Cytokines and Therapy in COPD*

A Promising Combination?

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COPD is a major health problem, with patients showing a progressively declining, largely irreversible, change in lung function. This is associated with chronic airways inflammation and structural remodeling, including loss of alveolar walls, and goblet cell metaplasia with mucus hypersecretion. Inflammatory cells may contribute to the airway remodeling via secretion of proteases, fibrotic or mitogenic growth factors, and cytokines. In turn, airway remodeling may contribute to the clinical symptoms of COPD. Currently available therapies are directed to improvement of clinical symptoms and reduction of the airways inflammation. The commonly used glucocorticosteroids are expected to reduce the inflammation by acting on kinases or transcription factors necessary for expression of pro-inflammatory cytokines or chemokines. However, several long-term and short-term studies showed that glucocorticosteroids are rather ineffective in improving lung function and reducing the airway inflammation in patients with COPD. New therapeutic strategies may reduce the inflammation and alleviate the clinical symptoms of COPD. Tumor necrosis factor-α, interleukin-8, and monocyte chemoattractant protein-1 are important chemotactic proteins for macrophages and neutrophils, the predominant inflammatory cells associated with COPD. As lung levels of these cytokines are higher in COPD compared to non-COPD patients, they may represent targets for novel therapies. (CHEST 2002; 121:209S–218S)

Key words: antagonists; chemokines; COPD; cytokines; interleukin-8; monocyte chemoattractant protein-1; receptors; therapy; tumor necrosis factor

Abbreviations: GRO = growth-regulated oncogene; IFN = interferon; IL = interleukin; MCP = monocyte chemoattractant protein; MIP = macrophage inflammatory protein; MMP = matrix metalloproteinase; SLPI = secretory leukocyte proteinase inhibitor; TNF = tumor necrosis factor; TNFR = tumor necrosis factor receptor

COPD is a major health problem, ranking among the most common causes of death in Western societies. It is defined by a progressive declining lung function that is only partly reversible by bronchodilator drugs. Although epidemiologic studies demonstrated a close association with cigarette smoking, only 10 to 20% of smokers develop COPD. The disease can be subdivided into three distinct pulmonary disorders: chronic bronchitis, small airway disease (bronchiolitis), and emphysema, which show different features such as goblet cell metaplasia and mucous hypersecretion in chronic bronchitis, and destruction of alveolar septae in emphysema. It has been recognized that COPD is characterized by chronic inflammation in the airways or alveoli that differs from that seen in asthma, involving increased numbers of neutrophils, macrophages, CDS+ T cells, and/or mast cells in the airway walls, alveolar compartments, and vascular smooth muscle. In a subpopulation of COPD patients with chronic bronchitis, the obstruction seems to be partially reversible and is accompanied by the presence of airway eosinophils. Activation of inflammatory cells is thought to be involved in the airway and alveolar remodeling. For example, neutrophils and eosinophils possess granules containing matrix-degrading proteases. Activated neutrophils also produce reactive oxygen free radicals such as H2O2. Proteases and free radicals can damage the epithelium and underlying basement membrane. This is normally followed by a repair process that includes the secretion of antiproteases, such as secretory leukocyte proteinase inhibitor (SLPI) and tissue inhibitor of metalloproteinases by epithelial cells in order to regulate the proteolytic processes. Activated macrophages, T cells, and mast cells also produce and secrete matrix metalloproteinases (MMPs) that can damage the epithelial barrier. The repair process is thought to be disturbed in COPD due to an imbalance in the protease-antiprotease balance.

Hence, inflammatory cells may be directly involved in airway wall remodeling.

