (Ig)G1 antibody binding to the extracellular domain of HER1, blocking ligand binding. Although approved by the FDA for use in metastatic colon cancer, it is in clinical trial for different carcinomas. One of the adverse effects is the development of interstitial lung disease, which is not favorable for patients with COPD. Another antagonist against the extracellular domain of HER1 is h-R3, a humanized antibody IgG1. It inhibits proliferation of carcinoma cells both in vitro and in vivo and VEGF expression, angiogenesis,

Table 2
Examples of Antagonists in Clinical Trial: Potentially Useful Drugs
for the Treatment of Chronic Obstructive Pulmonary Disease (COPD)

Name	Type of compound	Function	Trial phase (disease)
EGFR/HER2			
Cetuximab (IMC-225; Erbitux)	Chimeric IgG1 human/mouse Antibody binding to extracellular domain of EGFR	blocking ligand binding	¹ (CC)
h-R3	Humanized IgG1 antibody binding to extracellular domain of EGFR	blocking, ligand binding	IIb (HNC)
ABX-EGF	Human IgG2 monoclonal antibody against human EGFR	blocking ligand binding	II (RCC)
Erlotinib (Tarceva; OSI-774)	Oral 4-anilinoquinazoline	inhibiting EGFR tyrosine kinase	¹ (NSCLC)
Gefitinib (Iressa; ZD1839)	Oral 4-anilinoquinazoline	inhibiting EGFR tyrosine kinase	¹ (NSCLC)
Lapatinib (GW572016)	Small molecule inhibitor	inhibiting EGFR & HER2 tyrosine kinase	III (BC; cancer)
PKI166	Oral pyrrolo-pyrimidine derivate	inhibiting EGFR& HER2 tyrosine kinase	II (ST)
Canertinib (CI1033;PD 183805)	4-Anilinoquinazoline derivate	inhibiting pan-HER tyrosine kinase	I (BC)
TNF-α			
Infliximab	Chimeric IgG1 human/mouse anti-TNF- α antibody	Neutralization TNF- α	¹ (RA;CD) (<i>Remicade</i>) III (AS; psoriasis); II (COPD; asthma)
Etanercept (<i>Enbrel</i>)	Human TNFR p75 :Fc IgG1 fusion protein	Neutralization TNF- α	¹ (RA;AS); II (asthma)
Adalimumab (<i>Humira</i>)	Human IgG1 anti-TNF- α antibody	Neutralization TNF- α	¹ (RA); III (AS;CD); II (psoriasis)
Afelimomab (<i>Segard</i>)	Mouse F(ab') ₂ anti- TNF- α fragment	Neutralization TNF- α	III (sepsis)
CDP-571 (<i>Humicade</i>)	Humanised mouse anti-TNF- α antibody	Neutralization TNF- α	III (CD)
CDP-870	Polyethylene-glycolated-human F(ab') anti-TNF- α fragment	Neutralization TNF- α	III (RA;CD)
Onercept	Rec. human sTNFR p55	Neutralization TNF- α	II (CD; psoriasis)
PEG sTNF—RI	Polyethylene-glycolated-human rec. soluble TNF- receptor p55	Neutralization TNF- α	II (RA)
CytoTab	Sheep F(ab') anti-TNF- α antibody	Neutralization TNF- α	IIb (sepsis); I (CD)
ISIS-104838	Antisense TNF- α methoxy-oligonucleotide	Inhibition TNF- α transcription	II (RA;CD)
BMS-561392	TACE & MMP Inhibitor	Blocks pre-TNF- α processing	II (RA;CD)
Marimastat	TACE & MMP Inhibitor	Blocks pre-TNF- α processing	III (cancer)
CCL2/CCR2			
MLN-1202	humanized mouse anti CCR2 antibody	Inhibits binding CCL2 to CCR2	II (RA)

(continues)

Table 2 (continued)

