

# Mediators of Chronic Obstructive Pulmonary Disease

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**Abstract**—Chronic obstructive pulmonary disease (COPD) is a major and increasing global health problem that is now a leading cause of death. COPD is associated with a chronic inflammatory response, predominantly in small airways and lung parenchyma, which is characterized by increased numbers of macrophages, neutrophils, and T lymphocytes. The inflammatory mediators involved in COPD have not been clearly defined, in contrast to asthma, but it is now apparent that many lipid mediators, inflammatory peptides, reactive oxygen and nitrogen species, che-

mokines, cytokines, and growth factors are involved in orchestrating the complex inflammatory process that results in small airway fibrosis and alveolar destruction. Many proteases are also involved in the inflammatory process and are responsible for the destruction of elastin fibers in the lung parenchyma, which is the hallmark of emphysema. The identification of inflammatory mediators and understanding their interactions is important for the development of anti-inflammatory treatments for this important disease.

## I. Introduction

Chronic obstructive pulmonary disease (COPD<sup>1</sup>) is a major and increasing global health problem. It is predicted by the World Health Organization to become the third most common cause of death and the fifth most common cause of disability in the world by 2020 (Lopez and Murray, 1998). Indeed, COPD is already the fourth most common cause of death and the only common cause of death in the United States that has increased over the last 30 years (Murray et al., 2001). Although there have been major advances in the understanding and management of asthma, COPD has been relatively neglected, and there are no current therapies that reduce the inevitable progression of this disease. However, because of the enormous burden of disease and escalating health care costs, which now exceed those of asthma by more than 3-fold, there is now renewed interest in the under-

lying cellular and molecular mechanisms (Barnes, 2000a) and a search for new therapies (Barnes, 2001c), resulting in a reevaluation of the disease (Barnes, 2002b, 2003).

Although many inflammatory mediators (now more than 100) have been identified in asthma (Barnes et al., 1998; Chung and Barnes, 1999), there is much less information about the production and role of mediators in COPD. COPD is a complex inflammatory disease that involves many different types of inflammatory and structural cells, all of which have the capacity to release multiple inflammatory mediators (Figs. 1 and 2). This suggests that mediator antagonists may have some potential as new therapies for COPD. However, because of the redundant effects of many inflammatory mediators, it is unlikely that antagonism of a single mediator will provide major clinical benefit, as is the case in asthma. Although asthma and COPD both involve chronic inflammation in the respiratory tract, there are marked differences in the types of inflammatory cells involved and in the site of inflammation, making it likely that different patterns of mediators are involved. There is much less information available about the mediators of COPD than those of asthma. Unlike asthma, some patients with COPD also have systemic features of the disease, and these are also likely to be mediated via inflammatory mediators.

<sup>1</sup>Abbreviations: COPD, chronic obstructive pulmonary disease; BAL, bronchoalveolar lavage; NE, neutrophil elastase; MMP, matrix metalloproteinase; IL, interleukin; LT, leukotriene; GM-CSF, granulocyte-macrophage colony stimulating factor; FEV<sub>1</sub>, forced expiratory volume in 1 s; NF- $\kappa$ B, nuclear factor- $\kappa$ B; TNF, tumor necrosis factor; HDAC, histone deacetylase; TGF, transforming growth factor; Tc1, interferon- $\gamma$  producing; Tc2, IL-4 producing; IFN, interferon; NK, natural killer (cell); VEGF, vascular-endothelial growth factor; SLPI, secretory leukoprotease inhibitor; PG, prostaglandin; COX, cyclo-oxygenase; MUC, mucin gene; Tx, thromboxane; PAF, platelet-activating factor; ROS, reactive oxygen species; O<sub>2</sub><sup>-</sup>, superoxide anion; H<sub>2</sub>O<sub>2</sub>, hydrogen peroxide; NO, nitric oxide; SOD, superoxide dismutase; MAP, mitogen-activated protein; ERK, extracellular regulated kinase; OH<sup>-</sup>, hydroxyl radical; EGFR, epidermal growth factor receptor(s); iNOS, inducible NO synthase; ET, endothelin; C5a, anaphylatoxin; LPS, lipopolysaccharides; GRO- $\alpha$ , growth-related oncogene- $\alpha$ ; ENA-78, epithelial cell neutrophil-activating protein-78; IP-10, IFN- $\gamma$  activated protein-10; Mig, monokine induced by interferon- $\gamma$ ; ITAC, IFN-inducible T cell- $\alpha$  chemoattractant; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; TACE, TNF- $\alpha$  converting enzyme; TIMP, tissue inhibitor of MMPs; CTGF, collagen tissue growth factor; FGF, fibroblast growth factor;  $\alpha$ 1-AT,  $\alpha$ 1-antitrypsin; LY29311, 2-[2-propyl-3-[3-[2-ethyl-4-(4-fluorophenyl)-5-hydroxyphenoxy]propoxy]phenoxy]benzoic acid; MR889, midesteine, 2-[2-thiophenecarboxythio]-N-[dihydro-2-(3H)-thiophenone-3-yl]propionamide.

## II. Chronic Obstructive Pulmonary Disease as an Inflammatory Disease

### A. What is Chronic Obstructive Pulmonary Disease?

COPD is characterized by slowly progressive development of airflow limitation that is poorly reversible, in sharp contrast to asthma where there is variable airflow obstruction that is usually reversible spontaneously or with treatment. A new definition of COPD has recently been adopted by the Global Initiative on Obstructive Lung Disease: "a disease state characterized by airflow limitation that is not fully reversible. The airflow limi-

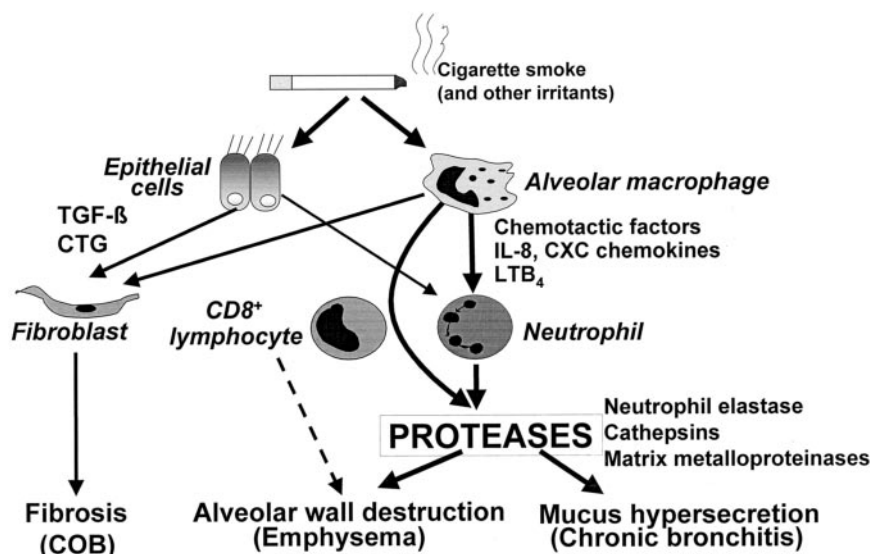


FIG. 1. Inflammatory mechanisms in COPD. Cigarette smoke (and other irritants) activate macrophages in the respiratory tract that release neutrophil chemotactic factors, including IL-8 and LTB<sub>4</sub>. These cells then release proteases that break down connective tissue in the lung parenchyma, resulting in emphysema, and also stimulate mucus hypersecretion. These enzymes are normally counteracted by protease inhibitors, including  $\alpha_1$ -antitrypsin, SLPI, and TIMP. Cytotoxic T cells (CD8<sup>+</sup>) may also be recruited and may be involved in alveolar wall destruction. Fibroblasts may be activated by growth factors released from macrophages and epithelial cells. CTG, connective tissue growth factor; COB, chronic obstructive bronchitis.

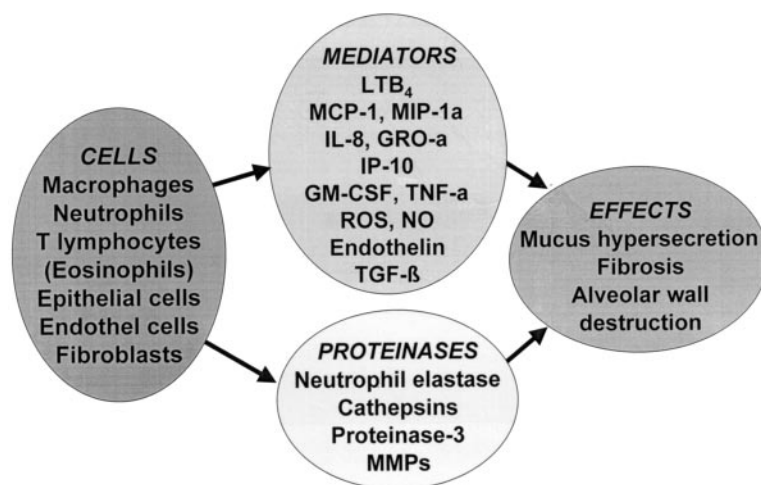


FIG. 2. Inflammation in COPD is complex, with many activated inflammatory and structural cells that release multiple mediators, including lipid mediators such as LTB<sub>4</sub>, which is chemoattractant for neutrophils; chemokines such as MCP-1 and MIP-1 $\alpha$ , which attract monocytes; IL-8 and GRO- $\alpha$ , which attract neutrophils and monocytes; IP-10, which attracts CD8<sup>+</sup> cells, ROS, and NO; GM-CSF, which prolongs neutrophils' survival; TNF- $\alpha$ , which amplifies inflammation by switching on multiple inflammatory genes and may also account for some of the systemic effects of the disease; and endothelin and TGF- $\beta$ , which induce fibrosis. In addition, multiple proteinases are released that result in elastolysis, including the serine proteinases neutrophils elastase and proteinase C, cathepsins, and MMPs. This combination of mediators that attract and activate inflammatory cells and proteinases, which cause elastolysis and mucus hypersecretion, results in the typical pathophysiology of COPD.

tation is usually progressive and associated with an abnormal inflammatory response of the lungs to noxious particles and gases" ([www.goldcopd.com/workshop/index.html](http://www.goldcopd.com/workshop/index.html)). For the first time, this definition encompasses the idea that COPD is a chronic inflammatory disease, and much of the recent research has focused on the nature of this inflammatory response.

COPD includes chronic obstructive bronchitis with fibrosis and obstruction of small airways, and emphysema with enlargement of airspaces and destruction of lung parenchyma, loss of lung elasticity, and closure of small airways (Fig. 3). The obstruction of peripheral

airways due to inflammatory cell infiltration and fibrosis, together with inflammatory exudates in the lumen, correlate best with the severity of airflow obstruction, indicating the importance of chronic inflammation in COPD (Hogg et al., 2004). Chronic bronchitis, by contrast, is defined by a productive cough of more than 3 months' duration for more than two successive years; this reflects mucus hypersecretion and is not necessarily associated with airflow limitation. Most patients with COPD have all three pathological mechanisms (chronic obstructive bronchitis, emphysema, and mucus plugging) as all are induced by smoking, but they may differ

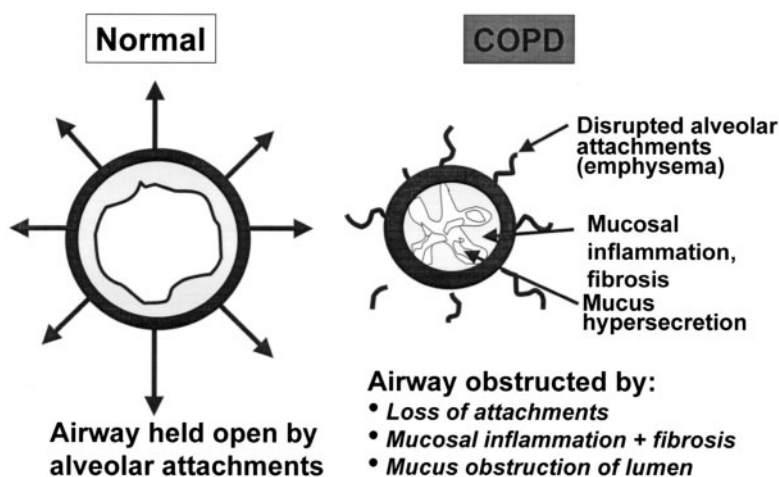


FIG. 3. Mechanisms of airflow limitation in COPD. The airway in normal subjects is distended by alveolar attachments during expiration, allowing alveolar emptying and lung deflation. In COPD these attachments are disrupted because of emphysema, thus contributing to airway closure during expiration, trapping gas in the alveoli and resulting in hyperinflation. Peripheral airways are also obstructed and distorted by airway inflammation and fibrosis (chronic obstructive bronchiolitis) and by occlusion of the airway lumen by mucus secretions, which may be trapped in the airways because of poor mucociliary clearance.

in the proportion of emphysema and obstructive bronchitis. In developed countries, cigarette smoking is by far the most common cause of COPD, but there are several other risk factors, including air pollution (particularly indoor air pollution from burning fuels), poor diet, and occupational exposure. COPD is characterized by acceleration in the normal decline of lung function seen with age (Fig. 4). The slowly progressive airflow limitation leads to disability and premature death and is quite different from the variable airway obstruction and symptoms in asthma, which rarely progresses in severity.

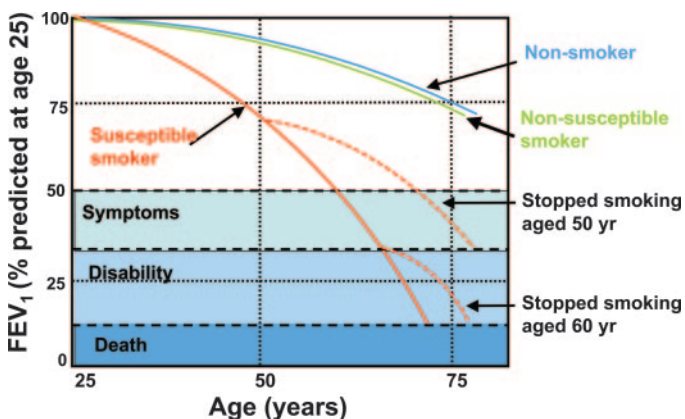


FIG. 4. Natural history of COPD. Annual decline in airway function shows accelerated decline in susceptible smokers and effects of smoking cessation. Patients with COPD usually show an accelerated annual decline in FEV<sub>1</sub>, often greater than 50 ml/year, compared with the normal decline of approximately 20 ml/year, although this is variable between patients. Only 10 to 20% of cigarette smokers are susceptible to this rapid decline. However, with longer follow-up, more smokers may develop COPD. The propensity to develop COPD among smokers is only weakly related to the amount of cigarettes smoked, and this suggests that other factors play an important role in determining susceptibility. Most evidence points toward genetic factors, although the genes determining susceptibility have not yet been determined.

*B. Differences from Asthma*

Although COPD and asthma both involve inflammation in the respiratory tract, there are marked differences in the nature of the inflammatory process, with differences in inflammatory cells, mediators, response to inflammation, anatomical distribution, and response to anti-inflammatory therapy (Barnes, 2000b; Saetta et al., 2001) (Fig. 5). Some patients appear to share the characteristics of COPD and asthma, however. Rather than this representing a graded spectrum of disease, it is more likely that these patients have both of these common diseases at the same time.

Histopathological studies show a predominant involvement of peripheral airways (bronchioles) and lung parenchyma, whereas asthma involves inflammation in all airways but without involvement of the lung parenchyma. There is obstruction of bronchioles, with fibrosis and infiltration with macrophages and T lymphocytes. There is destruction of lung parenchyma, as well as an increased number of macrophages and CD8<sup>+</sup> (cytotoxic) T lymphocytes (Saetta et al., 1998). Bronchial biopsies show similar changes with an infiltration of macrophages and CD8<sup>+</sup> cells and an increased number of neutrophils in patients with severe COPD (Di Stefano et al., 1998). Bronchoalveolar lavage (BAL) fluid and induced sputum demonstrate a marked increase in macrophages and neutrophils (Keatings et al., 1996; Pesci et al., 1998). In contrast to asthma, eosinophils are not prominent except during exacerbations or when patients have concomitant asthma (Fabbri et al., 1998, 2003).