Cytokines and Chemokines

Migration and activation of inflammatory cells is regulated by cytokines and chemokines, small proteins secreted by a variety of structural cells, such as epithelial, endothelial, smooth muscle, and fibroblasts, as well as by inflammatory cells. Cytokines associated with COPD include tumor necrosis factor (TNF)-α, interferon (IFN)-γ, and interleukin (IL)-1β and IL-6. The chemokines are chemotactic cytokines showing 2, 4, or 6 conserved cysteine residues. Based on the number and spacing of conserved cysteines, chemokines are assigned to four families: α- (CXC), β- (CC), CXXXC, and Ĉ chemokines in which X denotes the number of noncysteine residues between the first two conserved cysteines. At least 28 CC, 15 CXC, 2 XC, and 1 CX3C chemokines have been described (Table 1). Cytokines and chemokines act via binding to one or more cellular transmembrane receptors. For TNF-α, this includes TNF-α receptors (TNFRs) and 2 (TNFR p55) and 2 (TNFR p75). For mammalian chemokines, a summary of the seven-transmembrane, G protein-coupled receptors is provided in Table 2. The Duffy and D6 chemokine receptors are not shown as they bind chemokines in a nonspecific manner, and do not transduce intracellular signals. Significant redundancy is observed for several chemokines with respect to receptor binding. That is, in some cases, one receptor subtype can bind several chemokines, whereas a given chemokine can bind to several receptor subtypes (Table 1). Thus, if one...
According to the recent guidelines for COPD,23 regular clinical treatment of COPD includes the use of bronchodilators (β2-adrenoceptor agonists, anticholinergic drugs, and methylxanthines such as theophylline), and oral or inhaled corticosteroids. Alternative therapies currently being explored include phosphodiesterase 4 inhibitors, leukotriene receptor antagonists, and inhibitors of 5-lipoxygenase and cyclooxygenase. More specific details on some of these agents are provided, respectively, by Sturton and Fitzgerald, and Kilfeather in this supplement. Such treatments are normally expected to improve the quality of life by (subjective) improvement of lung function, dyspnea, and reduced inflammation. Studies24–28 in vitro have shown that corticosteroids reduce inflammatory responses by intracellular inhibition of transcription or translation of pro-inflammatory cytokines and chemokines. Hence, corticosteroid therapy may inhibit the increased expression of TNF-α, monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein (MIP)-1α, and IL-8 observed in COPD.7,19,20,29

In contrast to the positive effects in asthmatics and a subpopulation of patients with COPD, ie, those with bronchial hyperresponsiveness and eosinophilia, regular...
Corticosteroid treatment of patients with COPD has been disappointing. Some studies\textsuperscript{31-33} showed that long-term therapy with inhaled corticosteroids leads to an improvement in FEV\textsubscript{1} only during the first 3 to 6 months of treatment, whereas after that period, the FEV\textsubscript{1} declines at the same rate as in the placebo-treated subjects. Another study\textsuperscript{34} did not show any improvement in FEV\textsubscript{1}. Short-term treatment (2 to 4 weeks) with corticosteroids does not seem to affect the airways inflammation (numbers of neutrophils, macrophages, lymphocytes, eosinophils) or cytokine expression of cytokines (TNF-\textalpha, IL-8, MCP-1, and MIP-1\textalpha), whose expression levels have been demonstrated to increase in sputum,\textsuperscript{19} BAL fluid,\textsuperscript{20} plasma,\textsuperscript{31} or lung tissues\textsuperscript{32-33} from patients with COPD. Also, increased numbers of IFN-\gamma-positive T cells in peripheral blood were reported in patients with COPD.\textsuperscript{35} Although many cytokines, chemokines, and arachidonic acid metabolites may be involved in neutrophil and monocyte/macrophage effector functions, some studies suggest that TNF-\textalpha, IL-8, MCP-1, and MIP-1\textalpha, in particular, play important roles in this regard. These proteins, therefore, are the primary focus in ensuing sections.

**TNF-\textalpha and TNFR-Based Therapies**

Studies have shown that TNF-\textalpha expression levels in patients with COPD may be higher, due either to induction by eg, cigarette smoking or genetic aberrations. For example, TNF-\textalpha is secreted by cultured bronchial epithelial cells on exposure to cigarette smoke or its condensate.\textsuperscript{42} Alternatively, other studies reported the presence of gene-activating TNF-\textalpha polymorphism in patients with COPD,\textsuperscript{43-45} resulting in a constitutive higher expression of TNF-\textalpha.\textsuperscript{46} TNF-\textalpha has multiple pro-inflammatory actions, including neutrophil degranulation accompanied by release of proteolytic enzymes like lysozyme and stimulation of the respiratory burst\textsuperscript{47-49} (Fig 1).