Name	Type of compound	Function	Trial phase (disease)
INCB3284	CCR2 antagonist	Inhibits binding CCL2 to CCR2	I (DTH, RA)
RS-504393	spiropiperidine	Inhibits binding CCL2 to CCR2	Preclinical
MCP-1 ₍₉₋₇₆₎	CCL2 peptide equal to amino acids 9 to 76	Inhibits binding CCL2 to CCR2	Preclinical
CXCR2			
SB332235	CXCR2 antagonist	Inhibits binding CXCL8 to CXCR2	I (COPD)
SB656933	CXCR2 antagonist	Inhibits binding CXCL1 to CXCR2	I (COPD)
SB265610	biphenolic urea derivate	Inhibits CXCL1/8 to CXCR2	Preclinical
SB225002	biphenolic urea derivate	Inhibits CXCL1/8 to CXCR2	Preclinical
GROa _[8573]	CXCL1 peptide equal to amino acids 8 to 73	Inhibits CXCL1 binding to CXCR2	Preclinical

AS, ankylosing spondylitis; BC, breast tumours; CC, colon cancer; CD, Crohn's disease; HNC, head and neck cancer; DTH, delayed type hypersensitivity; HIV, human immunodeficiency virus; MS, multiple sclerosis; NSCLC, non-small-cell lung cancer; RA, rheumatoid arthritis; RCC, renal cell cancer; ST, solid tumors; ¹, approved by the US Food and Drug Administration (FDA).

and tumorigenesis in mice and man (73). In contrast to the small-molecule HER1 inhibitor ZD1839, h-R3 does not stimulate epithelial apoptosis (73). Potentially, this drug may reduce goblet cell hyperplasia as seen in smokers with COPD, or vascular remodeling or angiogenesis as can be observed in patients with emphysema. Trastuzumab (Herceptin) is a recombinant humanized murine anti-HER2 monoclonal antibody. It is approved by the FDA for treatment of metastatic breast carcinoma. It induces HER2 receptor downmodulation, inhibits critical signaling pathways (i.e., Ras-Raf-MAPK and PI3K/Akt), blocks cell cycle progression by inducing the formation of p27/Cdk2 complexes, and inhibits HER2 cleavage and angiogenesis (74). 2C4 is a humanized monoclonal antibody targeting the role of HER2 as a co-receptor by sterically hindering HER2 recruitment in heterodimers with other HER receptors (74).

Among the small molecules against HER1 is gefitinib (Iressa; ZD1839). This is a 4-anilinoquinazoline targeting the ATP-binding site in the intracellular tyrosine phosphorylation site of growth factor receptors including HER1 and HER2 (75). It has been approved by the FDA for use in local or metastatic non-small-cell lung carcinoma (NSCLC). Although capable of reducing tumor growth, it stimulates caspase-dependent apoptosis of lung epithelial cells *in vitro* (76). This is an effect that is not favorable in case of emphysema, in which alveolar epithelial cells are being lost presumably via apoptosis. PKI166 is an oral pyrrolo-pyrimidine derivate

inhibiting HER1 and HER2 tyrosine kinase activity. PKI166 is capable of reducing tumor growth but also stimulates epithelial intercellular adhesion and inhibits angiogenesis associated with an impaired VEGF and CXCL8 expression as well as endothelial apoptosis in human carcinoma growing in mice (77). Especially the re-establishment of epithelial adhesion, reduction of CXCL8 expression, and inhibition of angiogenesis may be favorable in treatment of patients with COPD, potentially leading to intact airway epithelium, reduced neutrophilic inflammation, and less vascular remodeling.

The water-soluble, orally available 4-anilinoquinazoline canertinib (CI-1033) is a specific pan-HER1-4 tyrosine kinase inhibitor, thereby potentially impairing HER functioning. All of these compounds may reduce excessive proliferation and mucin synthesis as seen in chronic bronchitis. On the other hand, further epithelial and endothelial apoptosis, as seen with trastuzumab, should be avoided in patients with emphysema. However, no data have yet been published on its efficacy for reducing tissue remodeling in the treatment of nononcological disorders.