*C. Inflammatory Cells*

COPD is a complex inflammatory disease that involves several types of inflammatory cells (Barnes et al., 2003). Although abnormal numbers of inflammatory

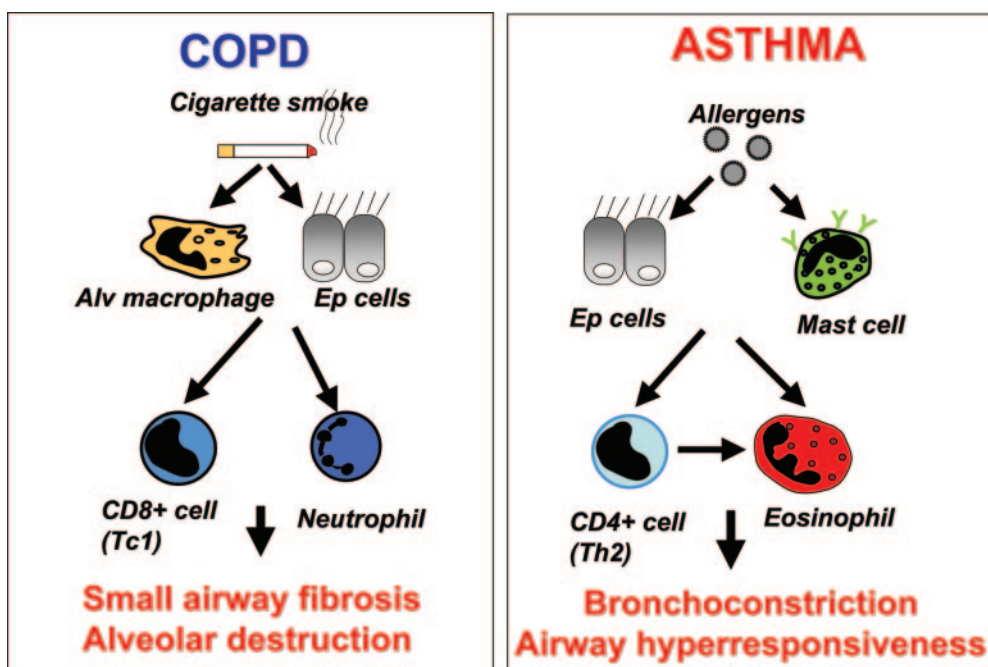


FIG. 5. COPD versus asthma. The pattern of inflammation in COPD and asthma are markedly different, and this underlies the different symptoms, clinical presentation, and response to treatment of these diseases. In COPD the predominant inflammatory cells are neutrophils, macrophages, and CD8<sup>+</sup> (Tc1) lymphocytes, whereas eosinophils, mast cells, and CD4<sup>+</sup> (T helper 2 cell) lymphocytes predominate in asthma. In COPD this inflammatory pattern leads to slowly progressive airflow limitation, whereas in asthma the inflammation results in variable bronchoconstriction and airway hyperresponsiveness. Alv, alveolar; Th2, T helper 2 cell; ep, epithelial.

cells have been documented in COPD, the relationship between these cell types and the sequence of their appearance and their persistence are largely unknown. Most studies have been cross-sectional based on selection of patients with different stages of the disease, and comparisons have been made between smokers without airflow limitation (normal smokers) and those with COPD who have smoked a similar amount. There are no serial studies, and selection biases (such as selecting tissue from patients suitable for lung volume reduction surgery) may give misleading results. Analysis of the cell profile in alveoli and small airways shows an increase in all of the cell types implicated in COPD, including macrophages, T lymphocytes, B lymphocytes, and neutrophils (Retamales et al., 2001).

**1. Neutrophils.** Increased numbers of activated neutrophils are found in sputum and BAL fluid of patients with COPD (Lacoste et al., 1993; Keatings et al., 1996), yet they are increased relatively little in the airways or lung parenchyma (Finkelstein et al., 1995). This may reflect their rapid transit through the airways and parenchyma. Neutrophils secrete serine proteases, including neutrophil elastase (NE), cathepsin G, and proteinase-3, as well as matrix metalloproteinase (MMP)-8 and MMP-9, which may contribute to alveolar destruction. These serine proteases are also potent mucus stimulants. Neutrophil recruitment to the airways and parenchyma involves adhesion to endothelial cells, and E-selectin is up-regulated on endothelial cells in the airways of COPD patients (Di Stefano et al., 1994). Ad-

herent neutrophils then migrate into the respiratory tract under the direction of neutrophil chemotactic factors, which include interleukin (IL)-8 and leukotriene B<sub>4</sub> (LTB<sub>4</sub>). Neutrophil survival in the respiratory tract may be increased by cytokines, such as granulocyte-macrophage colony stimulating factor (GM-CSF) and granulocyte colony stimulating factor.

The role of neutrophils in COPD is not yet clear. There is a correlation between the number of circulating neutrophils and fall in forced expiratory volume in 1 s (FEV<sub>1</sub>) (Sparrow et al., 1984). Neutrophil numbers in bronchial biopsies and induced sputum are correlated with COPD disease severity (Keatings et al., 1996; Di Stefano et al., 1998) and with the rate of decline in lung function (Stanescu et al., 1996). Smoking has a direct stimulatory effect on granulocyte production and release from the bone marrow, possibly mediated by GM-CSF and granulocyte colony stimulating factor released from lung macrophages (Terashima et al., 1997). Smoking may also increase neutrophil retention in the lung (MacNee et al., 1989). There is no doubt that the neutrophils recruited to the airways of COPD patients are activated, since there are increased concentrations of granule proteins, such as myeloperoxidase and human neutrophil lipocalin, in the sputum supernatant (Keatings and Barnes, 1997; Yamamoto et al., 1997; Peleman et al., 1999). These neutrophils also show an increase in the respiratory burst response, which correlates with the degree of airflow limitation (Richards et al., 1989).

Neutrophils have the capacity to induce tissue damage through the release of serine proteases and oxidants. Priming is a prerequisite for degranulation and superoxide anion generation in neutrophils (Condliffe et al., 1998). Neutrophils in the peripheral circulation show evidence of priming in COPD (Noguera et al., 2001), but this may result from, rather than contribute to, lung pathophysiology. There are several chemotactic signals that have the potential for neutrophil recruitment in COPD, including LTB<sub>4</sub>, IL-8, and related CXC chemokines, which are increased in COPD airways (Tanino et al., 2002; Traves et al., 2002). These mediators may be derived from alveolar macrophages and epithelial cells, but the neutrophil itself is a major source of IL-8 (Bazzone et al., 1991).

Neutrophils from the circulation marginate in the pulmonary circulation and adhere to endothelial cells in the alveolar wall before passing into the alveolar space (Hogg and Walker, 1995). The precise route for neutrophil migration in large airways is less certain, but it is more likely that they reach the airway from the tracheobronchial circulation and migrate across postcapillary venules (Pettersen and Adler, 2002). The cellular mechanisms underlying neutrophil adhesion and transmigration differ between systemic and pulmonary circulations, and this might confer different properties on the neutrophils arriving from the alveolar or bronchial compartments. There may be significant differences in neutrophil transit times in different areas of the lung that may account for differential distribution of emphysema, for example, the upper lobe predominance in centrilobular emphysema. Little is known about survival and apoptosis of neutrophils in COPD airways. Theoretically,

GM-CSF may prolong neutrophil survival, but it has proven difficult to culture neutrophils from sputum samples.

However, although neutrophils have the capacity to cause elastolysis, this is not a prominent feature of other pulmonary diseases where chronic airway neutrophilia is even more prominent, including cystic fibrosis and bronchiectasis. This suggests that other factors are involved in the generation of emphysema. Indeed, there is a negative association between the number of neutrophils and the amount of alveolar destruction in COPD (Finkelstein et al., 1995), and neutrophils are not a prominent feature of parenchymal inflammation in COPD. However, it is likely that airway neutrophilia is linked to mucus hypersecretion in chronic bronchitis. Serine proteases from neutrophils, including neutrophil elastase, cathepsin G, and proteinase-3, are all potent stimulants of mucus secretion from submucosal glands and goblet cells in the epithelium (Sommerhoff et al., 1990; Witko-Sarsat et al., 1999).

**2. Macrophages.** Macrophages appear to play a pivotal role in the pathophysiology of COPD and can account for most of the known features of the disease (Shapiro, 1999; Barnes, 2004) (Fig. 6).

There is a marked increase (5- to 10-fold) in the numbers of macrophages in airways, lung parenchyma, BAL fluid, and sputum in patients with COPD. A careful morphometric analysis of macrophage numbers in the parenchyma of patients with emphysema showed a 25-fold increase in the numbers of macrophages in the tissue and alveolar space compared with normal smokers (Retamales et al., 2001). Furthermore, macrophages are localized to sites of alveolar wall destruction in pa-

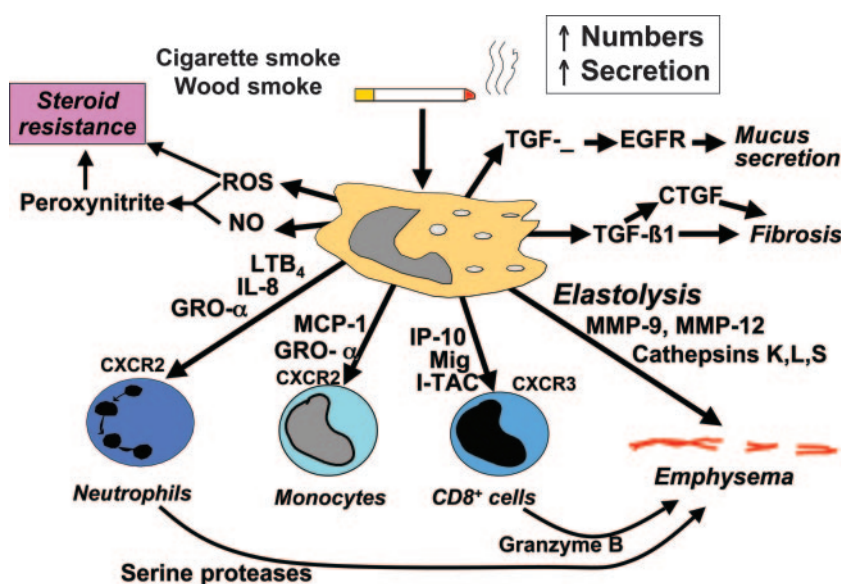


FIG. 6. Macrophages in COPD. Macrophages may play a pivotal role in COPD as they are activated by cigarette smoke extract and secrete many inflammatory proteins that may orchestrate the inflammatory process in COPD. Neutrophils may be attracted by IL-8, GRO-α, LTB<sub>4</sub>, MCP-1, and CD8<sup>+</sup> lymphocytes by IP-10, Mig, and I-TAC. Release of elastolytic enzymes including MMPs and cathepsins causes elastolysis and release of TGF-β1 and CTGF. Release of TGF-α activates EGFR, which stimulates mucus hypersecretion. Macrophages also generate ROS and NO, which together form peroxynitrite and may contribute to steroid resistance.

tients with emphysema (Finkelstein et al., 1995; Meshi et al., 2002). There is a correlation between macrophage numbers in the airways and the severity of COPD (Di Stefano et al., 1998).

Macrophages may be activated by cigarette smoke extract to release inflammatory mediators, providing a cellular mechanism that links smoking with inflammation in COPD. Alveolar macrophages also secrete elastolytic enzymes, including MMP-2, MMP-9, MMP-12, cathepsins K, L, and S, and neutrophil elastase taken up from neutrophils (Punturieri et al., 2000; Russell et al., 2002b). Alveolar macrophages from patients with COPD secrete more inflammatory proteins and have a greater elastolytic activity at baseline than those from normal smokers, and this is further increased by exposure to cigarette smoke (Lim et al., 2000b; Russell et al., 2002a, b). Macrophages demonstrate this difference even when maintained in culture for 3 days and therefore appear to be intrinsically different from the macrophages of normal smokers and nonsmoking normal control subjects (Russell et al., 2002b). The predominant elastolytic enzyme secreted by alveolar macrophages in COPD patients is MMP-9. Most of the inflammatory proteins that are up-regulated in COPD macrophages are regulated by the transcription factor nuclear factor- $\kappa$ B (NF- $\kappa$ B), which is activated in alveolar macrophages of COPD patients, particularly during exacerbations (Di Stefano et al., 2002; Caramori et al., 2003).

The increased numbers of macrophages in smokers and COPD patients may be due to increased recruitment of monocytes from the circulation in response to monocyte-selective chemokines and T lymphocytes via the release of lymphocyte chemotactic factors. The increased numbers of macrophages in COPD may also be due to increased proliferation and prolonged survival in the lungs. Macrophages have a very low proliferation rate in the lungs, but we have demonstrated that there is some increase in cell proliferation measured by proliferative cell nuclear antigen (Tomita et al., 2002). Macrophages have a long survival time, so this is difficult to measure directly. However, in macrophages from smokers, there is markedly increased expression of the antiapoptotic protein Bcl-X<sub>L</sub> and increased expression of p21<sup>CIP/WAF1</sup> in the cytoplasm (Tomita et al., 2002). This suggests that macrophages may have a prolonged survival in smokers and patients with COPD.

Corticosteroids are ineffective in suppressing inflammation, including cytokines, chemokines, and proteases, in patients with COPD (Keatings et al., 1997; Culpitt et al., 1999). In vitro the release of IL-8, TNF- $\alpha$ , and MMP-9 from macrophages from normal subjects and normal smokers are inhibited by corticosteroids, whereas corticosteroids are ineffective in macrophages from patients with COPD (Culpitt et al., 2003). Curiously, this does not apply to GM-CSF, which does not appear to have increased secretion in COPD and is suppressed by corticosteroids, albeit to a lesser extent than in macro-

phages from normal smokers. The reasons for resistance to corticosteroids in COPD and, to a lesser extent, macrophages from smokers may be the marked reduction in activity of histone deacetylase (HDAC) (Ito et al., 2001a), which is recruited to activated inflammatory genes by glucocorticoid receptors to switch off inflammatory genes (Ito et al., 2000). The reduction in HDAC activity in macrophages is correlated with increased secretion of cytokines like TNF- $\alpha$  and IL-8 and reduced response to corticosteroids. The reduction of HDAC activity on COPD patients may be mediated through oxidative stress and peroxynitrite formation.

Macrophages are phagocytic for bacteria and play an important role in host defense. The phagocytic potential of macrophages from COPD patients has not been explored, but it is possible that impaired phagocytosis may result in the increased bacterial load in the respiratory tract of patients with COPD. Macrophages recognize apoptotic cells via expression of phosphatidylserine, which interacts with specific receptors on the macrophage surface (Fadok et al., 2000). Ingestion of apoptotic granulocytes by macrophages induces the secretion of transforming growth factor (TGF)- $\beta$ 1 (Huynh et al., 2002). Neutrophil elastase cleaves the phosphatidylserine receptor and may thus impair the ability of macrophages to take up apoptotic neutrophils, resulting in increased numbers of apoptotic neutrophils in the airways (Vandivier et al., 2002).

3. *T Lymphocytes.* There is an increase in the total numbers of T lymphocytes in lung parenchyma and peripheral and central airways of patients with COPD, with the greater increase in CD8<sup>+</sup> than in CD4<sup>+</sup> cells (Finkelstein et al., 1995; O'Shaughnessy et al., 1997; Saetta et al., 1999; Majo et al., 2001; Retamales et al., 2001). There is a correlation among the number of T cells, the amount of alveolar destruction, and the severity of airflow obstruction. There is also an increase in the absolute number of CD4<sup>+</sup> T cells, but the ratio of CD4<sup>+</sup>/CD8<sup>+</sup> cells is reversed in COPD. This is mainly found in smokers with COPD rather than smokers without evidence of airflow limitation (Majo et al., 2001). It is not known whether these cells are classified as Tc1 (interferon- $\gamma$  producing) or Tc2 (IL-4 producing) subtypes (Vukmanovic-Stejić et al., 2000), but there is evidence that the majority of T cells in COPD airways are of the Tc1 subtype (Saetta et al., 2002). CD8<sup>+</sup> and CD4<sup>+</sup> T cells show increased expression of activation markers compared with T cells in the circulation, although there is no clear difference between patients with COPD and normal controls (Leckie et al., 2003).

The mechanisms by which CD8<sup>+</sup> and, to a lesser extent, CD4<sup>+</sup> cells accumulate in the airways and lungs of patients with COPD are not yet understood. However, homing of T cells to the lung must depend on some initial activation followed by adhesion and selective chemotaxis. T cells in peripheral airways of COPD patients show increased expression of CXCR3, and there is in-



creased secretion of CXCR3-activating chemokines in COPD airways (Saetta et al., 2002).

There is also an increase in the numbers of CD8<sup>+</sup> cells in the circulation in COPD patients who do not smoke (de Jong et al., 1997; Kim et al., 2002) and an increase in Th1 type [interferon (IFN)- $\gamma$  producing] CD4<sup>+</sup> cells in COPD patients (Majori et al., 1999). This indicates that there may be chronic immune stimulation via antigens presented via the human leukocyte antigen class 1 pathway. Dendritic Cells may migrate from the airways to regional lymph nodes and stimulate proliferation of CD8<sup>+</sup> and CD4<sup>+</sup> T cells. CD8<sup>+</sup> cells are typically increased in airway infections, and it is possible that the chronic colonization of the lower respiratory tract of COPD patients by bacterial and viral pathogens is responsible for this inflammatory response (Hill et al., 2000). It is also possible that protease-induced lung injury may uncover previously sequestered autoantigens or that cigarette smoke itself may damage airway epithelial cells and make them antigenic (Cosio et al., 2002).

The role of increased numbers of CD4<sup>+</sup> cells in COPD, particularly in severe disease, is also unknown (Retamales et al., 2001); it is possible that they have immunological memory and play a role in perpetuating the inflammatory process in the absence of cigarette smoking. Natural killer (NK; CD56<sup>+</sup>) cells are the first line of defense against viral infections. Circulating NK cells are reduced in patients with COPD and have reduced phagocytic activity (Prieto et al., 2001), and similar findings are found in normal smokers (Zeidel et al., 2002), although no difference in NK cells was found in lung parenchyma of COPD patients (Majo et al., 2001). There is an increase in  $\gamma/\delta$  T cells in alveoli of smokers, whether they have airway obstruction or not (Majo et al., 2001).

The role of T cells in the pathophysiology of COPD is not yet certain. CD8<sup>+</sup> cells have the capacity to cause cytolysis and apoptosis of alveolar epithelial cells through release of perforins, granzyme-B, and TNF- $\alpha$  (Hashimoto et al., 2000). There is an association between CD8<sup>+</sup> cells and apoptosis of alveolar cells in emphysema (Majo et al., 2001). In a mouse model of cigarette-induced emphysema, there is a predominance of T cells, which are directly related to the severity of emphysema (Takubo et al., 2002).