In addition to its pro-inflammatory actions, TNF-\textalpha has also been reported to have direct effects on epithelial cells. TNF-\textalpha is capable of inducing airway mucous cell metaplasia and hypersecretion \textit{in vitro} and \textit{in vivo}, features reminiscent of the goblet cell metaplasia observed in chronic bronchitis.\textsuperscript{50,51} Other effects include decreased interepithelial binding and cell death \textit{in vitro},\textsuperscript{52,53} emphysematous lesions and alveolar collagen deposition in murine alveolar walls,\textsuperscript{54,55} induction of IL-1, TNF-\textalpha, IL-8, and MCP-4 expression,\textsuperscript{56-59} and of IFN-\gamma receptors on epithelial cells.\textsuperscript{60} IFN-\gamma in turn inhibits the proliferation and decreases desmosome formation of epithelial cells\textsuperscript{63} and may, therefore, be involved in destruction of epithelial integrity and formation of emphysematous lesions. Targeted overexpression of IFN-\gamma in type II pneumocytes in mice resulted in emphysema, higher numbers of activated pulmonary neutrophils and macrophages, in addition to increased activity of MMP-9 and MMP-12. Antiprotease SLPI levels were decreased.\textsuperscript{61} Such data indicate that TNF-\textalpha has direct and indirect (via IFN-\gamma) effects on epithelial barrier functions, eg, via inducing cell death and emphysema, and clearance function (replacement of ciliated cells by goblet cells), and may contribute to the clinical deterioration seen in COPD. The induced pro-inflammatory cytokine expression and protease release can perpetuate the inflammatory cell influx and activation, causing distortion of the airways architecture. Anti-TNF-\textalpha or anti-TNFR therapies may, therefore, provide more specific means to impair inflammation and epithelial remodeling.

Studies \textit{in vitro} in mice and humans have revealed that TNF-\textalpha is involved in the recruitment of macrophages to sites of inflammation. Thus, in chronic colitis (Crohn's disease, Crohn's disease)

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*The receptors are grouped according to their ligand binding into CCR, CXCR, XCR, and CX3CR. Receptors expressed in human lungs as well as cell types expressing them are shown in bold. B = B cell; NK = natural killer cell; see Table 1 for expansion of other abbreviations.
disease) and rheumatoid arthritis, diseases characterized by the presence of macrophages, T cells, and neutrophils, therapy with neutralizing antibodies directed against TNF-α reduces the inflammation, whereas clinically the patients improved, showing reduced symptoms and an improved quality of life. In addition, in Crohn’s disease, >30% of the fistulae closed. The infiltration of macrophages as well as the expression of IL-8 and MCP-1 were also reduced in patients with rheumatoid arthritis after a single dose of anti-TNF-α. Similar effects were seen in animals and patients treated with a chimeric ligand-binding domain of TNFR p75 linked to the Fc portion of human IgG1. With regard to chronic lung diseases, clinical trials have begun, including a phase II trial with the TNFR-Fc chimera in patients with atopic asthma, at the National Heart, Lung, and Blood Institute, Bethesda, MD.

As a caution, however, anti-TNF-α treatment may be disadvantageous in some conditions such as endotoxemia or sepsis. For example, following a single dose of anti-TNF-α, plasma levels of IL-1, IL-6, and IL-8 were not reduced in patients with severe sepsis, whereas TNF-α levels were only reduced transiently. Also, the clinical aspects of sepsis were not affected by this treatment. Anti-TNF-α treatment of chimpanzees that were injected with endotoxin reduced TNF-α and IL-8 levels but did not impair neutrophilia and lymphopenia, indicating that TNF-α is not a key regulator for neutrophilic inflammation in this model. As COPD patients are prone to bacterial infections, therapy with anti-TNF-α or TNFR-Fc during infectious exacerbations may have only limited effectiveness. To date, few side effects of the anti-TNF-α therapies are reported, including local reactions at the injection site, hypersensitivity reactions, and minor upper airway infections. Minor events include aplastic anemia and demyelination syndrome by TNFR-Fc. Support for demyelination syndrome was provided by Liu et al, where mice lacking TNF-α were more susceptible to neurologic changes and inflammation than their wild-type counterparts.

**CXC Chemokine and CXCR-Based Therapy**

IL-8 and growth-regulated oncogene (GRO)-α are expressed by lung epithelium, fibroblasts, endothelial cells, and alveolar macrophages, and their expression can be induced by stimuli such as cigarette smoke, endotoxin, or TNF-α. Several studies in vivo and in vitro have suggested that IL-8 and GRO-α, acting via their receptors, CXCR1 and CXCR2, are important mediators of neutrophil chemotaxis, endothelial cell adhesion, and degranulation. Evidence for neutrophil chemoattractant
roles of IL-8 and GRO-α was provided in several animal studies. For example, treatment with CXCR2 antagonist GRO-α(5–73) or a neutralizing anti-IL-8 antibody reduced the neutrophilic inflammation and alveolar damage and decreased mortality associated with endotoxinemia, acid aspiration, and in a skin air pouch model.79,82,83 In addition, CXCR2-deficient mice show an impaired neutrophil influx and myeloperoxidase activity in wounds after skin injury.84