VEGF AND VEGFR, FGF AND FGFR.

Several studies have pointed to the involvement of VEGF/VEGFR in pulmonary and vascular remodeling and inflammation. VEGF transgenic mice showed alveolar vascular and airspace remodeling as well as

increased numbers of alveolar macrophages, or a Th2-type airway inflammation in a murine asthma model (78). An *in vitro* study demonstrated that VEGF induced monocyte migration via VEGFR1 expressed by monocytes (78,79). Therefore, such inhibitors may be of potential use for treatment of vascularization, as seen in asthma or in specific subtypes of COPD, such as chronic bronchitis, but not emphysema. Several drugs have been developed to reduce tumor growth and metastasis by impairing the neovascularization. Antagonists include those in clinical trials and in pre-clinical investigations. Among those in clinical trial are VEGF-Trap, bevacizumab (or: Avastin), CEP-7055, VEGF Trap, and PTK787 (79).

Avastin is a humanized monoclonal IgG1 antibody against VEGF preventing binding of VEGF to VEGFR1 and VEGFR2. The FDA has approved it for intravenous treatment of metastatic colon and rectal cancer. Clinical studies have pointed out that this antibody has little clinical activity as a single drug, but in combination with chemotherapy in the treatment of a variety of cancers, it reduced tumor growth and angiogenesis (80). Side effects of Avastin were noted to include thrombosis and hypertension and, in combination with chemotherapy, cerebrovascular and cardiovascular infarctions (80). CEP-7055 is a small-molecule, water-soluble indenopyrrolocarbazole derivate inhibitor of pan-VEGFR tyrosine kinase activity. CEP-7055 is reported to inhibit pulmonary VEGFR2 activation in mice, capillary formation by human and rat endothelial cells *in vitro* and *in vivo* in mice, granuloma formation and vascularization in a chronic inflammation model in mice, and tumorigenesis (80,81). It is currently being evaluated in phase-I trials for treating patients with solid tumors. VEGF Trap is a soluble VEGFR constructed from VEGFR1- and VEGFR2-binding domains linked to an IgG1 constant region. Different *in vivo* mouse models demonstrated that VEGF Trap reduced hemangiogenesis, lymphangiogenesis, and tumor growth and size (81). VEGF Trap entered clinical trial phase-I studies for treatment of solid tumors. However, little is known yet of side effects or its efficacy in treatment of chronic inflammatory diseases.

SU6668 is a IgG2a fusion protein inhibiting tyrosine kinase activity of FGFR1, VEGFR2, and platelet growth factor receptor b. SU6668 was demonstrated to inhibit tumor growth, lung cancer metastasis, and tumor vascularization (82). Its broader spectrum of inhibitory effects may be more effective in treating airway remodeling in COPD. Many other small-molecule VEGFR inhibitors are being developed for clinical therapy (38,82). Despite these data, nothing has been published yet on such treatments in asthma or COPD. Also, the redundancy of the VEGF-VEGFR system may temper applicability and efficacy of VEGF/VEGFR inhibitors (83). In addition, in, e.g.,

patients with emphysema, inhibitors of apoptosis are needed whereas in contrast to chronic bronchitis, expression of hypoxia-inducible factor, VEGF, and VEGFR2 may be stimulated to overcome endothelial and epithelial cell death and improve vascular restoration (84). Hence, treatment with VEGF/VEGFR inhibitors should be further investigated and may be restricted to specific subtypes of chronic lung diseases.

TGF-B AND TGF-BR

Both pulmonary TGF- β 1 and FGFR1 expression have been reported to be enhanced in COPD as compared to smokers without COPD. The expression patterns were reported to be related to either inflammation or tissue remodeling (15,32,47). One of the involved mechanisms may include TGF- β 1, which can induce FGFR1 and FGFR2, but not HER1, expression in human lung fibroblasts *in vitro* (85). The enhanced expression of TGF- β 1- or TGF- β -mediated pathways may hence be a target for therapeutic intervention.