**4. Eosinophils.** The role of eosinophils in COPD is uncertain. There are some reports of increased numbers of inactive eosinophils in the airways and lavage of patients with stable COPD, whereas others have not found increased numbers in airway biopsies, BAL, or induced sputum (Turato et al., 2001). The presence of eosinophils in patients with COPD predicts a response to corticosteroids and may indicate coexisting asthma (Brightling et al., 2000; Papi et al., 2000). Increased numbers of eosinophils have been reported in bronchial biopsies and BAL fluid during acute exacerbations of chronic bronchitis

(Saetta et al., 1994, 1996). Surprisingly, the levels of eosinophil basic proteins in induced sputum are as elevated in COPD as in asthma, despite the absence of eosinophils, suggesting that they may have degranulated and are no longer recognizable by microscopy (Keatings and Barnes, 1997). Perhaps this is due to the high levels of neutrophil elastase that have been shown to cause degranulation of eosinophils (Liu et al., 1999).

**5. Dendritic Cells.** Dendritic cells play a central role in the initiation of the innate and adaptive immune response (Banchereau et al., 2000). The airways and lungs contain a rich network of dendritic cells that are localized near the surface, so they are ideally located to signal the entry of foreign substances that are inhaled (Holt and Stumbles, 2000). Dendritic cells can activate a variety of other inflammatory and immune cells, including macrophages, neutrophils, and T and B lymphocytes (Huang et al., 2001). It therefore likely that the dendritic cell may play an important role in the pulmonary response to cigarette smoke and other inhaled noxious agents and may therefore be a key cellular element in COPD. The mechanism by which tobacco smoke activates the immune system is not yet understood, but a glycoprotein isolated from tobacco has powerful immunostimulatory actions (Francus et al., 1988). There is an increase in the number of dendritic cells in rat lungs exposed to cigarette smoke (Zeid and Muller, 1995) and in the airways and alveolar walls of smokers (Casolaro et al., 1988; Soler et al., 1989). Pulmonary histiocytosis is a disease caused by dendritic cell granulomata in the lung and is characterized by destruction of the lung parenchyma, which resembles emphysema (Tazi et al., 1999, 2000). The adult form of the disease occurs almost exclusively in smokers. In mice exposed to chronic cigarette smoke, there is an increase in dendritic cells in the airways and lung parenchyma (D'Hulst et al., 2002). The role of dendritic cells in recruiting other effector cells in COPD deserves further study.

**6. Epithelial Cells.** Airway and alveolar epithelial cells may be an important source of inflammatory mediators and proteases in COPD. Epithelial cells are activated by cigarette smoke to produce inflammatory mediators, including TNF- $\alpha$ , IL-1 $\beta$ , GM-CSF, and IL-8 (Mio et al., 1997; Hellermann et al., 2002; Floreani et al., 2003). Epithelial cells in small airways may be an important source of TGF- $\beta$ , which then induces local fibrosis (Takizawa et al., 2001). Vascular-endothelial growth factor (VEGF) appears to be necessary to maintain alveolar cell survival, and blockade of VEGF receptors (VEGFR2) in rats induces apoptosis of alveolar cells and an emphysema-like pathology (Kasahara et al., 2000).

Airway epithelial cells are also important in defense of the airways. Mucus produced from goblet cells traps bacteria and inhaled particulates (Adler and Li, 2001). Epithelial cells secrete defensins and other cationic peptides with antimicrobial effects and are part of the innate defense system but are also involved in tissue re-

pair processes (Aarbiou et al., 2002). They secrete antioxidants as well as antiproteases, such as secretory leukoprotease inhibitor (SLPI). Epithelial cells also transport IgA and are therefore involved in adaptive immunity (Pilette et al., 2001). It is possible that cigarette smoke and other noxious agents impair these innate and adaptive immune responses of the airway epithelium, thereby increasing susceptibility to infection.

The airway epithelium in chronic bronchitis and COPD often shows squamous metaplasia, which may result from increased proliferation of airway epithelial cells. Proliferation in basal airway epithelial cells, measured by proliferative cell nuclear antigen, is increased in some normal smokers but is markedly increased in patients with chronic bronchitis and correlates with the degree of squamous metaplasia (Demoly et al., 1994). The nature of the growth factors involved in epithelial cell proliferation, cell cycle, and differentiation in COPD are not yet known. Epithelial growth factor receptors show increased expression in airway epithelial cells of smokers and may contribute to basal cell proliferation, resulting in squamous metaplasia and an increased risk of bronchial carcinoma (Franklin et al., 2002).

### III. Lipid Mediators

As in asthma, lipid mediators derived from arachidonic acid may play an important role in the pathophysiology of COPD.

#### A. Prostanoids

1. *Prostaglandin E<sub>2</sub>*. There is an increase in the concentration of prostaglandin (PG)E<sub>2</sub> in exhaled breath of COPD patients (Montuschi et al., 2003) (Fig. 7). This is likely to be derived from cyclooxygenase-2 (COX-2), which is expressed in alveolar macrophages (Jiang et al., 2003). There is increased COX-2 expression in alveolar macrophages from patients with COPD compared with normal control subjects (Taha et al., 2000). This is presumably a result of induction by inflammatory cytokines

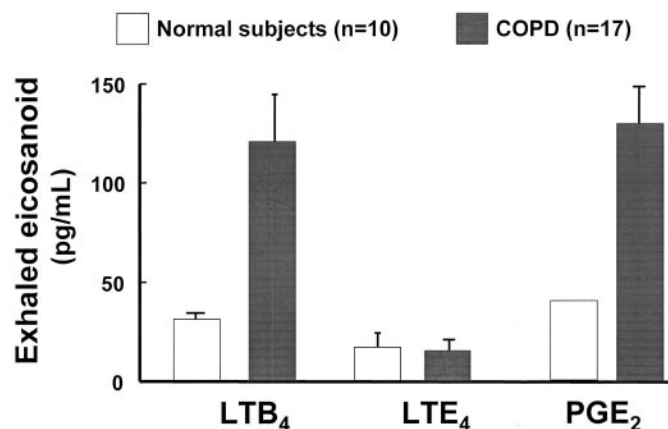


FIG. 7. Increased concentrations of LTB<sub>4</sub> and PGE<sub>2</sub> but not the cysteinyl-leukotriene LTE<sub>4</sub> in exhaled breath condensate of patients with COPD. (Adapted from Montuschi et al., 2003).

such as TNF- $\alpha$  and IL-1 $\beta$ , which activate NF- $\kappa$ B, the key regulator of COX-2 (Newton et al., 1997). Inflammatory cytokines may also activate sphingomyelinase in the cell membrane to generate ceramide, which may also up-regulate COX-2 independently of NF- $\kappa$ B (Newton et al., 2000).

PGE<sub>2</sub> is a bronchodilator of human airways (Pavord and Tattersfield, 1995) and inhibits the release of proinflammatory cytokines from monocytes (Meja et al., 1997) and acetylcholine release from airway cholinergic nerves (via prostaglandin E<sub>3</sub> receptors) (Spicuzza et al., 1998), suggesting that it may have beneficial effects in COPD airways. Furthermore, PGE<sub>2</sub> markedly enhances the anti-inflammatory actions of phosphodiesterase-4 inhibitors, which are in clinical development as anti-inflammatory therapy for COPD (Au et al., 1998). However, PGE<sub>2</sub> also has potentially detrimental effects in stimulating mucus secretion and expression of mucin genes (MUC5AC, MUCB) (Borchers et al., 1999) and in sensitizing and activating airway sensory nerves to enhance coughing (Stone et al., 1992; Lee et al., 2002). Inhalation of the nonselective COX inhibitor indomethacin is reported to reduce mucus hypersecretion in patients with COPD (Tamaoki et al., 1992), but long-term trials of COX inhibitors (and in particular COX-2 inhibitors) have not yet been undertaken.

2. *Prostaglandin F<sub>2 $\alpha$</sub>* . PGF<sub>2 $\alpha$</sub>  is also increased in exhaled breath condensate of COPD patients (Montuschi et al., 2003). PGF<sub>2 $\alpha$</sub>  is a bronchoconstrictor and also activates airway sensory nerves to produce cough (Nichol et al., 1990).

3. *Thromboxane*. Thromboxane (Tx) B<sub>2</sub> concentrations are not increased in exhaled breath of patients with COPD (Montuschi et al., 2003). However, the concentration of the major metabolite of thromboxane 11-dehydro-TxB<sub>2</sub> is increased in the urine of patients with COPD, and this is correlated with the degree of hypoxia and reversed by supplementary oxygen therapy (Davi et al., 1997). The elevated concentrations of 11-dehydro-TxB<sub>2</sub> are almost normalized by low doses of aspirin, indicating that they are likely to be derived from platelets. Thromboxane is a potent pulmonary vasoconstrictor and may contribute to the pulmonary hypertension in hypoxic COPD patients. A thromboxane receptor antagonist seratrodist reduces urinary 11-dehydro-TxB<sub>2</sub> in COPD patients and is reported to reduce symptoms of dyspnea, but it has no effect on airway function (Horiguchi et al., 2002).

#### B. Leukotrienes

1. *Leukotriene B<sub>4</sub>*. Human alveolar macrophages express cytosolic phospholipase A<sub>2</sub> and release LTB<sub>4</sub> and platelet-activating factor on activation (Shamsuddin et al., 1997). LTB<sub>4</sub> is increased in exhaled breath condensate of patients with stable COPD (Montuschi et al., 2003) (Fig. 7) and is further increased during exacerbations (Biernacki et al., 2003). LTB<sub>4</sub> is also increased in

the sputum of patients with COPD, particularly during exacerbations (Hill et al., 1999; Woolhouse et al., 2002; Beeh et al., 2003c). Plasma concentrations of LTB<sub>4</sub> are also reported to be increased in COPD patients (Seggev et al., 1991). The cellular source of LTB<sub>4</sub> in COPD is likely to be from alveolar macrophages and neutrophils.

LTB<sub>4</sub> is a potent chemoattractant of neutrophils through the activation of BLT<sub>1</sub> receptors that are expressed predominantly on neutrophils. BLT<sub>2</sub> receptors are expressed on T lymphocytes (Yokomizo et al., 2000). BLT<sub>1</sub> antagonists, such as LY29311 (2-[2-propyl-3-[3-[2-ethyl-4-(4-fluorophenyl)-5-hydroxyphenoxy]propoxy]phenoxy]benzoic acid), have now been developed for the treatment of neutrophilic inflammation (Silbaugh et al., 2000). BLT<sub>1</sub>-receptor antagonists inhibits the neutrophil chemotactic activity of sputum from COPD patients, indicating the potential clinical value of such drugs (Crooks et al., 2000; Beeh et al., 2003c), but they only give about 25% inhibition, indicating that other neutrophil chemotactic factors are also involved. LTB<sub>4</sub> antagonists have also been shown to reverse lipopolysaccharide-induced survival of neutrophils from COPD patients (Lee et al., 2000).

2. *Cysteinyl-leukotrienes.* Cysteinyl-leukotrienes are increased in asthma and largely derived from mast cells, but there is no evidence that they are increased in COPD. Thus, exhaled breath condensate shows an increase in concentration of cysteinyl-LTs in adults and children with asthma, but not in patients with COPD (Csoma et al., 2002; Montuschi and Barnes, 2002b; Montuschi et al., 2003). There is no scientific rationale for the use of cysteinyl-leukotriene receptor antagonists, such as montelukast, in the treatment of COPD. However, it has recently been reported that montelukast improves some of the symptoms of COPD, although

there is no improvement in objective lung function measurements (Rubinstein et al., 2004). This might indicate an effect on some other aspects of COPD, such as mucus secretion.

C. *Platelet-Activating Factor*

Platelet-activating factor (PAF) is a potent chemoattractant and activator of neutrophils. PAF is also produced by and activates alveolar macrophages (Shindo et al., 1998). There are no reports of PAF production in COPD patients, and there are no studies of PAF antagonists, so the role of PAF in COPD remains unknown.

IV. **Reactive Oxygen Species**

Oxidative stress is an important feature of COPD and there is increasing evidence that it is involved in its pathophysiology (Fig. 8). Oxidative stress occurs when reactive oxygen species (ROS) are produced in excess of the antioxidant defense mechanisms and result in harmful effects, including damage to lipids, proteins, and DNA. There is increasing evidence that oxidative stress is an important feature in COPD (Repine et al., 1997; Henricks and Nijkamp, 2001; MacNee, 2001).

A. *Formation*

Inflammatory and structural cells that are activated in the airways of patients with COPD produce ROS, including neutrophils, eosinophils, macrophages, and epithelial cells (MacNee, 2001). Superoxide anions (O<sub>2</sub><sup>•-</sup>) are generated by NADPH oxidase, and this is converted to hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) by superoxide dismutases. H<sub>2</sub>O<sub>2</sub> is then dismuted to water by catalase. O<sub>2</sub><sup>•-</sup> and H<sub>2</sub>O<sub>2</sub> may interact in the presence of free iron to form the highly reactive hydroxyl radical (•OH). O<sub>2</sub><sup>•-</sup>

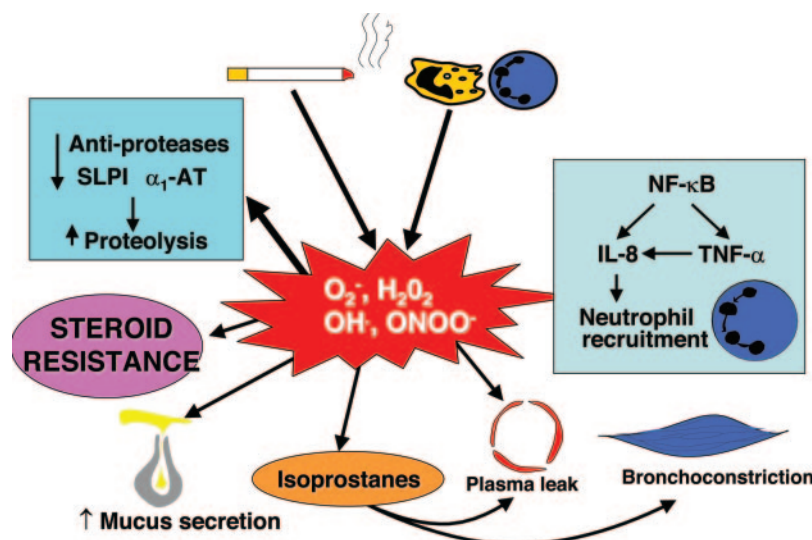


FIG. 8. Oxidative stress in COPD. Oxidative stress plays a key role in the pathophysiology of COPD and amplifies the inflammatory and destructive process. Reactive oxygen species from cigarette smoke or from inflammatory cells (particularly macrophages and neutrophils) result in several damaging effects in COPD, including decreased antiprotease defenses such as α1-AT and SLPI, activation of NF-κB, resulting in increased secretion of the cytokines IL-8 and TNF-α, increased production of isoprostanes, and direct effects on airway function. In addition, recent evidence suggests that oxidative stress induces steroid resistance.

may also combine with NO to form peroxynitrite, which also generates  $\cdot\text{OH}$  (Beckman and Koppenol, 1996). Oxidative stress leads to the oxidation of arachidonic acid and the formation of a new series of prostanoid mediators called isoprostanes, which may exert significant functional effects (Morrow, 2000), including bronchoconstriction and plasma exudation (Kawikova et al., 1996; Okazawa et al., 1997; Janssen, 2001).

Granulocyte peroxidases, such as myeloperoxidase in neutrophils, play an important role in the generation of oxidative stress. In neutrophils,  $\text{H}_2\text{O}_2$  generated from  $\text{O}_2^{\cdot-}$  is metabolized by myeloperoxidase in the presence of chloride ions to hypochlorous acid, which is a strong oxidant. Myeloperoxidase is also able to nitrate tyrosine residues, as can peroxynitrite (Eiserich et al., 1998; van der Vliet et al., 1999a; Gaut et al., 2002).

The lung is also exposed to exogenous oxidants, which presumably summate with endogenous ROS production to further enhance oxidative stress in the lungs. Cigarette smoke itself is a potent source of oxidants, and the gas phase has been estimated to contain over  $10^{15}$  free radicals (Pryor and Stone, 1993). Some forms of air pollution, including ozone, nitrogen dioxide, and diesel particulates, are also an oxidative stress and are associated with increased prevalence and increased numbers of exacerbations of COPD (Sunyer, 2001).

### B. Antioxidants

The normal production of oxidants is counteracted by several endogenous antioxidant mechanisms in the human respiratory tract (Cantin et al., 1990). Antioxidants may be enzymatic or nonenzymatic. The major enzymatic antioxidants in the airways are catalase, superoxide dismutase (SOD), glutathione peroxidase, glutathione *S*-transferase, xanthine oxidase, and thioredoxin. The nonenzymatic category of antioxidant defenses includes low molecular weight compounds such as glutathione, ascorbate, urate,  $\alpha$ -tocopherol, bilirubin, and lipoic acid. Concentrations of these antioxidants vary, depending on both subcellular and anatomic location. For example, glutathione is 100-fold more concentrated in the airway epithelial lining fluid compared with plasma (van der Vliet et al., 1999b). Oxidant stress activates the inducible enzyme heme oxygenase-1, converting heme and hemin to biliverdin with the formation of carbon monoxide (Choi and Alam, 1996). Biliverdin is converted via bilirubin reductase to bilirubin, which is a potential antioxidant. Heme oxygenase-1 is widely expressed in human airways (Lim et al., 2000a), and carbon monoxide production is increased in COPD (Montuschi et al., 2001). In the lung, intracellular antioxidants are expressed at relatively low levels and are not induced by oxidative stress, whereas the major antioxidants are extracellular (Comhair and Erzurum, 2002). Extracellular antioxidants, particularly glutathione peroxidase, are markedly up-regulated in response to cigarette smoke and oxidative stress. The

glutathione system is the major antioxidant mechanism in the airways. There is a high concentration of reduced glutathione in lung epithelial lining fluid (Cantin et al., 1990), and concentrations are higher in cigarette smokers. Extracellular glutathione peroxidase is an important antioxidant in the lungs and may be secreted by epithelial cells and macrophages, particularly in response to cigarette smoke or oxidative stress (Avissar et al., 1996). Extracellular glutathione peroxidase inactivates  $\text{H}_2\text{O}_2$  and  $\text{O}_2^{\cdot-}$  but also reactive nitrogen species (Comhair and Erzurum, 2002). Extracellular antioxidants also include the dietary antioxidants vitamin C (ascorbic acid) and vitamin E ( $\alpha$ -tocopherol), uric acid, lactoferrin, and extracellular SOD3. SOD3 is highly expressed in human lung, but its role in COPD is not yet clear (Bowler and Crapo, 2002).