In addition to neutrophil chemoattractant properties, IL-8 and GRO-α may be involved in wound repair and angiogenesis. Thus, skin, colon, and lung epithelial cells as well as endothelial cells express CXCR2.85–87 Secondly, activation of CXCR2 by IL-8 and GRO-α can stimulate epithelial proliferation, migration, endothelial migration, and neovascularization.85–87,90,93 CXCR2-deficient mice show delayed skin wound healing and neovascularization in vivo, and CXCR2-deficient keratinocyte cultures exhibit delayed repair that was not improved by mouse GRO-α.84 Also, only basally located, nondifferentiated keratinocytes in human skin wounds in vivo showed CXCR2, coinciding with high expression of IL-8 and GRO-α.85,86

Thus, IL-8 and GRO-α are primary mediators in neutrophilic inflammation acting via CXCR1 and CXCR2. In contrast, CXCR2 is involved in epithelial repair. Several receptor antagonists or anti-IL-8 antibodies have been developed, but these have so far been reported only in assays in vitro or animal models.78–80,82,85 Clinical trials in rheumatoid arthritis and psoriasis with humanized antibodies against IL-8, or CXCR2 antagonists are being conducted. Such agents may also represent potential therapeutic agents for COPD. As noted above, however, such agents may be contraindicated in patients with bacterial infections, as CXCR2 antagonist treatment of mice infected with Pseudomonas aeruginosa showed impaired pulmonary bacterial clearance.96

With regard to COPD, we observed that CXCR2 but not CXCR1 protein and messenger RNA are present in bronchial epithelial cells, mainly in injured areas (Fig 2). In the same patients, IL-8 expression was significantly higher in bronchial epithelium from COPD patients as compared to smokers without COPD.90 Preliminary functional analyses indicated that GRO-α but not IL-8 is mitogenic for bronchial epithelial cells, whereas both stimulate mitochondrial activity (unpublished observations). Given that IL-8 and GRO-α are capable of stimulating directly epithelial wound repair via CXCR2, such an antagonist therapy in COPD may impair this repair. CXCR1, although expressed primarily in neutrophils, is also expressed in macrophages, mast cells, and CD8+ T cells.97,98 Specific antagonists for CXCR1 inhibit both the respiratory burst and degranulation of neutrophils.78 Hence, CXCR1 antagonists, rather than CXCR2 antagonists, may be a more effective approach to reducing airways inflammation in COPD.

CC Chemokine and CCR2-Based Therapy

Macrophages and monocytes express several chemokine receptors, including CCR1, CCR2, and CCR5. Ligands for these receptors include MCP-1α, MCP-1β, MCP-1 to MCP-4, and RANTES (regulated on activation, normal T-cell expressed and secreted) [Table 1]. These chemokines stimulate monocyte/macrophage migration in vitro. Despite this chemokine and receptor redundancy, studies91–102 in vivo indicate that MCP-1 and CCR2 are important monocytes and macrophage chemoattractants. Mast cells and T cells can also be attracted and activated by MCP-1.103,104 CCR2 is the only known receptor for MCP-1,105,106 MCP-1 is produced by several cell types including alveolar macrophages, epithelial, endothelial, and smooth-muscle cells, and fibroblasts.30,107 MCP-1 expression can be induced by various cytokines, including TNF-α and IFN-γ.96,108 In contrast, the expression of CCR2 is inhibited by IFN-γ.109 This may represent an anti-inflammatory reaction preventing excessive influx of macrophages into the tissue. Different studies in vivo support specific roles of MCP-1 and CCR2 in macrophage migration. First, in mice with experiment peritonitis, the influx of monocytes and macrophages but not neutrophils, eosinophils, mast cells, or T cells, was impaired in MCP-1- or CCR2-deficient mice as well as in mice pretreated with antibody against CCR2.99–102 In addition, bacterial clearance was impaired in CCR2-deficient mice, pointing to the importance of macrophages for bacterial clearance.100 Secondly, transgenic mice with targeted overexpression of MCP-1 in type II pneumocytes showed increased numbers of monocytes, macrophages, and lymphocytes but not neutrophils in the lungs.109 Third, ovalbumin sensitized mice repeatedly exposed to ovalbumin showed an influx of monocytes/macrophages and lymphocytes into the lung coinciding with increased MCP-1 and MIP-1α expression. This influx was almost completely inhibited in mice pretreated with antibodies against MCP-1, but not with anti-MIP-1α.111 Also, bronchial hyperreactivity was reduced by anti-MCP-1. Finally, Hantamaki et al.112 showed, in a murine emphysema model, that intratracheal MCP-1 increased both the numbers of lung macrophages and the smoke-induced emphysema, presumably via macrophage-derived MMP-12. These studies support the specificity of the MCP-1-CCR2 system rather than MIP-1α in recruitment of monocytes and macrophages.