A few inhibitors have been reported to reduce TGF β ₁ expression, scavenge TGF β , interfere with binding of TGF β to its receptors, or to inhibit TGF β R signal transduction. All of these antagonists are still in preclinical development. The adenoviral 5 vector encoding for TGF- β 1 antisense RNA molecules was reported to reduce TGF- β 1 expression, leading to impaired collagen and smooth muscle cell actin in liver cells in mice (86). The recombinant TGF- β 1 peptide coding for amino acids 41 to 65 antagonizes TGF- β 1 binding to its receptor. The peptide stimulated re-epithelialization of skin wounds in mice with concomitant reduction of wound contraction and scarring (87). As lung epithelial damage is often seen in smokers with or without COPD, and epithelial TGF- β 1 expression is higher in airway epithelium in COPD, molecules such as TGF- β 1_(AA41-65) may be useful in epithelial repair. Soluble TGF- β receptor molecules TGF- β RII and TGF- β RIII were shown to reduce mammary tumor metastases or airway fibrosis while TGF- β function in normal tissues remained intact (87,88). However, Lutgens et al. (89) found that sTGF- β RII reduced fibrosis in an arteriosclerosis mouse model while increasing T-cell inflammation. As patients with COPD also show higher numbers of T-cells, modulating TGF- β RII functioning may have adverse effects in patients with COPD.

CYTOKINES

As pointed out previously, cytokines and chemokines are not only involved in innate and acquired immunity processes but also may be involved in tissue remodeling in chronic lung diseases like COPD. We recently reviewed

potential antagonists regarding cytokine therapy for COPD patients summarized a plethora of molecules (60). Important potential targets for therapy include TNF- α , CCL2, CXCL1, and CXCL8 and their respective receptors. This section outlines drugs that may be potential for therapy of patients with COPD.

The pro-inflammatory cytokine TNF- α activates and stimulates migration of monocytes, macrophages, neutrophils, and T-cells. Higher levels of TNF- α have been observed in induced sputum and plasma in patients with COPD (Table 1). Studies on gene-activating polymorphisms suggested that TNF- α polymorphisms rely on different populations (e.g., Japanese vs Caucasian) and the location (e.g., -376G/A, -308, -238G/A, +489G/A) (6,90). However, polymorphisms seem to be associated with a poorer prognosis (90). TNF- α was shown to play a role in cigarette smoke-induced emphysema and airway inflammation with neutrophils and macrophages in mice (91). In addition, following exposure to cigarette smoke, TNFR p55- and p75-deficient mice had threefold less increase in airspace enlargement, 65% fewer neutrophils, and no increase in macrophage numbers in the bronchoalveolar lavage (BAL) fluid as compared with wild-type mice (92).

Drugs aimed at reducing TNF- α levels have been developed for treating chronic diseases such as rheumatoid arthritis, psoriasis, and inflammatory bowel disease (especially Crohn's disease) (Table 2). Some are in clinical trial (phase II) for asthma or COPD (Table 2). TNF- α and TNFR antagonists include antibodies (infliximab, afelimomab, CytoTab, adalimumab, CDP-571, CDP-870), soluble TNFR (Onercept, Etanercept), small molecules (ISIS-104838), and inhibitors of TNF- α -converting enzyme (TACE).