### C. Evidence for Increased Oxidative Stress

There is considerable evidence for increased oxidative stress in COPD (Repine et al., 1997; MacNee, 2001). As discussed above, oxidant stress is derived from cigarette smoke and from inflammatory cells, such as activated macrophages and neutrophils. Epidemiological evidence indicates that reduced dietary intake of antioxidants may be a determinant of COPD, and population surveys have linked a low dietary intake of the antioxidant ascorbic acid with declining lung function (Britton et al., 1995; Schunemann et al., 2001).

*1. Pulmonary Oxidative Stress.* There is abundant evidence for increased oxidative stress in the lungs of patients with COPD. Using electromagnetic spin trapping, a marked increase in xanthine/xanthine oxidase activity has been detected in BAL fluid of COPD patients (Pinamonti et al., 1998). A specific marker lipid peroxidation, 4-hydroxy-2-nonenal, which forms adducts with basic amino acid residues in proteins, can be detected by immunocytochemistry and has been detected in lungs of patients with COPD (Rahman et al., 2002). This signature of oxidative stress is localized to airway and alveolar epithelial cells, endothelial cells, and neutrophils.

*2. Exhaled Markers of Oxidative Stress.* There are several markers of oxidative stress that may be detected in the breath, and several studies have demonstrated increased production of oxidants in exhaled air or breath condensates (Kharitonov and Barnes, 2001; Montuschi and Barnes, 2002a; Paredi et al., 2002). Ethane, a volatile hydrocarbon formed through lipid peroxidation, is increased in the breath of COPD patients, and the concentration correlates with disease severity (Paredi et al., 2000). There is an increased concentration of  $\text{H}_2\text{O}_2$  in exhaled breath condensate of patients with COPD, particularly during exacerbations (Dekhuijzen et al., 1996; Nowak et al., 1999). There is also an increase in the concentration of 8-iso prostaglandin  $\text{F}_{2\alpha}$  (8-isoprostane) in exhaled breath condensate, which is found even in

patients who are ex-smokers (Montuschi et al., 2000) and is increased further during acute exacerbations (Biernacki et al., 2003). 8-Isoprostane is also increased in the breath of normal smokers, but to a lesser extent than in COPD, suggesting that there is an exaggeration of oxidative stress in COPD. Malondialdehyde and thiobarbituric acid reactive substances, which are markers of lipid peroxidation, are also increased in exhaled breath condensate of patients with COPD (Nowak et al., 1999; Corradi et al., 2003).

3. *Systemic Markers of Oxidative Stress.* There is also evidence for increased systemic markers of oxidative stress in patients with COPD as measured by biochemical markers of lipid peroxidation (Rahman et al., 1996). Increased plasma concentrations of malondialdehyde have been reported in COPD patients (Calikoglu et al., 2002). 8-Isoprostane is increased in the urine of patients with COPD and further increased during exacerbations (Pratico et al., 1998). The interaction of  $O_2^{\cdot-}$  and NO forms peroxynitrite, which forms stable 3-nitrotyrosine adducts, as a footprint of oxidative stress, as discussed below.

#### D. Effects on Airway Function

ROS have several effects on the airways, which would have the effect of increasing the inflammatory and destructive response in COPD. These effects may be mediated by direct actions of ROS on target cells in the airways but may also be mediated indirectly via activation of signal transduction pathways and transcription factors and via the formation of oxidized mediators, such as isoprostanes and hydroxyl-nonenal.

1. *Effects on Transcription Factors.* ROS activate NF- $\kappa$ B, which switches on multiple inflammatory genes resulting in amplification of the inflammatory response (Barnes and Karin, 1997). The molecular pathways by which oxidative stress activates NF- $\kappa$ B have not been fully elucidated, but there are several redox-sensitive steps in the activation pathway (Janssen-Heininger et al., 2000). Many of the stimuli that activate NF- $\kappa$ B appear to do so via the formation of ROS, particularly  $H_2O_2$ . ROS activate NF- $\kappa$ B in an epithelial cell line (Adcock et al., 1994) and increase the release of proinflammatory cytokines from cultured human airway epithelial cells (Rusznak et al., 1996). Oxidative stress results in activation of histone acetyltransferase activity, which opens up the chromatin structure and is associated with increased transcription of multiple inflammatory genes (Rahman, 2003; Tomita et al., 2003).

Another transcription factor that activates inflammatory genes is activator protein-1, a heterodimer of Fos and Jun proteins. As with NF- $\kappa$ B, there are several redox-sensitive steps in the activation pathway (Xanthoudakis and Curran, 1996).

2. *Effects on Signal Transduction Pathways.* Oxidants also activate mitogen-activated protein (MAP) kinase pathways.  $H_2O_2$  is a potent activator of extracellu-

lar regulated kinases (ERK) and p38 MAP kinase pathways that regulate the expression of many inflammatory genes and survival in certain cells and spreading of macrophages (Ogura and Kitamura, 1998). Indeed, many aspects of macrophage function are regulated by oxidants through the activation of multiple kinase pathways (Forman and Torres, 2002).

3. *Effects on Target Cells.*  $H_2O_2$  directly constricts airway smooth muscle in vitro (Rhoden and Barnes, 1989), and hydroxyl radicals ( $OH^{\cdot}$ ) potentially induce plasma exudation in airways (Lei et al., 1996). 8-Isoprostane (or 8-epi-prostaglandin  $F_{2\alpha}$ ), the predominant isoprostane formed by the nonenzymatic oxidation of arachidonic acid in humans, is a potent constrictor of animal and human airways in vitro, an effect that is largely mediated via thromboxane receptors (Kawikova et al., 1996). In rat airways, oxidant stress increases cholinergic nerve-induced bronchoconstriction, an effect that may be due to oxidant damage of acetylcholinesterase (Ohrui et al., 1991). 8-Isoprostane also has direct effects on airway nerves (Spicuzza et al., 2001).

Little is known about the effects of ROS on the vasculature.  $\cdot OH$  potentially induces plasma exudation in rodent airways (Lei et al., 1996), and 8-isoprostane is a potent inducer of plasma exudation in airways (Okazawa et al., 1997), but their effects on pulmonary vessels are not known.

In rats, oxidative stress increases airway mucus secretion, an effect that is blocked by cyclo-oxygenase inhibitors (Adler et al., 1990). The effect of oxidative stress may be mediated via the activation of epidermal growth factor receptors (EGFR) on submucosal glands (Takeyama et al., 2000). Neutrophil elastase is a potent stimulant of mucus secretion and increases the expression of mucin genes (MUC5AC); its effects are inhibited by dimethylthiourea, a purported scavenger of  $\cdot OH$  (Fischer and Voynow, 2000). Oxidative stress may induce proliferation of airway epithelial cells, and this effect also appears to be mediated via activation of EGFR (Tamaoki et al., 2004).

4. *Effects on Inflammatory Response.* The increased oxidative stress in the airways of COPD patients may play an important pathophysiological role in the disease by amplifying the inflammatory response in COPD. This may reflect the activation of NF- $\kappa$ B and activator protein-1, which then induce a neutrophilic inflammation via increased expression of IL-8 and other CXC chemokines, TNF- $\alpha$  and MMP-9. Oxidative stress may therefore serve to amplify the ongoing chronic inflammatory response in COPD and may be an important mechanism leading to increased inflammation during acute exacerbations.

5. *Effect on Proteases.* Oxidative stress may also impair the function of antiproteases such as  $\alpha_1$ -antitrypsin and SLPI and thereby accelerates the breakdown of elastin in lung parenchyma (Taggart et al., 2000).

6. *Effect on Steroid Responsiveness.* Corticosteroids are much less effective in COPD than in asthma and do not reduce the progression of the disease (Pauwels et al., 1999; Vestbo et al., 1999; Burge et al., 2000; Lung Health Study Research Group, 2000). In contrast to patients with asthma, those with COPD do not show any significant anti-inflammatory response to corticosteroids (Keatings et al., 1997; Culpitt et al., 1999). Alveolar macrophages from patients with COPD show a marked reduction in responsiveness to the anti-inflammatory effects of corticosteroids, compared with cells from normal smokers and nonsmokers (Culpitt et al., 2003). Recent studies suggest that there may be a link between oxidative stress and the poor response to corticosteroids in COPD. Oxidative stress impairs binding of glucocorticoid receptors to DNA and the translocation of these receptors from the cytoplasm to the nucleus (Hutchison et al., 1991; Okamoto et al., 1999). Corticosteroids switch off inflammatory genes by recruiting HDAC2 to the active transcription site, and by deacetylating the hyperacetylated histones of the actively transcribing inflammatory gene, they are able to switch off its transcription and thus suppress inflammation (Ito et al., 2000; Barnes et al., 2003). In cigarette smokers and patients with COPD, there is a marked reduction in activity of HDAC and reduced expression of HDAC2 in alveolar macrophages (Ito et al., 2001a) and an even greater reduction in HDAC2 expression in peripheral lung tissue (Ito et al., 2001b). This reduction in HDAC activity is correlated with reduced expression of inflammatory cytokines and a reduced response to corticosteroids. This may result directly or indirectly from oxidative stress and is mimicked by the effects of H<sub>2</sub>O<sub>2</sub> in cell lines (Ito et al., 2001b).

7. *Effects on Apoptosis.* Oxidative stress may also induce apoptosis in endothelial and epithelial cells (Haddad, 2004). Apoptosis of type 1 pneumocytes may be contributory to the development of emphysema, and this might be induced by cytotoxic T lymphocytes or by inhibition of vascular-endothelial growth factor receptors (Kasahara et al., 2000; Majo et al., 2001). ROS may induce apoptosis by activating the NF- $\kappa$ B pathway, by direct DNA damage via activation of poly-ADP-ribose, and via the generation of 4-hydroxy-nonenal. Apoptosis signal-regulating kinase-1 is held in an inactive conformation by thioredoxin, and when oxidized by ROS, this triggers apoptotic pathways (Gotoh and Cooper, 1998).

8. *Systemic Effects.* The systemic oxidative stress in COPD may contribute to the systemic effects seen in severe disease. For example, impaired redox balance in skeletal muscle cells may be contributory to the muscle weakness, fatigability, and wasting seen in some patients (Langen et al., 2003).

#### E. Effects of Antioxidants

In view of the persuasive evidence presented above that oxidative stress is important in the pathophysiology

of COPD, antioxidants are a logical approach to therapy (MacNee, 2000; Barnes, 2001c).

Several antioxidants have also been administered to patients with COPD to explore their effects on lung function. *N*-Acetyl cysteine was developed as a mucolytic agent but also acts as an antioxidant by increasing the formation of glutathione. Although small-scale trials failed to demonstrate any clear clinical benefit, more recent meta-analyses have shown a small but significant clinical benefit in COPD, particularly in reducing exacerbations (Grandjean et al., 2000; Poole and Black, 2001). This benefit is not shared by other mucolytics and is therefore likely to be due to the antioxidant effect of *N*-acetyl cysteine. These results should encourage the development of more effective antioxidants in the future. Currently available antioxidants are rather weak, but more potent drugs, including spin-trap antioxidants (nitrones) and stable glutathione analogs, are currently in clinical development (Cuzzocrea et al., 2001).

## V. Nitric Oxide

### A. Formation

1. *Nitric Oxide.* NO is generated in COPD from the enzyme inducible NO synthase (iNOS), which is expressed in macrophages and lung parenchyma of patients with COPD, particularly in patients with severe disease (Ichinose et al., 2000; Maestrelli et al., 2003). NO is markedly increased in exhaled breath of patients with mild asthma, reflecting the inflammatory process in the airways, but in patients with COPD exhaled NO levels are little raised above normal (Maziak et al., 1998; Corradi et al., 1999; Rutgers et al., 1999) but are more clearly increased during exacerbations (Maziak et al., 1998; Agusti et al., 1999).

2. *Peroxynitrite.* The reason why exhaled NO may be elevated in COPD as much as in asthma may be because exhaled NO levels are depressed by cigarette smoking and oxidative stress, since NO combines avidly with superoxide anions to form peroxynitrite. This is supported by the fact that nitrate concentrations, formed by metabolism of peroxynitrite, are increased in breath condensate and sputum of cigarette smokers and patients with COPD (Corradi et al., 2001; Kanazawa et al., 2003c). Generation of superoxide anions from neutrophils also decreases the amount of NO formed by an epithelial cell line in vitro, as NO is consumed to form peroxynitrite (Jones et al., 1998). There is also a reduction in the undefined "peroxynitrite inhibitory activity" in sputum of COPD patients (Kanazawa et al., 2003c). Peroxynitrite reacts with tyrosine residues in certain proteins to form 3-nitrotyrosine, which may be detected immunologically. There is increased 3-nitrotyrosine immunoreactivity in sputum macrophages from patients with COPD (Ichinose et al., 2000). There is also an increase in the tyrosine nitration of proteins in sputum of COPD patients compared with normal controls, and

this is correlated with disease severity (Sugiura et al., 2004).

Oxidative stress and peroxynitrite may also reduce HDAC2 levels, thereby inducing resistance to the anti-inflammatory actions of corticosteroids (Barnes et al., 2004). This may be the result of nitration of critical tyrosine residues in the structure of HDAC2, which impairs its enzymatic activity (Ito et al., 2004). Peroxynitrite appears to induce steroid resistance in vitro through this molecular mechanism. Nitration of other regulatory proteins may also be an important mechanism of disease in COPD that has not yet been explored. Peroxynitrite infusion causes a neutrophilic inflammatory response in rabbit lungs (Farshid et al., 2002). It may also induce cell damage. Peroxynitrite activates MAP kinase pathways in airway epithelial cells and may induce apoptosis of epithelial cells, particularly through the activation of the ERK pathway (Nabeyrat et al., 2003). Peroxynitrite is a potent pulmonary vasoconstrictor in an isolated perfused rat lung preparation, but it does not affect hypoxic vasoconstrictor responses (Nosaman et al., 2004).

### B. Inhibition

Inhibitors of iNOS may inhibit formation of peroxynitrite and may be of value in therapy in view of the potential detrimental role of peroxynitrite. Selective, potent, and long-lasting inhibitors of iNOS are now in clinical development (Hansel et al., 2003). The selenium-containing antioxidant ebselen is reported to be effective as an efficient scavenger of peroxynitrite (Sies and Masumoto, 1997) but does not appear to be in clinical development.

## VI. Peptide Mediators

### A. Endothelins

**1. Formation.** There is an increased concentration of endothelin-1 (ET-1) in induced sputum and bronchoalveolar lavage fluid of patients with COPD (Chalmers et al., 1999; Bacakoglu et al., 2003), particularly during exacerbations (Roland et al., 2001). Plasma ET-1 concentrations are also elevated in COPD patients, particularly in patients who develop nocturnal hypoxemia during the night (Trakada et al., 2001; Spiropoulos et al., 2003) and in patients with hypoxemia (Faller et al., 1998), but it is not correlated with secondary pulmonary hypertension (Bacakoglu et al., 2003). This may reflect the release of ET-1 by hypoxemia, suggesting that the release of ET-1 may contribute to pulmonary vasoconstriction and pulmonary hypertension in COPD patients.

**2. Effects.** ET-1 is a potent vasoconstrictor and also causes vascular smooth muscle hyperplasia. There is increased expression of ET-1 in pulmonary endothelial cells of patients with COPD who have secondary pulmonary hypertension (Giaid et al., 1993), suggesting that

ET-1 may contribute to the vascular remodeling associated with hypoxic pulmonary hypertension.

**3. Therapeutic Potential.** There is increasing evidence that endothelin antagonists (ET<sub>A</sub>-receptor antagonists) reduce pulmonary hypertension in pulmonary hypertension (Kenyon and Nappi, 2003). Several endothelin antagonists are in clinical development, and the nonselective ET-receptor antagonist bosentan is now used in the therapy of primary pulmonary hypertension. It is not yet certain whether it is clinically useful in the secondary pulmonary hypertension that occurs in severe COPD.

### B. Bradykinin

Although the roles of bradykinin and related kinins in asthma have been extensively explored (Barnes, 1992), there is very little information about kinins in COPD. It is likely that kinins are generated in the airways of COPD patients as plasma exudation occurs. Furthermore, proinflammatory cytokines that are found in COPD airways increase the expression of bradykinin B<sub>1</sub>- and B<sub>2</sub>-receptors in pulmonary cells (Tsukagoshi et al., 1995; Trevisani et al., 1999; Haddad et al., 2000). Bradykinin is a potent bronchoconstrictor of human airways, particularly small airways (Hulsmann et al., 1994), stimulates mucus secretion (Nagaki et al., 1996), and potently potentiates cough by an effect on unmyelinated sensory nerve endings in the airways (Fox et al., 1996).