Other effects of MCP-1 include stimulation of endothelial wound healing by inducing endothelial migration,113 angiogenesis,114 induction of vascular smooth-muscle hyperplasia,115 collagen and transforming growth factor-β expression by fibroblasts,116 and expression of adhesion molecules CD11c and CD11b as well as IL-1 and IL-6 by blood monocytes.117 Our own studies30 revealed expression of CCR2 on human bronchial epithelial cells. Preliminary data indicated that a signal transduction enzyme, mitogen-activated protein kinase p42/44, is phosphorylated in bronchial human epithelial cells upon MCP-1 treatment in vitro, and MCP-1 slightly but significantly induced epithelial proliferation. This indicates that CCR2 receptors are functional in airway epithelial cells and, moreover, that MCP-1 may have an autocrine effect on epithelial cells. These data further support a major role for MCP-1 and CCR2 in airway remodeling and inflammation directly or via macrophages. Antagonists of CCR2 or MCP-1 may, therefore, be an attractive approach to therapeutic treatment of COPD.
Several antagonist of MCP-1 and CCR2 have been described\textsuperscript{102,118,119} These include nonpeptide CCR2-specific antagonists,\textsuperscript{119} CCR2 neutralizing antibodies,\textsuperscript{120} MCP-1 peptide analogs,\textsuperscript{118} and commercially available MCP-1 neutralizing antibodies. In several mouse models, these molecules show improvements of clinical and histologic symptoms in peritonitis,\textsuperscript{102} arthritis,\textsuperscript{121} allergic airways inflammation and hyperresponsiveness,\textsuperscript{111} and bacterial clearance.\textsuperscript{100} However, clinical trials with these molecules have not been reported. In diseases such as \textit{Mycobacterium tuberculosis} infection, however, these antagonists may not be effective, as seen in infected MCP-1 deficient mice.\textsuperscript{101} In addition, as MCP-1 seems to be involved in wound repair, inhibition of MCP-1 may also retard the healing.

\textbf{Conclusion}

As Scanlon et al\textsuperscript{122} described, sustained smoking cessation improves lung function as compared to subjects who continue to smoke. However, for many smokers, stopping smoking is difficult. New therapies may prove to be more

\textbf{Figure 2.} Expression of CXCR2 in airway epithelium. CXCR2 protein expression as detected by immunohistochemistry is shown in human bronchial tissue in intact epithelium (top, A) and damaged epithelium (bottom, B). Note the intense staining in regenerating epithelium (bottom, B) as compared to the virtual absence in intact epithelium (top, A). The brown (3,3’-diaminobenzidine)-stained cells in the airway lumen (top, A) are neutrophils. L = airway lumen (original \times 200).
effective than therapies such as glucocorticosteroids. Although chemokines show extensive redundancy, several studies in vitro demonstrate the specificity of MCP-1 migration and activation of macrophages and monocytes, of IL-8 and GRO-α to neutrophils, and TNF-α to macrophages and neutrophils. Thus, treatment of COPD with chemokine or cytokines inhibitors may provide advantages over glucocorticosteroids. In order to reduce both macrophage and neutrophil numbers and activation, combinations of antagonists may be necessary. Long-term efficacy and safety studies with the anti-TNF-α therapies in humans are, however, lacking. Case reports may provide insight into side effects of these treatments. In addition, any impairment of pulmonary bacterial clearance may indicate a need for concomitant administration of antibiotics. Furthermore, from the human studies with anti-TNF-α agents performed so far, it can be concluded that such treatments should be continual as the disease activity is only suppressed during treatment. Future clinical trials may provide encouraging data on novel treatments for COPD with one or more chemokine or cytokine antagonists.

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