Recent studies have shown that treating patients with COPD with infliximab over 6 to 24 wk did not improve lung function, body weight, quality of life scores, airway inflammation, or cytokine levels (93–95). One study reported higher exhaled nitric oxide (NO) levels (93), whereas another study noted a tendency to improved 6-min walking distance (94). Subgroup analysis of COPD patients in different GOLD stages may reveal more details. In asthma, short-term treatment with etanercept enhanced TNFR p75 levels in the bronchial epithelial lining fluid but did not change airway eosinophilia or hyperresponsiveness (96). In a 10-wk treatment study, Berry et al. (97) reported that etanercept improved the hyperresponsiveness by methacholine testing (PC₂₀) and FEV₁ (postbronchodilator) as well as the quality of life scores as compared to treatment by placebo. However, no changes in inflammatory or total cell counts in sputum were noted.

TNF- α antagonists not only reduce inflammation but also tissue destruction by downregulation of MMP expression or inhibition of MMP activation (98,99).

Mucin 5AC (MUC5AC) is an important constituent of mucus seen in chronic bronchitis. A potential therapeutic effect of infliximab was described by inhibiting the TNF- α -induced expression of MUC5AC by an airway epithelial cell line (100). Other experimental TNF- α antagonists include the inhibitors of TACE such as Marimastat and BMS-561392 (Table 2). These molecules inhibit both pre-TNF- α cleavage into mature TNF- α and are inhibitors of MMPs. Recent studies demonstrated that cigarette smoke and lipopolysaccharide (LPS)-induced MUC5AC production in vitro via a TACE-dependent mechanism (101). Inhibition of TACE via TACE-specific drugs would appear to be potentially beneficial, especially in COPD/chronic bronchitis or asthma. However, side effects may include musculoskeletal pain, inflammatory polyarthritis, and hand contraction, potentially precluding its applicability (102). The concomitant inhibition of MMP activity may be important in restoration of the protease-antiprotease balance in COPD, which favors proteolytic breakdown of tissue at higher MMP and elastase activities. Among the preclinical drugs is TMI-1, a thiomorpholinecarboxamide derivative, which specifically inhibits LPS-induced TNF- α secretion, but not secretion of other proinflammatory cytokines such as IL-1 β , IL-6, or IL-8, both from human cell lines and in human explants (103). In vivo, TMI-1 reduced clinical symptoms in a collagen-induced arthritis model in mice (103).

Given the side effects, smaller noninjectable, bioactive molecules with long half-life and high bioavailability that are capable of regulating TNF- α expression, synthesis, and/or secretion seem to be needed (104). The antisense oligonucleotide for TNF- α (ISIS-104838) is an example of an orally available molecule in clinical trial for RA and Crohn's disease (Table 2). ISIS-104838 inhibits TNF- α mRNA translation into pre-TNF- α proteins, finally resulting in reduced extracellular TNF- α levels. Furthermore, TACE-specific siRNA, which blocks TACE mRNA synthesis, may prove to be useful, but little is known yet about the clinical effects. Inhalation antisense formulations are less likely to reduce systemic pulmonary inflammation in COPD, but may be useful in treating local pulmonary inflammation, and hence reduce tissue remodeling and mucus hyperproduction. In conclusion, given the selective efficacy of TNF- α antagonists, clinical trials for COPD with different antagonists other than infliximab seem worthwhile for determining efficacy.

CHEMOKINES

Macrophages migrate toward a gradient of CCL2, CCL3, or CCL4 via their receptors CCR2 or CCR5. Neutrophils are mainly attracted by CXCL1 and CXCL8