### C. Tachykinins

**1. Formation.** Increased substance P concentrations have been reported in induced sputum of patients with chronic bronchitis (Tomaki et al., 1995); this is presumably derived from sensory nerve endings, although non-neuronal sources of tachykinins are now recognized. For example, rat alveolar macrophages express preprotachykinin-1 and synthesis substance P in response to endotoxin stimulation (Killingsworth et al., 1997), and human sputum macrophages express substance P in response to endotoxin (Germonpre et al., 1999). Tachykinin receptors are expressed in the human respiratory tract with NK<sub>1</sub>- and NK<sub>2</sub>-receptors localized to submucosal glands, blood vessels, and airway smooth muscle, whereas NK<sub>2</sub>-receptors are also expressed on inflammatory cells, including macrophages and T lymphocytes (Mapp et al., 2000). There is no difference in the distribution of receptors between normal subjects and smokers with or without airway obstruction.

Tachykinins are metabolized by neutral endopeptidase 24.11, which is strongly expressed in airway epithelial cells (Nadel, 1990). Although a reduction in neutral endopeptidase activity and expression has been implicated in worsening of asthma, there have been no studies of the role of this enzyme in COPD.

**2. Effects.** Tachykinins are potent stimulants of submucosal gland and goblet cell secretion. The effects of

cigarette smoke on mucus secretion is also blocked by tachykinin antagonists in experimental animals, indicating that tachykinin release from sensory nerves mediates these effects (Tokuyama et al., 1990). Substance P stimulates secretion from human airways in vitro, and this effect is mediated via NK<sub>1</sub>-receptors (Rogers et al., 1989). In porcine airways, tachykinins elicit submucosal gland secretion via NK<sub>1</sub>-receptors in gland cells, but also via NK<sub>3</sub>-receptors on cholinergic nerve terminal (Phillips et al., 2003). Neurokinin-A activates alveolar macrophages of smokers via NK<sub>2</sub>-receptors (Brunelleschi et al., 1996), suggesting that tachykinins have the potential to enhance inflammation in COPD airways.

3. *Therapeutic Potential.* Tachykinin antagonists have therapeutic potential in COPD, particularly for reducing neurogenic mucus secretion stimulated by cigarette smoke exposure (Joos and Pauwels, 2001; Barnes, 2002a). There are no reported studies of tachykinin receptor antagonists in COPD patients.

#### D. Complement Fragments

Activation of complement generates several complement fragments that have potent chemotactic activity. C5a (anaphylatoxin) is derived from cleavage of complement protein C5 when the classical complement cascade is activated. Once formed, C5a can bind immediately to neutrophils in the circulation and acts as a potent chemoattractant (Ward, 2004). C5 is also produced by tissue macrophages and type II pneumocytes in the lung as a component of the alternative complement cascade (Strunk et al., 1988). The two pathways promote an inflammatory gradient enhancing subsequent migration. C5a enhances adhesion molecule expression (especially intercellular adhesion molecule-1) in airway epithelial cells, and this effect was exaggerated in the presence of cigarette smoke (Floreani et al., 2003). Sputum concentrations of C5a, but not C3a or C4a, are elevated in COPD patients (Marc et al., 2004), suggesting that this may contribute to the neutrophil chemotactic activity of sputum in COPD patients.

## VII. Chemokines

Over 50 different chemokines are now recognized, and they activate up to 20 different surface receptors (Rossi and Zlotnik, 2000). Chemokine receptors belong to the 7 transmembrane receptor superfamily of G-protein-coupled receptors, and this makes it possible to discover small molecule inhibitors, which has not been possible for classical cytokine receptors (Proudfoot, 2002). Some chemokines appear to be selective for single chemokine receptors, whereas others are promiscuous and mediate the effects of several related chemokines. Four different families of chemokines are now differentiated, based on differences in the position of critical cysteine residues; CC, CXC, Cm, and CX<sub>3</sub>C chemokines are recognized. Each chemokine molecule binds to a single or several

receptors expressed on target inflammatory cells, resulting in the activation of signal transduction pathways that then result in chemotaxis or other cellular activities that include proliferation, differentiation, and survival. Chemokines appear to act in sequence in determining the final inflammatory response, so inhibitors may be more or less effective depending on the kinetics of the response (Gutierrez-Ramos et al., 2000).

Chemokines play a critical role in orchestrating inflammatory and immune responses by regulating the trafficking of inflammatory and immune cells to target organs (Olson and Ley, 2002). Several chemokines are involved in the recruitment of inflammatory cells in COPD (Lukacs, 2001). There is considerable interest in identifying chemokines in COPD as small molecule chemokine receptor inhibitors are now in development for COPD (Barnes, 2002b; Panina-Bordignon and D'Ambrosio, 2003).

#### A. Interleukin-8

1. *Formation.* The CXC chemokine IL-8 (CXCL8) is a potent chemoattractant of neutrophils, and it is not surprising that it has been implicated in COPD.

IL-8 levels are markedly increased in induced sputum of patients with COPD and correlate with the increased proportion of neutrophils (Keatings et al., 1996; Yamamoto et al., 1997). The concentrations of IL-8 are even more elevated in patients with emphysema due to  $\alpha$ 1-antitrypsin deficiency (Woolhouse et al., 2002). The concentrations of IL-8 in induced sputum are further increased during acute exacerbations, which presumably contributes to the increased numbers of neutrophils and the increased purulence of the sputum (Crooks et al., 2000; Aaron et al., 2001; Gompertz et al., 2001). There is a correlation between IL-8 concentrations and the bacterial colony count in sputum, indicating that bacterial infection may induce neutrophilic inflammation, at least in part, via induction of IL-8 release in the airways (Hill et al., 2000; Patel et al., 2002). IL-8 is also increased in BAL fluid of patients with COPD and correlates with numbers of neutrophils (Nocker et al., 1996; Soler et al., 1999). The concentrations of IL-8 are significantly higher in smokers with emphysema than in matched smokers without airflow limitation, whereas the concentrations of other CXC chemokines in BAL do not appear to discriminate between these groups (Tanino et al., 2002). IL-8 concentrations are also increased in hospitalized COPD patients and are correlated with skeletal muscle weakness (Spruit et al., 2003).

IL-8 is not stored and is synthesized in several cell types, predominantly epithelial cells, macrophages, and neutrophils, on cell stimulation with various agents (Mukaida, 2003). The cellular source of IL-8 in COPD is not completely certain. Airway epithelial cells secrete IL-8 in response to several stimuli, including TNF- $\alpha$ , IL-1 $\beta$ , bacterial products, lipopolysaccharides (LPS), certain viruses, oxidative stress, and cigarette smoke



extract (Nakamura et al., 1991a,b; DeForge et al., 1993; Kwon et al., 1994; Johnston et al., 1998; Schulz et al., 2004). Interestingly, cultured airway epithelial cells and alveolar macrophages from COPD patients produce more IL-8 than cells from normal smokers, indicating an amplified response (Culpitt et al., 2003; Schulz et al., 2004). IL-8 protein and mRNA are increased in bronchiolar epithelial cells of patients with COPD (de Boer et al., 2000), and there is increased basal release of IL-8 from airway epithelial cells of patients with COPD (Profita et al., 2003; Schulz et al., 2003). Alveolar macrophages also secrete IL-8 in response to the same stimuli, and cells derived from patients with COPD secrete more IL-8 than those from normal smokers, who in turn secrete more macrophages than do normal nonsmokers (Culpitt et al., 2003). Neutrophils themselves also release IL-8 and attract more neutrophils; therefore, a self-perpetuating inflammatory state may be established (Bazzoni et al., 1991). The secretion of IL-8 is regulated transcriptionally by several transcription factors, among which NF- $\kappa$ B is predominant (Fig. 9). MMP-9 appears to increase the activity of IL-8 by up to 10-fold by truncating the amino terminal (Van Den Steen et al., 2000). Release of IL-8 is regulated mainly by increased transcription in response to the transcription factor NF- $\kappa$ B (Carter et al., 1998; DiMango et al., 1998) and is inhibited through inhibition of the NF- $\kappa$ B activating kinase IKK2 (inhibitor of NF- $\kappa$ B kinase-2) (Jazrawi et al., 2003). The activation of NF- $\kappa$ B results in histone hyperacetylation, which results in local unwinding of DNA and increased transcription of the IL-8 gene (Tomita et al., 2003). IL-8 secretion also appears to be regulated through p38 MAP kinase and ERK pathways (Wang et al., 2003).

**2. Effects.** Neutralization of IL-8 with a blocking antibody significantly reduces the neutrophil chemotactic activity of sputum from patients with COPD (Crooks et

al., 2000; Beeh et al., 2003c). The reduction in neutrophil chemotactic activity is only of the order of approximately 30%, however, indicating that other neutrophil chemotactic factors are also involved and that blocking IL-8 alone may be insufficient as a therapeutic strategy to reduce neutrophil inflammation in the respiratory tract.

IL-8 acts via two receptors: CXCR1, which is a low-affinity receptor that is specific for IL-8, and CXCR2, which has high affinity and is shared by other CXC chemokines (Fig. 10). It is likely that CXCR2 mediates the chemotactic response of neutrophils and monocytes to IL-8, whereas CXCR1 may mediate the effects of IL-8 on release of mediators and proteases. There is a marked up-regulation of CXCR2 in airway epithelial cells during acute exacerbations of COPD, and this is correlated with the increased numbers of neutrophils in the airway (Qiu et al., 2003).

Binding of IL-8 to CXCR1 and -2 activates protein kinase B (Akt) and GTPases, which lead to enhanced neutrophil adherence to endothelial cells (by increasing expression of  $\beta_2$ -integrins) and directed cell migration. Protein kinase B activates phosphoinositide 3 kinase, which induces F-actin polymerization, resulting in pseudopod formation and chemotaxis (Chodniewicz and Zhelev, 2003). There is also activation of Ras and MAP kinases in neutrophils, causing degranulation. These effects can be down-regulated by intracellular regulator of G-protein signaling proteins, which decrease the half-life of the active GTP-bound state of CXCR, leading to reduced IL-8-induced neutrophil migration and adherence (Bowman et al., 1998).

**3. Therapeutic Potential.** As discussed above, anti-IL-8 antibodies reduce the neutrophil chemotactic activity of COPD sputum (Crooks et al., 2000; Beeh et al., 2003b), but this is a partial effect, making it unlikely that blocking IL-8 alone would have a major clinical impact in COPD. This is because other CXC chemokines and other neutrophil chemotactic mediators such as LTB<sub>4</sub> and C5a are also involved. A monoclonal antibody to IL-8 has been developed (Yang et al., 1999) and has been tested in COPD without reported success. The chemotactic response of neutrophils and monocytes to IL-8 is mediated via CXCR2, and as other CXC chemokines implicated in COPD (see section VII.D.) also act through this receptor, antagonism of CXCR2 may be a more effective strategy. Several small molecule inhibitors of CXCR2 are now in clinical development for the treatment of COPD (White et al., 1998; Hay and Sarau, 2001). In CXCR2 knockout mice, there is a marked reduction in mucus secretion in response to viral infection, implicating this receptor in mucus hypersecretion (Miller et al., 2003).

**B. Growth-Related Oncogene- $\alpha$**

Growth-related oncogene- $\alpha$  (GRO- $\alpha$ ; CXCL1) is another CXC chemokine that is involved in COPD. GRO- $\alpha$

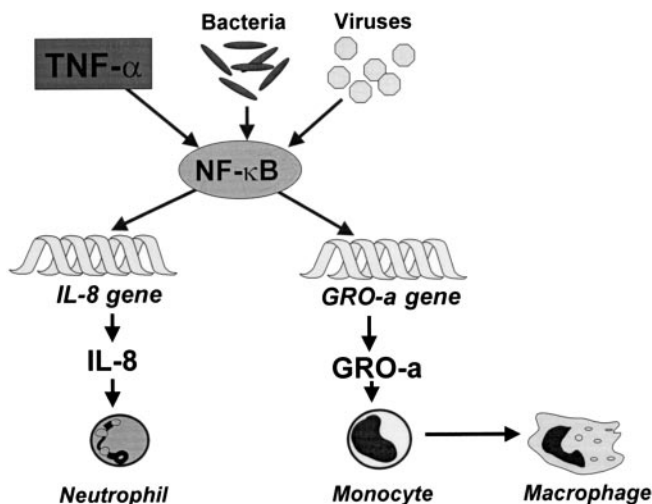


FIG. 9. NF- $\kappa$ B plays a pivotal role in the regulation of chemokine genes such as IL-8 and GRO- $\alpha$ . NF- $\kappa$ B may be activated by cigarette smoke and during exacerbations by bacterial and viral infections.

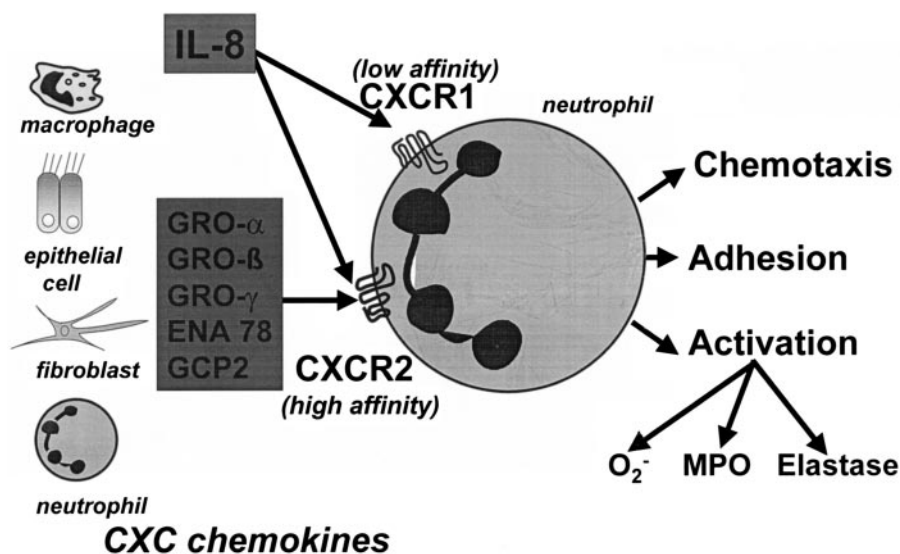


FIG. 10. CXC chemokine receptors on neutrophils. IL-8 binds with low affinity to CXCR1, resulting in adhesion and activation and to CXCR2 with high affinity resulting in chemotaxis. CXCR2 is also activated by other CXC chemokines, including GRO- $\alpha$ , - $\beta$ , and - $\gamma$ , ENA-78, and granulocyte chemotactic protein (GCP)-2.

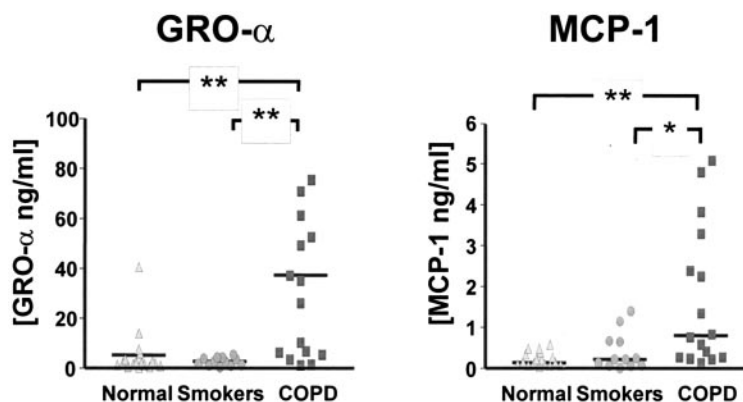


FIG. 11. Elevated concentrations of GRO- $\alpha$  and MCP-1 in induced sputum of patients with COPD (Traves et al., 2002).

is secreted by alveolar macrophages and airway epithelial cells in response to stimulation with TNF- $\alpha$  and IL-17 (Jones and Chan, 2002; Prause et al., 2003; Schulz et al., 2004). Epithelial cells from COPD patients produce greater amounts of GRO- $\alpha$  than cells from normal smokers (Schulz et al., 2004). GRO- $\alpha$  activates neutrophils, monocytes, basophils, and T lymphocytes via CXCR2 (Geiser et al., 1993) (Fig. 9). The concentrations of GRO- $\alpha$  were markedly elevated in induced sputum and BAL of patients with COPD compared with normal smokers and nonsmokers (Traves et al., 2002) (Fig. 11). GRO- $\alpha$  is released in greater amounts from BAL cells from smokers compared with nonsmokers (Morrison et al., 1998). GRO- $\alpha$  selectively activates CXCR2 and is chemotactic for neutrophils and monocytes. There is an increase in the monocyte chemotactic response to GRO- $\alpha$  in COPD patients, and this may be related to increased turnover of CXCR2 in monocytes from COPD patients (Traves et al., 2004). It is possible that the increased chemotactic response of monocytes to GRO- $\alpha$  is one of the mechanisms leading to increased numbers of alveo-

lar macrophages in the lungs of patients with COPD (Retamales et al., 2001) and could be one of the molecular mechanisms of susceptibility to cigarette smoking.

### C. Epithelial Cell-Derived Neutrophil-Activating Peptide-78

Epithelial cell-derived neutrophil-activating peptide-78 (ENA-78; CXCL5) is derived predominantly from epithelial cells and also activates CXCR2 (Imaizumi et al., 1997), although monocytes do not appear to show an increased chemotactic response to this chemokine as they do to GRO- $\alpha$  (Traves et al., 2004). ENA-78 is increased in BAL fluid of COPD patients compared with normal subjects, but there is no difference between patients with emphysema and normal smokers (Tanino et al., 2002). BAL cells from smokers release more ENA-78 than cells from nonsmokers (Morrison et al., 1998). A marked increase in expression of ENA-78 has been reported in epithelial cells during exacerbations of COPD (Qiu et al., 2003).

D. CXC3 Chemokines

The mechanisms by which CD8<sup>+</sup> and, to a lesser extent, CD4<sup>+</sup> cells accumulate in the airways and lungs of patients with COPD are not yet understood. However, homing of T cells to the lung depends on initial activation followed by adhesion and selective chemotaxis. T cells in peripheral airways of COPD patients show increased expression of CXCR3, a receptor activated by interferon- $\gamma$  inducible protein of 10 kDa (IP-10; CXCL10), monokine induced by interferon- $\gamma$  (Mig; CXCL9), and interferon-inducible T cell- $\alpha$  chemoattractant (I-TAC; CXCL11). All three chemokines activate CXCR3, although I-TAC has the highest affinity (Clark-Lewis et al., 2003). CXCR3 is expressed on T lymphocytes, particularly of the CD8<sup>+</sup> subtype. There is increased expression of IP-10 by bronchiolar epithelial cells and airway smooth muscle cells, and this could therefore contribute to the accumulation of CD8<sup>+</sup> cells, which preferentially express CXCR3 (Saetta et al., 2002; Panzner et al., 2003; Hardaker et al., 2004). It is of interest that interferon- $\gamma$  stimulates dendritic cells to produce IP-10 and Mig, which then enhance their ability to attract CD8<sup>+</sup> cells (Padovan et al., 2002). Alveolar macrophages also have the capacity to produce IP-10 and Mig and thus attract CD8<sup>+</sup> T cells (Agostini et al., 2000). Since CD8<sup>+</sup> Tc1 cells produce interferon- $\gamma$ , this provides a potential feed-forward amplification loop. The role of CD8<sup>+</sup> T cells in COPD is not yet certain, but because they have the capacity to produce perforins and granzyme B, they might induce apoptosis in alveolar epithelial and endothelial cells, thereby contributing to emphysema (Majo et al., 2001; Cosio et al., 2002) (Fig. 12).

E. Monocyte Chemoattractant Protein-1

1. Production in Chronic Obstructive Pulmonary Disease. Monocyte chemoattractant protein-1 (MCP-1; CCL2) is a CC-chemokine that activates CCR2 on monocytes and T lymphocytes (Rose et al., 2003). CCR2 may play a

role in COPD, as MCP-1 levels are increased in sputum, bronchoalveolar lavage, and lungs of patients with COPD (Fig. 11), and MCP-1 is expressed in alveolar macrophages, T lymphocytes, and epithelial cells (Capelli et al., 1999; de Boer et al., 2000; Traves et al., 2002). MCP-1 is also secreted basally by type II pneumocytes in culture, and release is stimulated by LPS but inhibited by cigarette smoke extract (Witherden et al., 2004). CCR2 are down-regulated via Toll-like receptors 2 and 4 and other inflammatory receptors, which may provide a mechanism for terminating the chemotactic response in the lungs, resulting in an accumulation of monocytes (Parker et al., 2004).

2. Effects in Chronic Obstructive Pulmonary Disease. MCP-1 is a potent chemoattractant of monocytes and may therefore be involved in the recruitment of macrophages in COPD. Indeed, the chemoattractant effect of induced sputum from patients with COPD is almost completely abrogated by an antibody to CCR2. Since macrophages appear to play a critical role in COPD as a source of elastases and neutrophil chemoattractants, blocking CCR2 may be a useful therapeutic strategy in COPD. Several small molecule inhibitors and blocking antibodies are in development (initially for the treatment of rheumatoid arthritis) (Mirzadegan et al., 2000).

F. Macrophage Inflammatory Protein

Macrophage inflammatory protein (MIP)-1 $\alpha$  is released by macrophages and has chemotactic activity for monocytes and neutrophils via CCR1. However, concentrations are not apparently increased in BAL fluid of smokers with or without chronic bronchitis, whereas MIP-1 $\beta$  concentrations are increased in patients with chronic bronchitis but not in asymptomatic smokers (Capelli et al., 1999).

G. Eosinophil-Selective Chemokines

As discussed above, eosinophils are increased in COPD airways and lungs, although they are not the predominant inflammatory cells as they are in asthma. Several chemokines have chemoattractant effects on eosinophils; this is mediated via CCR3, which is expressed predominantly on eosinophils. In COPD there is a small increase in eosinophils and eosinophil basic proteins in induced sputum and bronchoalveolar lavage fluid, and an increase in eosinophils has been described in exacerbations of chronic bronchitis (Saetta et al., 1994; Keatings et al., 1996; Pesci et al., 1998). This suggests that eosinophil chemoattractants may play some role, particularly during exacerbations. RANTES (released by activated normal T cells expressed and secreted; CCL5) activates CCR3 and is strongly expressed in airway epithelial cells of patients with chronic bronchitis exacerbations (Zhu et al., 2001). Eotaxin (CCL11) and CCR3 show increased expression in the bronchi of patients with exacerbations of chronic bronchitis and are corre-

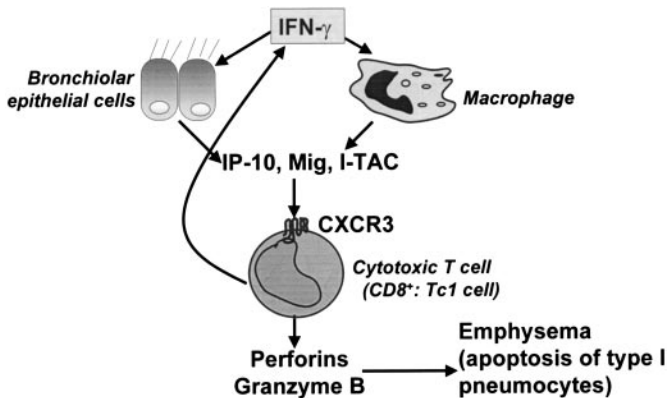


FIG. 12. Chemotaxis of cytotoxic (CD8<sup>+</sup>) T lymphocytes via activation of CXCR3 by the CXC chemokines IP-10, Mig, and I-TAC. CD8<sup>+</sup> cells may release perforins and granzyme B, which may induce apoptosis in alveolar cells and release IFN- $\gamma$ , which in turn activates the release of these chemokines.

lated with increased numbers of eosinophils (Bocchino et al., 2002).

#### H. Lymphocyte-Selective Chemokines

CCR4, CCR8, and CXCR4 are selectively expressed on Th2 cells and are activated by the chemokines macrophage-derived chemokine (CCL22), thymus- and activation-dependent chemokine (TARC, CCL17), and stromal cell-derived factor 1 $\alpha$  (CXCL12), respectively (Lloyd et al., 2000). However, Th2 cells are not prominent in COPD, so it is unlikely that these receptors are relevant.

#### I. Dendritic Cell-Selective Chemokines

CCR7 plays a role in the migration of dendritic cells to regional lymph nodes, and therefore blocking this receptor might suppress antigen presentation (Sallusto and Lanzavecchia, 2000). There is an increase in the number of dendritic cells in rat lungs exposed to cigarette smoke (Zeid and Muller, 1995; D'Hulst et al., 2002) and in the airways and alveolar walls of smokers (Casolaro et al., 1988; Soler et al., 1989), but the chemotactic factors involved have not yet been determined. MIP-3 $\alpha$  (CCL20) acts on CCR6, which is expressed by immature dendritic cells, is a potent chemoattractant of dendritic cells and is expressed by airway epithelial cells in response to IFN- $\gamma$  (Reibman et al., 2003).

#### J. CX<sub>3</sub>C Chemokines

The unique CX<sub>3</sub>C chemokine fractalkine, which is tethered to cell surfaces, shows increased expression in human airway epithelial cells after stimulation with IFN- $\gamma$  and may be involved in recruitment and adhesion of monocytes, T lymphocytes, and natural killer cells to epithelial surfaces (Fujimoto et al., 2001). Whether fractalkine or its receptor CX<sub>3</sub>CR1 is increased in COPD is not yet known.

### VIII. Cytokines

Since chronic inflammation is a prominent feature of COPD, it is not surprising that cytokines play a key role in its pathophysiology. Several cytokines have been implicated in COPD (Chung, 2001).

#### A. Tumor Necrosis Factor- $\alpha$

1. *Increased Production.* TNF- $\alpha$  is present in high concentration in the sputum of COPD patients (Keatings et al., 1996), particularly during exacerbations (Aaron et al., 2001). There is also an increase in soluble TNF receptors in sputum (Vernooy et al., 2002). Measurement of TNF- $\alpha$  in sputum is difficult, since dithiothreitol interferes with the antibody-based assay, and it is only reliably detected when sputum supernatant is prepared by ultracentrifugation.

There has been considerable interest in polymorphisms of the TNF- $\alpha$  gene in the pathogenesis of COPD, especially the A/G polymorphism at -308 (TNF2). This

polymorphism has been associated with enhanced TNF transcription and therefore production of greater concentrations of TNF- $\alpha$  than of controls following activation (Kroeger et al., 2000). This TNF2 polymorphism has been associated with increased susceptibility of COPD in some studies (Huang et al., 1997; Kucukaycan et al., 2002; Sakao et al., 2002), but not in others (Higham et al., 2000; Ishii et al., 2000b; Ferrarotti et al., 2003). This may be related to the type of COPD studied and has been associated with more severe disease (Keatings et al., 2000) and with extent of emphysema on high-resolution computed tomography scan (Sakao et al., 2002). These discrepancies may reflect ethnic variations in the pathogenesis of COPD or may suggest that a combination of predisposing factors is required to develop the disease.

TNF- $\alpha$  is normally synthesized as a 26-kDa precursor (pro-TNF- $\alpha$ ) that is stored in a membrane-bound form. On stimulation with an appropriate stimulus (such as lipopolysaccharide), the precursor is converted to pro-TNF- $\alpha$ , a 17-kDa, biologically active form, ready for release. Pro-TNF- $\alpha$  is finally converted to active TNF- $\alpha$  by a membrane-bound metalloproteinase called TNF- $\alpha$  converting enzyme (TACE), although other matrix metalloproteinases also have a greater or lesser degree of TNF- $\alpha$  converting potential (Gearing et al., 1994). In vitro MMP-12 also releases active TNF- $\alpha$  from a synthetic pro-form, and murine models suggest that active TNF- $\alpha$  release from macrophages after acute smoke exposure is dependent on both TACE and MMP-12 (Churg et al., 2003).

Serum concentrations of TNF- $\alpha$  and stimulated TNF- $\alpha$  production from peripheral blood monocytes are increased in weight-losing COPD patients, suggesting that it may play a role in the cachexia of severe COPD (Di Francia et al., 1994; de Godoy et al., 1996; Pitsioui et al., 2002). In one study (Calikoglu et al., 2004), serum concentrations of TNF- $\alpha$  were increased compared with normal subjects, although this difference is not statistically significant, but they increased significantly during exacerbations when there was a correlation with plasma leptin concentrations. This may indicate that the increased TNF- $\alpha$  formation during exacerbations may contribute to loss of body weight. Plasma concentrations of TNF- $\alpha$  are also increased slightly in COPD patients compared with normal controls during exercise, but this is not associated with any increase in TNF- $\alpha$  expression in skeletal muscle (Rabinovich et al., 2003).

2. *Effects.* Studies with TNF receptor knockout suggest that TNF may account for 70% of cigarette smoke-induced emphysema in mice (Churg et al., 2004). TNF- $\alpha$  activates NF- $\kappa$ B, which switches on the transcription of inflammatory genes, including cytokines, chemokines, and proteases, in epithelial cells and macrophages. It similarly activates p38 MAP kinase, which in turn may activate a similar array of genes and may interact with the NF- $\kappa$ B pathway. This suggests a role for TNF- $\alpha$  in

amplifying the inflammation of COPD. TNF- $\alpha$  has a broad spectrum of inflammatory effects relevant to COPD, resulting in activation of neutrophils, monocytes, macrophages, epithelium, mucus secretion, and destruction of lung parenchyma through release of proteinases (Fig. 13).

TNF- $\alpha$  inhibits the expression of skeletal muscle proteins via activation of NF- $\kappa$ B (Langen et al., 2001). This suggests that inhibitors of TNF- $\alpha$  might be useful in reversing the skeletal wasting seen in COPD as well as reducing the airway inflammatory response (Barnes, 2001a).

3. *Inhibition.* Blocking antibodies (such as infliximab) and soluble receptors (such as etanercept) have proven to be very useful in the treatment of severe rheumatoid arthritis and inflammatory bowel disease, even in patients who are relatively unresponsive to steroids (Palladino et al., 2003). TNF- $\alpha$  inhibitors are therefore a logical approach to COPD therapy, and clinical trials are now underway. However, there are some concerns about potential long-term adverse effects, such as increased susceptibility to infections. Because antibody-based therapies have to be given by injection, small molecule inhibitors of TNF- $\alpha$  would be beneficial, because they may be given orally. TACE is a matrix metalloproteinase-related enzyme critical for the release of TNF- $\alpha$  from the cell surface. Small-molecule TACE inhibitors are in development as oral TNF- $\alpha$  inhibitors, but cell-associated TNF- $\alpha$  may exert residual effects (Rabinowitz et al., 2001).

*B. Interleukin-1 $\beta$*

IL-1 $\beta$  has similar actions to TNF- $\alpha$  and is a potent activator of alveolar macrophages from COPD patients (Russell et al., 2002b). Bronchial epithelial cells in culture release more IL-1 $\beta$  than do cells from normal subjects after stimulation with cigarette smoke (Rusznak et al., 2000). However, elevated levels of IL-1 in COPD have not yet been reported. IL-1 receptor antagonist

(IL-1RA) is an endogenous inhibitor of IL-1 effects and has been reported to be reduced in asthma. In COPD macrophages, a reduced secretion of IL-1RA compared with normal macrophages in response to *Chlamydia* infection has been described (Rupp et al., 2003). IL-1 $\beta$  stimulates the expression of elastolytic MMPs, including MMP-9, from various cell types (Kusano et al., 1998).

Recently, the role of IL-1 $\beta$  in the development of emphysema has been studied comparing an IL-1 $\beta$  type 1 receptor knockout mouse with double-TNF- $\alpha$  receptor-deficient, combined IL-1 $\beta$ - and TNF- $\alpha$  receptor-deficient, and wild-type mice after intratracheal instillation of porcine pancreatic elastase. Emphysema continued to progress for more than 10 days after clearance of the elastase. After 21 days, emphysema was reduced in all knockout mice compared with the wild strain. The combined IL-1 $\beta$  and TNF- $\alpha$  receptor-deficient mice showed a significant reduction in emphysema compared with the wild strain and also the single knockout animals. The authors (Lucey et al., 2002) suggest that 27% of emphysema could be related to the IL-1 $\beta$  type 1 receptor, 36% to TNF- $\alpha$  type 1 and 2 receptors, and 81% to combination of the two receptor groups, which is more than additive. This animal model suggests that both IL-1 $\beta$  and TNF- $\alpha$  are important in the pathogenesis of COPD with some evidence of a synergistic interaction.

*C. Interleukin-6*

IL-6 concentrations are increased in induced sputum, bronchoalveolar lavage, and exhaled breath condensate of COPD patients, particularly during exacerbations (Bhowmik et al., 2000; Song et al., 2001; Bucchioni et al., 2003). IL-6 is also increased in the plasma of COPD patients (Debigare et al., 2003; Godoy et al., 2003; Hageman et al., 2003), especially during exacerbations (Wedzicha et al., 2000). Monocytes from COPD patients release more IL-6 in response to stimulation with LPS than cells from normal subjects (Aldonyte et al., 2003). IL-6 is a marker of inflammation, since it is activated by

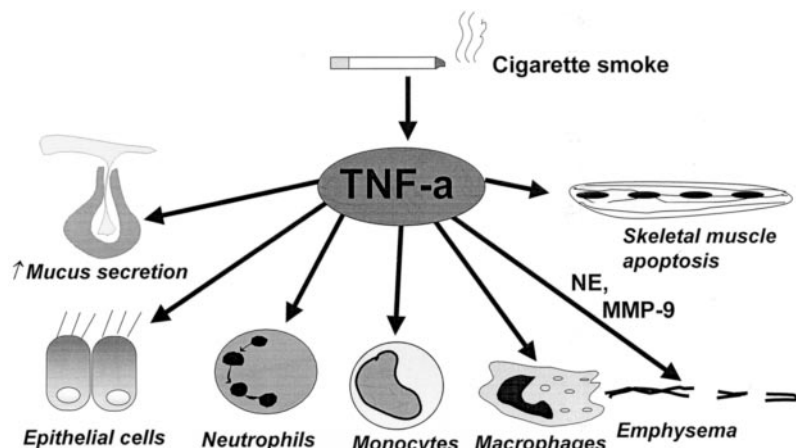


FIG. 13. TNF- $\alpha$  may play a pivotal role in COPD and amplifies the inflammatory response, resulting in activation of epithelial cells, monocytes, macrophages, and neutrophils. It may induce emphysema through the release of proteinases, including NE and MMP-9, stimulate mucus secretion, and induce apoptosis of skeletal muscle cells.

NF- $\kappa$ B, but its role in inflammation is uncertain, as it has both anti-inflammatory and proinflammatory actions and its effects may be determined by the presence of other cytokines.

#### D. Interleukin-9

IL-9 is a cytokine that is normally released from T helper 2 cells and has an amplifying effect on allergic inflammation (Shimbara et al., 2000). Surprisingly, it shows a marked increase in expression in T lymphocytes in bronchial biopsies of patients with COPD (Panzner et al., 2003). IL-9 is a potent inducer of mucus production, causing increased expression of the MUC5AC gene (Reader et al., 2003).