via CXCR1 and CXCR2, of which CXCR1 has a larger affinity for CXCL8 than for CXCL1. CXCR1 is involved in neutrophilic degranulation, protease release, and oxidative stress. T-cells may have receptors for CCL2–4, and a subset also has CXCL10 receptors (CXCR3). In COPD, levels of some of these chemokines and their receptors may be associated with lung function (Table 1), and seem to be involved in the pathogenesis of COPD (60). As previously pointed out, chemokines seem to be involved in tissue repair processes (18,61). Therefore, chemokine antagonists might have potential as specific treatments for both inflammation and tissue remodeling in COPD. A few of the many chemokine and chemokine receptor antagonists are in clinical trials for inflammatory diseases (Table 2) (60). These include an anti-CCR2 antibody (MLN1202) and small-molecule inhibitors INCB3284, SB332235, and SB656933 (60). For most drugs, no peer-reviewed data on effects on tissue remodeling have yet been published. However, RS-504393—a pre-clinical CCR2 spiroperidine antagonist—was shown to be capable of blocking CCR2-dependent renal fibrosis apart from reducing macrophage infiltration and activation and CCL2 expression in mice (105). Among the CXCL1 and CXCL8 antagonists that prevent binding to CXCR2, SB332235 and SB656933 are being evaluated in clinical trial phase I for COPD (Table 2). Whereas both drugs impair CXCR2-mediated effects on neutrophils, no data on human lung tissue remodeling or inflammation have yet been published for both drugs.

In contrast, SB225002 and SB265610 inhibit binding of CXCL1 and CXCL8 to CXCR2 by interfering with the chemokine binding sites (60). SB265610 treatment inhibited skin wound repair and neutrophil recruitment in a skin wound healing model in mice (61). Although inflammation can be reduced, a potential drawback of cytokine antagonists may be caused by the lung epithelial expression of functional chemokine receptors CCR2 and CXCR2, which are probably involved in epithelial migration and wound healing (18,65). Inhibition of these receptors may impair wound healing following smoke exposure and hence, may increase the risk of infection and concomitant inflammation. In conclusion, chemokine antagonists may reduce pulmonary inflammation, but they may hamper epithelial repair upon smoke-induced damage. Further studies are needed to resolve this.

TRANSCRIPTION FACTORS AND SIGNALING MOLECULES

An alternative and recently explored route either to reduce growth factor, cytokine, or chemokine levels or to impair their effects is to interfere with gene expression or intracellular signal transduction. For growth

factors, receptor tyrosine kinases are needed in order to activate downstream acting Ras, Raf, and MAPK p42 and p44 proteins. For cytokines and chemokines, signal transduction molecules such as NF- κ B and MAPK p38 are needed. NF- κ B is both a signal transduction and a transcription factor. In its inactive state, it is bound to another protein, Inhibitor of κ B (I κ B). Upon phosphorylation of I κ B by I κ B Kinase (IKK), I κ B dissociates from the complex and becomes ubiquitinated and subsequently degraded. Then, activated NF- κ B is translocated to the nucleus and binds to NF- κ B binding sites in the promoter region of genes including cytokines, chemokines, and MMPs. MAPK p38 is activated via upstream signaling proteins including G protein-coupled receptors. MAPK p38 is involved in the regulation of transcription factors and a variety of enzymes including those involved in histone remodeling (106). Studies have suggested that NF- κ B and MAPK p38 are involved in the pathogenesis of COPD (106,107). The therapeutic effects of antagonists may be achieved at the transcription level of these signaling molecules by blockade of intracellular translocation of NF- κ B, reduction of signaling of NF- κ B by inhibition of I κ B degradation, and inhibition of enzyme activity (108). Several antagonists of MAPK p38 (SB681323, RWJ67657, SCIO-469), NF- κ B (siRNA), or IKK (BMS345541) are in preclinical or clinical trial phase I or II for inflammatory diseases including allergic rhinitis and COPD (60,109). As MAPK p38 has four isozymes and each MAPK p38 inhibitor has its own specificity toward one or more of these isozymes, this specificity may cause differential effects. With respect to NF- κ B, several studies have indicated the involvement of NF- κ B in tissue remodeling. Using an *in vivo* model for intestinal disease, Lawrence et al. (110) reported that treatment of mice with antisense NF- κ B p65 reduced the intestinal fibrosis.