#### E. Granulocyte-Macrophage Colony Stimulating Factor

The concentrations of GM-CSF in BAL fluid are increased in stable COPD but markedly elevated during exacerbations (Balbi et al., 1997). GM-CSF is important for neutrophil survival and priming, and it may play an enhancing role in neutrophilic inflammation. Like other proinflammatory cytokines, it is predominantly regulated by NF- $\kappa$ B. Interestingly, the spontaneous and induced secretion of GM-CSF from alveolar macrophages of COPD is no different than that from macrophages of smokers, whereas there is a marked increase in TNF- $\alpha$ , IL-8, and MMP-9 (Culpitt et al., 2003). Furthermore, GM-CSF secretion is suppressed by a corticosteroid, whereas the secretion of the other cytokines appears to be steroid resistant.

#### F. Interleukin-10

IL-10 is a potent anti-inflammatory cytokine that is released from monocytes and alveolar macrophages in response to inflammatory stimuli. IL-10 secretion is markedly reduced in alveolar macrophages from patients with asthma (Barnes, 2001b), and its concentrations are reduced in sputum of patients with asthma and COPD, suggesting that a similar abnormality may apply in COPD (Takanashi et al., 1999). IL-10 production appears to be increased in macrophages from normal smokers (Lim et al., 2000b), but it is not certain whether macrophages from COPD patients show a relatively reduced production, as in asthma, which may help to amplify inflammation. However, bronchial biopsies from COPD patients show increased IL-10 expression (Panzner et al., 2003).

IL-10 has therapeutic potential in COPD, as it has a broad spectrum of anti-inflammatory effects, many of which are mediated via inhibition of NF- $\kappa$ B via an effect on signal transducer and activator of transcription 3 (Williams et al., 2004). IL-10 suppresses the release of MMP-9 from monocytes of COPD patients and at the same time stimulates the release of its major endogenous inhibitor, tissue inhibitor of matrix metalloproteinases (TIMP-1) (Lacraz et al., 1995; Mostafa et al., 2001).

#### G. Interleukin-12

There is increased expression of IL-12 in bronchial biopsies of COPD patients accompanied by an increase in phosphorylated signal transducer and activator of transcription 4 (Di Stefano et al., 2004). By contrast, the transcription factor T-bet, which regulates IFN- $\gamma$  expression in response to IL-12 stimulation, did not increase.

#### H. Interleukin-13

Overexpression of IL-13 and also interferon- $\gamma$  in murine lungs unexpectedly results in emphysema that is mediated by increased expression of MMPs and cathepsins (Wang et al., 2000; Zheng et al., 2000). There is also an association between a promoter polymorphism in the IL-13 gene and COPD, as has been seen for asthma (van der Pouw Kraan et al., 2002). There is increased expression of IL-13 in bronchial biopsies of smokers with mucus hypersecretion compared with normal smokers (Miotto et al., 2003). This may be consistent with the fact that IL-13 is a potent stimulant of mucus secretion and stimulates the differentiation of goblet cells via activation of EGFR (Shim et al., 2001).

#### I. Interleukin-17

IL-17 is a cytokine that releases CXC chemokines from airway epithelial cells (Prause et al., 2003) but is not increased in sputum of COPD patients (Barczyk et al., 2003).

#### J. Interferon- $\gamma$

Overexpression of IFN- $\gamma$  in murine lungs results in emphysema (Wang et al., 2000). There is increased expression of IFN- $\gamma$  in bronchial biopsies of COPD patients (Panzner et al., 2003; Di Stefano et al., 2004). The CXCR3-positive T cells that are increased in small airways of COPD patients all express IFN- $\gamma$ , and IFN- $\gamma$  stimulates the expression of CXCR3 ligands such as IP-10, suggesting a positive feedback loop. CD8<sup>+</sup> IFN- $\gamma$ -positive cells (Tc1 cells) are increased in the sputum of COPD patients (Tzanakis et al., 2004). An increase in IFN- $\gamma$  secreting peripheral blood mononuclear cells has also been described in COPD patients (Majori et al., 1999).

### IX. Growth Factors

Marked structural changes are found in small airways and lung parenchyma, presumably as a result of chronic inflammation and the release of growth factors that induce fibrosis and cell proliferation.

#### A. Transforming Growth Factors

TGF- $\beta$ 1 shows increased expression in small airway epithelial cells and alveolar macrophages of patients with COPD and might play a role in the characteristic fibrosis in small airways (de Boer et al., 1998; Takizawa

et al., 2001). However, no increase in TGF- $\beta$  has been found in large airway biopsies from COPD patients (Aubert et al., 1994; Kokturk et al., 2003). Increased secretion of TGF- $\beta$  is reported in peripheral blood monocytes from COPD patients (Hodge et al., 2003). Increased TGF- $\beta$  expression in peripheral lung tissue of COPD patients has been correlated with immunoreactivity for 4-hydroxy-4-nonenal, a marker of oxidative stress (Rahman et al., 2002). TGF- $\beta$  induces the release of collagen tissue growth factor (CTGF), which mediates the fibrosis response to TGF- $\beta$  (Ihn, 2002). In a human epithelial cell line, latent adenovirus infection (which has been associated with COPD) induces increased expression of both TGF- $\beta$  and CTGF (Ogawa et al., 2004). TGF- $\beta$  is secreted in a latent form that is inactive but is potently activated by MMP-9 (Yu and Stamenkovic, 2000); this may be mediated via MMP-9-induced proteolytic cleavage of latent TGF-binding protein-1, resulting in release of active TGF- $\beta$ 1 (Dallas et al., 2002). This mechanism therefore could be a link between elastolysis induced by MMP-9 and simultaneous production of fibrosis by activation of TGF- $\beta$ 1 (Fig. 14). Alveolar macrophages recognize apoptotic cells via phosphatidylserine receptors, and this interaction results in the expression of TGF- $\beta$ 1, which exerts both an anti-inflammatory action and promotes fibrosis (Huynh et al., 2002).

TGF- $\beta$  potently down-regulates  $\beta_2$ -adrenergic receptors by inhibiting gene transcription in human cell lines (Mak et al., 2000; Takeyama et al., 2001) and markedly reduces the bronchodilator response to  $\beta$ -agonists in airway smooth muscle in vitro (Ishikawa et al., 2003).

A polymorphism in the promoter of the TGF- $\beta$ 1 gene (-509T) that is associated with increased production of TGF- $\beta$  has been associated with severe asthma (Pulleyn et al., 2001; Silverman et al., 2004). A polymorphism in the coding region of TGF- $\beta$ 1 that is associated with increased TGF- $\beta$  production surprisingly appears to be

less commonly associated with COPD, indicating a possible protective role of TGF- $\beta$  (Wu et al., 2004).

Small-molecule inhibitors of TGF receptor kinase are now in development, and TGF- $\beta$  antagonists (DaCosta et al., 2004) might be a means of preventing the small airway fibrosis that is characteristic of COPD.

Alveolar macrophages produce TGF- $\alpha$  in much greater amounts than they do TGF- $\beta$  (Toossi et al., 1996), and this may be a major endogenous activator of EGFR that plays a key role in regulating mucus secretion in response to many stimuli, including cigarette smoke. Cigarette smoke activates TACE in airway epithelial cells, which results in the shedding of TGF- $\alpha$  and the activation of EGFR, resulting in increased mucus secretion (Shao et al., 2004). The mucus secretory response to cigarette smoke is inhibited by knockdown of TGF- $\alpha$  and TACE by interference RNA.

### B. Epidermal Growth Factor

EGF and TGF- $\alpha$  activate EGFR, which appear to play a key role in the regulation of mucus secretion, expression of mucin (MUC) genes, and differentiation of mucus-secreting cells (Nadel and Burgel, 2001). EGFR are involved in the increased mucus secretory response to oxidative stress and cigarette smoke (Takeyama et al., 1999, 2000, 2001; Basbaum et al., 2002) (Fig. 15).

This suggests that inhibitors of EGFR may be of potential value in the treatment of the mucus hypersecretion of COPD. Several small-molecule inhibitors of EGFR tyrosine kinase, such as gefitinib, are now developed for the treatment of nonsmall-cell lung cancer (Wakeling, 2002). These treatments appear to be well tolerated and therefore might be suitable for the treatment of mucus hypersecretion.

### C. Vascular-Endothelial Growth Factor

VEGF is a major regulator of vascular growth and is likely to be involved in the pulmonary vascular remodel-

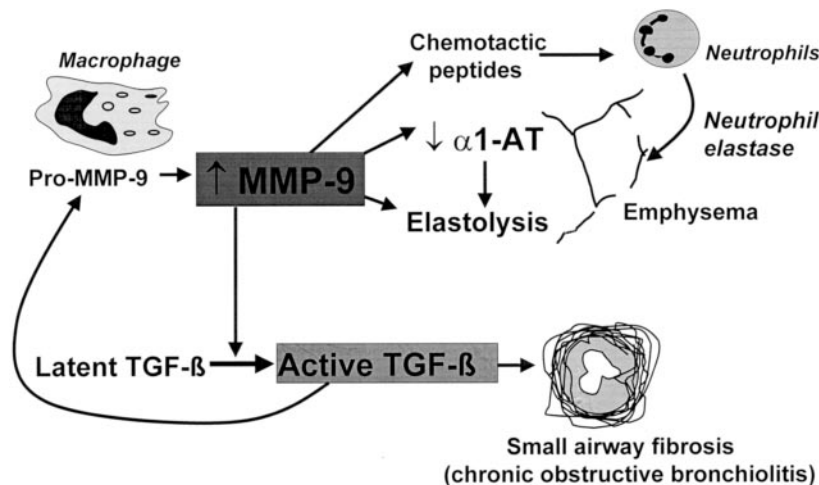


FIG. 14. Possible interrelationship between small airway fibrosis and emphysema in COPD. TGF- $\beta$  is activated by MMP-9, resulting in alveolar destruction and also inactivation of  $\alpha$ 1-AT, thus enhancing the effects of neutrophil elastase released from neutrophils that are attracted by chemotactic peptides generated by MMP-9. TGF- $\beta$  causes small airway fibrosis and also activates MMP-9.

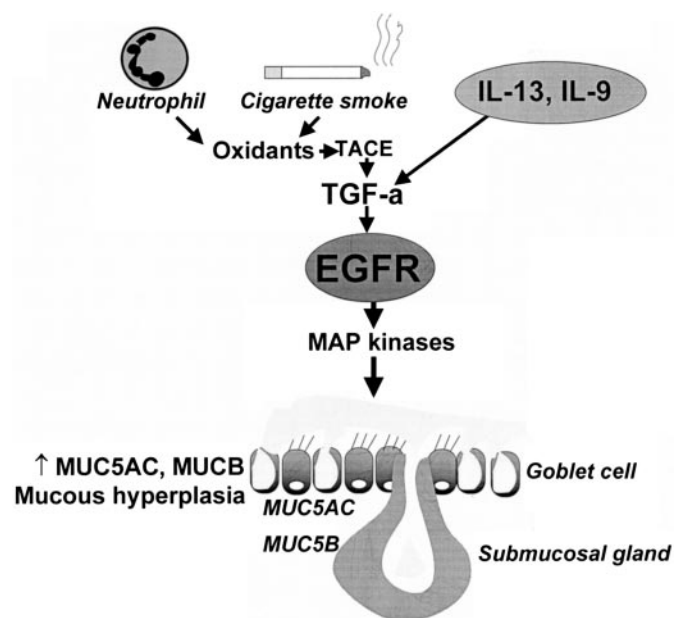


FIG. 15. EGFR appear to play a key role in the regulation of mucus hypersecretion, with increased expression of mucin genes (MUC5AC, MUCB) and differentiation of goblet cells and hyperplasia of mucus-secreting cells. These effects are mediated via the activation of MAP kinases. EGFRs are activated by TGF- $\alpha$ , which is in turn activated by TACE activated via release of oxidants from cigarette smoke and neutrophils. TGF- $\alpha$  may also be activated by IL-13 and IL-9.

eling that occurs as a result of hypoxic pulmonary vasoconstriction in severe COPD (Wagner, 2003). There is increased expression of VEGF in pulmonary vascular smooth muscle of patients with mild and moderate COPD, but paradoxically, a reduction in expression in severe COPD with emphysema (Santos et al., 2003). Inhibition of VEGF receptors in rats using a selective inhibitor induces apoptosis of alveolar endothelial cells resulting in emphysema (Kasahara et al., 2000), and this appears to be associated with oxidative stress (Tuder et al., 2003). Interestingly, the concentration of VEGF is increased in induced sputum of patients with asthma and chronic bronchitis but is significantly reduced in patients with COPD with emphysema (Kanazawa et al., 2003a,b). A common polymorphism of the VEGF gene that is associated with decreased VEGF expression (C936T) is not associated with any increased risk of COPD (Sakao et al., 2003).

#### D. Fibroblast Growth Factors

Fibroblast growth factor (FGF)-1, FGF-2, and FGF receptors are abnormally expressed in airway and pulmonary vascular smooth muscle and airway epithelial cells in peripheral lung of patients with COPD (Kranenburg et al., 2002). The increased expression of FGF is particularly correlated with vascular remodeling. Blocking endogenous FGF through transgenic expression of a soluble receptor is associated with emphysema in developing mice, but it has no effect in adult animals (Hokuto et al., 2003).

## X. Proteases

It has long been proposed that various proteases break down connective tissue components, particularly elastin, in lung parenchyma to produce emphysema and that there is an imbalance between proteases and endogenous antiproteases that should normally protect against protease-mediated effects (Fig. 16). Elastin may be the most important target for these enzymes, because there is a loss of elasticity in the lung parenchyma in patients with emphysema, and elastin cannot be regenerated in an active form. Evidence for elastin degradation in COPD is provided by the increased excretion of desmosine, derived from elastin cross-links, in smokers with rapid decline in lung function compared with those experiencing a normal decline (Gottlieb et al., 1996). Although early attention was focused on neutrophil elastase, many other proteases that have the capacity to degrade elastin have now been implicated (Stockley, 2001).

### A. Neutrophil Elastase

There has been particular emphasis on the role of NE since patients with inherited  $\alpha$ 1-antitrypsin ( $\alpha$ 1-AT) deficiency (PiZZ) were shown to develop early-onset emphysema. Furthermore, the demonstration that  $\alpha$ 1-AT may be inactivated by cigarette smoke exposure raised the possibility that neutrophil elastase may also be important in smokers with normal plasma  $\alpha$ 1-AT concentrations. This was supported by animal models in which tracheal instillation of NE induces emphysema, infiltration of neutrophils (Senior et al., 1977), and immunocytochemical localization of NE on elastin fibers in the lung parenchyma of patients with emphysema (Damiano et al., 1986). NE (E.C.3.4.21.37) is a serine protease that is inhibited by  $\alpha$ 1-AT in the lung parenchyma. It is stored in azurophilic granules in neutrophils, and it may be expressed on the cell surface in cells primed by cytokines (Owen et al., 1997). There is an increase in the

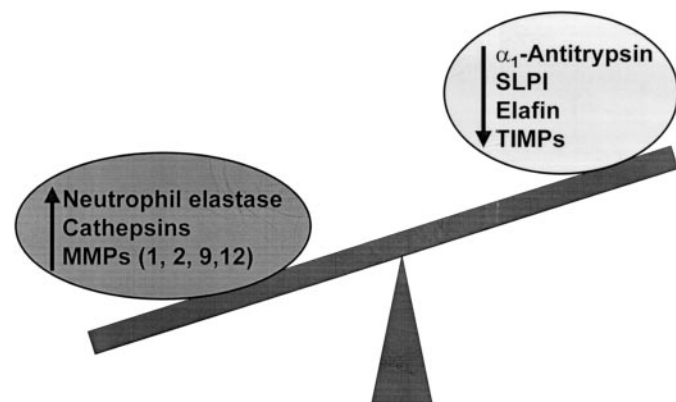


FIG. 16. Protease-antiprotease imbalance in COPD. In COPD the balance appears to be tipped in favor of increased proteolysis, either because of an increase in proteases, including neutrophil elastase, cathepsins, and MMPs, or a deficiency in antiproteases, which may include  $\alpha$ 1-antitrypsin, elafin, SLPI, and TIMPs.



amount of NE/ $\alpha$ 1-AT complexes in bronchoalveolar lavage fluid of COPD patients who are normal smokers (Yoshioka et al., 1995), and this is correlated with the rate of decline in FEV<sub>1</sub> (Betsuyaku et al., 2000).

NE has subsequently been shown to have several other actions relevant to its potential role on COPD. It is a potent mucus secretagogue of submucosal gland cells and goblet cells (Sommerhoff et al., 1990; Takeyama et al., 1998; Nadel, 2000). NE induces the expression of MUC5AC in an epithelial cell line, and this mechanism appears to be dependent on the generation of reactive oxygen species (Voynow et al., 1999; Fischer and Voynow, 2002). NE also induces the expression of some cytokines, including IL-8 in airway epithelial cells (Nakamura et al., 1992). NE cleaves the phosphatidylserine receptor on macrophages, thus impairing the macrophages' ability to clear apoptotic cells (Vandivier et al., 2002).

On the other hand, NE also inactivates CD14, a cell surface receptor for lipopolysaccharide, thus reducing the inflammatory response to endotoxin (Le Barillec et al., 1999). NE is likely to play a role in host defense, and NE<sup>(-/-)</sup> mice have increased susceptibility to overwhelming Gram-negative bacterial infections, but they do not appear to have any increase in spontaneous infections (Belaouaj et al., 1998; Shapiro, 2002).