In rat tracheal explants, the NF- κ B inhibitor SN50 inhibited both TGF- β 1 and procollagen I expression that was enhanced by exposure to diesel exhaust particles (111). Using siRNA directed against the NF- κ B subunit p65 in airway epithelial cell lines caused an impaired TNF- α -induced NF- κ B p65, IL-6, and CXCL8 expression (112). Hence, NF- κ B inhibitors may potentially reduce not only inflammation but also matrix remodeling in chronic inflammatory disease like COPD. An antisense antagonist for NF- κ B p65 is now in clinical trial phase I for Crohn's disease.

Another class of kinases regulate activation of signaling proteins. The biaryl-urea-derivate BAY 43-9006 is a dual inhibitor of Raf kinase and VEGFR being tested in clinical phase III studies for treatment of cancer (113,114). This drug impairs tumor growth and angiogenesis. Recently, Hewson et al. (115) showed that MUC5AC expression by airway epithelial cells needed

Ras and Raf activation downstream from EGFR activation. Hence, BAY 43-9006 may potentially reduce growth factor receptor tyrosine kinase-induced proliferation or MUC5AC expression as well as vascular remodeling as seen in COPD.

TGF- β R signal transduction involves the downstream-acting Smad transcription factors. This pathway includes Smad2 and Smad3, which activate TGF- β -responsive genes, and the inhibitory Smad6 and Smad7 proteins (116). Patients with COPD were observed to have an unchanged Smad3 expression in the airways, whereas expression of Smad6 and 7 was lower as compared to patients without COPD (117). In addition, cigarette smoke extract reduced the Smad6 and Smad7 expression in alveolar epithelial A549 cells in vitro (117). SB431542 and the more potent SB505124 inhibit Smad2 and 3 phosphorylation, their nuclear translocation, and TGF- β -induced collagen I synthesis and tumor cell death (117,118). These data point to the potential use of Smad2/3 antagonists or Smad6/7 agonists in the treatment of airway remodeling in COPD.

In conclusion, several inhibitors of MAPK p38 and NF- κ B are at a more advanced stage with respect to clinical trials, whereas antagonists for Raf, IKK, and Smad2/3 may also prove to be useful for therapy against remodeling and inflammation. The potential disadvantage of using inhibitors of cell signaling molecules is that these molecules are general signal transduction proteins involved in various physiological processes. Systemic administration of antagonists may have profound effects on other receptor-mediated pathways other than the pro-inflammatory and remodeling reactions in COPD. Moreover, inhibition of these molecules may also potentially impair defense and repair mechanisms resulting in increased infection risk.

PERSPECTIVES AND CONCLUDING REMARKS

Although imbalance mechanisms between proteases and antiproteases and oxidants and antioxidants are thought to prevail in COPD, pathogenetic mechanisms with regard to tissue remodeling are not yet clarified. Changes in ECM, epithelial metaplasia in chronic bronchitis and asthmatics or apoptosis in emphysema, and vascular changes can occur. Recent investigations also point to the potential molecules involved in these processes, including growth factors and cyto- or chemokines. Specific antagonist drugs capable of reducing growth factor and cytokine expression or signaling are available or being tested. Some seem to be effective in different inflammatory diseases or in reducing tumor growth. However, these antagonists have not yet been tested extensively with regard to reducing the remodel-

ing processes in chronic inflammatory lung diseases. Experimental models and expression studies suggest that anti-VEGFR strategies may be of use in patients with emphysema, whereas anti-HER1 directed strategies may be more useful in patients with pulmonary mucus hypersecretion as seen in chronic bronchitis and asthma. This suggests the need of stratification of antagonist therapy. However, a complete block of cytokine-driven or growth factor-dependent repair mechanisms may be disadvantageous, as defense reactions to infection may be impaired. Antibiotics may overcome bacterial infection but are not useful as a strategy for treating viral infections. The efficacy and bioavailability of biological drugs may be improved by reducing immunogenicity and improving routes of administration. Finally, restoration of lung tissue structure may be provided by other drugs such as retinoic acids. Such approaches, however, must await the first results of growth factor or cytokine antagonist therapy in chronic lung diseases.

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