The role of NE in COPD will only be established when the effect of NE inhibitors has been studied clinically (Ohbayashi, 2002). In guinea pigs exposed to cigarette smoke, a NE inhibitor markedly reduced emphysema and the neutrophil inflammatory response (Wright et al., 2002). Deletion of the gene for NE in mice (NE<sup>(-/-)</sup>) significantly protects the animals against the development of cigarette smoke-induced emphysema and also results in a reduction in the numbers of neutrophils in the lungs (Shapiro et al., 2003). Although several NE inhibitors have been tested in humans, there are few results reported. It is not certain whether the drugs failed or the clinical trials were not adequately designed. The NE inhibitor MR889 had no effect on urinary desmosine in unselected COPD patients, but a small reduction was seen in patients with a relatively mild disease load (Luisetti et al., 1996). Several small-molecule NE inhibitors are apparently in clinical development for COPD (Ohbayashi, 2002; Wark, 2002). The macrolide antibiotics erythromycin and flurithromycin have also been shown to inhibit NE activity (Gorrini et al., 2001), and this might account for their beneficial effect on mucus hypersecretion (Goswami et al., 1990). However, a trial of clarithromycin for 12 weeks failed to reduce NE concentrations or neutrophil count in sputum of COPD patients (Banerjee et al., 2004).

#### B. Other Serine Proteases

Neutrophils also store two other serine proteases, cathepsin G and proteinase 3, in their specific granules (Rao et al., 1991). These other serine proteases have

similar properties to NE and induce mucus secretion in a similar way (Sommerhoff et al., 1990; Witko-Sarsat et al., 1999). Proteinase-3 is potently expressed on the surface of neutrophils after activation with cytokines (Campbell et al., 2000). Proteinase 3 is potently inhibited by  $\alpha$ 1-AT (Duranton and Bieth, 2003), but it is only poorly inhibited by SLPI in comparison to NE, and indeed proteinase 3 destroys the activity of SLPI (Rao et al., 1993). The neutrophil elastase inhibitors currently in development also inhibit these other serine proteases (Ohbayashi, 2002).

#### C. Cysteine Proteases

Lysosomal cysteine proteases (cathepsins) may also be involved in COPD (Chapman et al., 1997; Turk et al., 2001). Cathepsin S expression is induced by IFN- $\gamma$  in several cell types, including smooth muscle cells. Overexpression of IFN- $\gamma$  induces emphysema in mice, and there is increased expression of cathepsins B, D, H, L, and S (Wang et al., 2000). Cathepsin inhibitors markedly reduce the emphysema induced in IL-13 transgenic mice, indicating the elastolytic potential of this cathepsin (Zheng et al., 2000). Several other cathepsins also have elastolytic activity, including cathepsins B, K, and L, which are expressed in alveolar macrophages (Reddy et al., 1995; Punturieri et al., 2000) and cathepsin W in CD8<sup>+</sup> T cells (Linnevers et al., 1997). Cathepsins B, L, and S inactivate SLPI (Taggart et al., 2001).

The role of cathepsins in COPD is uncertain. Increased concentrations of cathepsin L have been detected in BAL fluid of patients with emphysema (Takeyabu et al., 1998), and alveolar macrophages from patients with COPD secrete more cysteine protease activity than macrophages from normal smokers or non-smokers (Russell et al., 2002b). The endogenous inhibitors of cathepsins are cystatins and stefins, but little is known about their role in COPD. Cystatin C concentrations are increased in BAL fluid of patients with COPD (Takeyabu et al., 1998).

#### D. Matrix Metalloproteinases

MMPs are a large family of zinc-dependent proteinases that regulate the destruction of extracellular matrix components (Stamenkovic, 2003). It is now increasingly recognized that MMPs also play a key role in the regulation of cytokines, chemokines, and growth factors. There is increasing evidence for a role for MMPs in COPD (Shapiro and Senior, 1999). In patients with emphysema, there is an increase in bronchoalveolar lavage concentrations and macrophage expression of MMP-1 (collagenase) and MMP-9 (gelatinase B) (Finlay et al., 1997; Betsuyaku et al., 1999; Culpitt et al., 1999). There is an increase in activity of MMP-9 in the lung parenchyma of patients with emphysema (Ohnishi et al., 1998), and this is correlated with FEV<sub>1</sub> (Kang et al., 2003). MMP-1 expression is also increased in the lungs of patients with emphysema, with predominant localiza-

tion to type II pneumocytes (Imai et al., 2001). Alveolar macrophages from normal smokers express more MMP-9 than those from normal subjects (Lim et al., 2000b), and there is an ever greater increase in cells from patients with COPD (Russell et al., 2002a), which has greatly enhanced elastolytic activity (Russell et al., 2002b). Indeed, using the MMP inhibitor marimastat, it was shown that MMPs account for most of the elastolytic activity released from alveolar macrophages from COPD patients over prolonged periods (Russell et al., 2002b). MMP-9 and the ratio of MMP-9 to TIMP-1 are increased in induced sputum of patients with COPD (Cataldo et al., 2000; Beeh et al., 2003a). MMP-8 and MMP-9 do not only act as secreted enzymes, but they are also bound to cells where they exert elastolytic activity. Thus, approximately 80% of the MMP-8 and MMP-9 synthesized by neutrophils remains associated with the surface and is not neutralized by TIMPs, so they may play a critical role in elastolysis (Owen et al., 2003, 2004).

The interest in MMPs has been heightened by the demonstration that emphysema induced by chronic cigarette exposure is prevented in MMP-12<sup>-/-</sup> (macrophage metalloelastase) mice (Hautamaki et al., 1997). In MMP-12<sup>-/-</sup> mice, emphysema induced by IL-13 and IFN- $\gamma$  overexpression is reduced (Wang et al., 2000; Zheng et al., 2000), and there is a marked reduction in the recruitment of monocytes into the lung. This may be because MMPs generate chemotactic peptides that promote macrophage recruitment to the parenchyma and airways. MMPs activate the latent form of TGF- $\beta$  to its active form (Dallas et al., 2002). In addition, mice in which the integrin  $\alpha v \beta 6$  is deleted (Itgb6-null mice) fail to activate TGF- $\beta$  and develop age-related emphysema, which is prevented in MMP-12<sup>-/-</sup> mice and by overexpression of TGF- $\beta 1$  (Morris et al., 2003). This suggests that TGF- $\beta 1$  down-regulates MMP-12 under normal conditions, and absence of TGF- $\beta$  results in excessive MMP-12 and emphysema. MMP-9<sup>-/-</sup> mice are not protected against emphysema induced by cigarette smoke, but they are protected from small airway fibrosis (Lanone et al., 2002). TGF- $\beta 1$  is activated by MMP-9 (Yu and Stamenkovic, 2000); this may be mediated via MMP-9-induced proteolytic cleavage of latent TGF-binding protein-1, resulting in the release of TGF- $\beta 1$  (Dallas et al., 2002). Therefore, this mechanism could be a link between elastolysis induced by MMP-9 and simultaneous production of fibrosis by activation of TGF- $\beta 1$  (Fig. 14). Thus, MMP-12 is a prominent MMP in the mouse, and, while present in humans, it does not appear to be as important as MMP-9.

Various polymorphisms of MMP-1, MMP-9, and MMP-12 have been associated with emphysema (Mine-matsu et al., 2001; Joos et al., 2002; Wallace and Sandford, 2002). The increasing evidence for the involvement of MMP-9 in COPD suggests that inhibitors would be of value in preventing emphysema (Belvisi and Bottomley, 2003). A nonselective MMP inhibitor inhibits the devel-

opment of emphysema in cigarette smoke-exposed guinea pigs (Selman et al., 2003). Nonselective small-molecule MMP inhibitors, such as marimastat, appear to have considerable musculoskeletal side effects. It is possible that side effects could be reduced by increasing selectivity for specific MMPs or by targeting delivery to the lung parenchyma. Another approach is to reduce the expression of MMP-9 in pulmonary cells. Treatment of emphysema patients with retinoic acid appears to reduce the concentration of MMP-9 in circulation (Mao et al., 2003). TIMPs may also be used therapeutically, particularly if engineered for greater stability (Nagase and Brew, 2003).

### E. Antiproteases

Normally, proteases are counteracted by an excess of endogenous antiproteases. The major inhibitors of serine proteases are  $\alpha 1$ -AT in lung parenchyma and airway epithelium-derived SLPI in the airways. Other serine protease inhibitors include elafin and  $\alpha 1$ -antichymotrypsin. Serine protease inhibitors inactivate NE and other serine proteases such as proteinase-3 (Rooney et al., 2001).

1.  *$\alpha 1$ -Antitrypsin.* Multiple genetic variants of  $\alpha 1$ -AT are now recognized that give rise to reduced circulating active  $\alpha 1$ -AT concentrations (Mahadeva and Lomas, 1998; Carrell and Lomas, 2002). The best described deficiency that results in early-onset emphysema is the ZZ type (PiZZ), in which a single amino acid substitution (Gly342 $\rightarrow$ Lys) results in structural alterations in  $\alpha 1$ -AT, resulting in failure of its normal post-translational modification and secretion by hepatocytes, leading to very low plasma concentrations. Whether heterozygotes and other genetic variants that reduce circulating  $\alpha 1$ -AT concentrations to a lesser extent than the ZZ phenotype also predispose to emphysema is more debatable (Lomas and Mahadeva, 2002). ZZ  $\alpha 1$ -AT deficiency is a rare cause of emphysema accounting for less than 1% of patients, but it was proposed long ago that cigarette smoking may oxidize  $\alpha 1$ -AT, resulting in impaired antiprotease function and increased neutrophil elastase activity (Carp and Janoff, 1978). The mechanism appears to be due to oxidative stress, and oxidation of methionine at positions 351 or 358 impairs anti-NE activity of  $\alpha 1$ -AT (Taggart et al., 2000).

2. *Secretory Leukoprotease Inhibitor.* SLPI is the other major serine proteinase inhibitor in the airways (Vogelmeier et al., 1991). Like  $\alpha 1$ -AT, SLPI may be inactivated by oxidative stress, but also by cleavage through its active site by cathepsins L and S (Taggart et al., 2001). In patients with emphysema, proteolytic fragments of SLPI are found in BAL fluid, which contributes to the reduced anti-NE activity in these patients. This inactivation of SLPI not only impairs its anti-NE activity, but also its antimicrobial and anti-inflammatory roles. SLPI down-regulates LPS-induced TNF- $\alpha$  and MMP secretion from monocytes (Jin et al., 1997; Zhang

et al., 1997) and this may be mediated by an inhibitory effect on I $\kappa$ B $\alpha$  (inhibitor of NF- $\kappa$ B- $\alpha$ ), degradation, resulting in inhibition of NF- $\kappa$ B (Taggart et al., 2002). The role of elafin and  $\alpha$ 1-antichymotrypsin in COPD are less well defined (Ishii et al., 2000a; Sallenave, 2000).

3. *Tissue Inhibitor of Matrix Metalloproteinases.* Four tissue inhibitors of MMPs (TIMP1–4) counteract MMPs (Cawston et al., 2001). TIMP-1 secretion from alveolar macrophages is increased in response to inflammatory stimuli, but the increase is blunted in cells derived from COPD patients, thus favoring increased elastolysis (Russell et al., 2002a,b). An increased frequency of loss-of-function mutations of TIMP-2 has been described in patients with COPD (Hirano et al., 2001).

**XI. Conclusions**

It is clear that many inflammatory mediators are involved in the chronic inflammation and structural changes that occur in COPD. These mediators are derived not only from activated inflammatory cells, such as alveolar macrophages, neutrophils, and T lymphocytes that are recruited to the airways and lungs, but also from structural cells in the respiratory tract, including epithelial and endothelial cells and fibroblasts, which transform into mediator-producing cells. These mediators have complex effects in the airways, resulting in recruitment of inflammatory cells from the circulation, bronchoconstriction, vascular changes, mucus secretion, and structural changes in the airways and lung parenchyma. These mediators may also spill over into the systemic circulation to produce systemic changes such as cachexia and muscle wasting seen in severe disease. The therapeutic implications are that blocking the generation or receptors for these mediators will have a beneficial clinical effect, and several mediator antagonists are now in development (Barnes, 2002b). However, blocking a single mediator when so many are involved and with redundant effects, it is unlikely that this approach will produce major clinical benefit unless the mediator plays a pivotal or unique role and is high in a cascade of events. The only way to determine the importance of a mediator is to study the effect of a specific inhibitor in the disease, and this will require careful and prolonged clinical studies.

References

Aarbiou J, Rabe KF, and Hiemstra PS (2002) Role of defensins in inflammatory lung disease. *Ann Med* **34**:96–101.  
 Aaron SD, Angel JB, Lunau M, Wright K, Fex C, Le Saux N, and Dales RE (2001) Granulocyte inflammatory markers and airway infection during acute exacerbation of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* **163**:349–355.  
 Adcock IM, Brown CR, Kwon OJ, and Barnes PJ (1994) Oxidative stress induces NF- $\kappa$ B DNA binding and inducible NOS mRNA in human epithelial cells. *Biochem Biophys Res Commun* **199**:1518–1524.  
 Adler KB, Holden Stauffer WJ, and Repine JE (1990) Oxygen metabolites stimulate release of high-molecular-weight glycoconjugates by cell and organ cultures of rodent respiratory epithelium via an arachidonic acid-dependent mechanism. *J Clin Invest* **85**:75–85.  
 Adler KB and Li Y (2001) Airway epithelium and mucus: intracellular signaling pathways for gene expression and secretion. *Am J Respir Cell Mol Biol* **25**:397–400.  
 Agostini C, Facco M, Siviero M, Carollo D, Galvan S, Cattelan AM, Zambello R,

Trentin L, and Semenzato G (2000) CXC chemokines IP-10 and mig expression and direct migration of pulmonary CD8<sup>+</sup>/CXCR3<sup>+</sup> T cells in the lungs of patients with HIV infection and T-cell alveolitis. *Am J Respir Crit Care Med* **162**:1466–1473.  
 Agusti AG, Villaverde JM, Togores B, and Bosch M (1999) Serial measurements of exhaled nitric oxide during exacerbations of chronic obstructive pulmonary disease. *Eur Respir J* **14**:523–528.  
 Aldonnye R, Jansson L, Piitulainen E, and Janciauskiene S (2003) Circulating monocytes from healthy individuals and COPD patients. *Respir Res* **4**:11.  
 Au BT, Teixeira MM, Collins PD, and Williams TJ (1998) Effect of PDE4 inhibitors on zymosan-induced IL-8 release from human neutrophils: synergism with prostanooids and salbutamol. *Br J Pharmacol* **123**:1260–1266.  
 Aubert JD, Dalal BI, Bai TR, Roberts CR, Hayashi S, and Hogg JC (1994) Transforming growth factor  $\beta$  1 gene expression in human airways. *Thorax* **49**:225–232.  
 Avissar N, Finkelstein JN, Horowitz S, Willey JC, Coy E, Frampton MW, Watkins RH, Khullar P, Xu YL, and Cohen HJ (1996) Extracellular glutathione peroxidase in human lung epithelial lining fluid and in lung cells. *Am J Physiol* **270**:L173–L182.  
 Bacakoglu F, Atasever A, Ozhan MH, Gurgun C, Ozkicik H, and Guzelant A (2003) Plasma and bronchoalveolar lavage fluid levels of endothelin-1 in patients with chronic obstructive pulmonary disease and pulmonary hypertension. *Respiration* **70**:594–599.  
 Balbi B, Bason C, Balleari E, Fiasella F, Pesci A, Ghio R, and Fabiano F (1997) Increased bronchoalveolar granulocytes and granulocyte/macrophage colony-stimulating factor during exacerbations of chronic bronchitis. *Eur Respir J* **10**:846–850.  
 Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, Pulendran B, and Palucka K (2000) Immunobiology of dendritic cells. *Annu Rev Immunol* **18**:767–811.  
 Banerjee D, Honeybourne D, and Khair OA (2004) The effect of oral clarithromycin on bronchial airway inflammation in moderate-to-severe stable COPD: a randomized controlled trial. *Treat Respir Med* **3**:59–65.  
 Barczyk A, Pierzchala W, and Sozanska E (2003) Interleukin-17 in sputum correlates with airway hyperresponsiveness to methacholine. *Respir Med* **97**:726–733.  
 Barnes PJ (1992) Bradykinin and asthma. *Thorax* **47**:979–983.  
 Barnes PJ (2000a) Chronic obstructive pulmonary disease. *N Engl J Med* **343**:269–280.  
 Barnes PJ (2000b) Mechanisms in COPD: differences from asthma. *Chest* **117** (Suppl):10S–14S.  
 Barnes PJ (2001a) Cytokine modulators as novel therapies for airway disease. *Eur Respir J Suppl* **18**:67S–77S.  
 Barnes PJ (2001b) IL-10: a key regulator of allergic disease. *Clin Exp Allergy* **31**:667–669.  
 Barnes PJ (2001c) New treatments for chronic obstructive pulmonary disease. *Curr Opin Pharmacol* **1**:217–222.  
 Barnes PJ (2002a) Current and future therapies for airway mucus hypersecretion. *Novartis Found Symp* **248**:237–249.  
 Barnes PJ (2002b) New treatments for COPD. *Nat Rev Drug Discov* **1**:437–446.  
 Barnes PJ (2003) New concepts in COPD. *Annu Rev Med* **54**:113–129.  
 Barnes PJ (2004) Macrophages as orchestrators of COPD. *J COPD* **1**:59–70.  
 Barnes PJ, Chung KF, and Page CP (1998) Inflammatory mediators of asthma: an update. *Pharmacol Rev* **50**:515–596.  
 Barnes PJ, Ito K, and Adcock IM (2004) A mechanism of corticosteroid resistance in COPD: inactivation of histone deacetylase. *Lancet* **363**:731–733.  
 Barnes PJ and Karin M (1997) Nuclear factor- $\kappa$ B: a pivotal transcription factor in chronic inflammatory diseases. *N Engl J Med* **336**:1066–1071.  
 Barnes PJ, Shapiro SD, and Pauwels RA (2003) Chronic obstructive pulmonary disease: molecular and cellular mechanisms. *Eur Respir J* **22**:672–688.  
 Basbaum C, Li D, Gensch E, Gallup M, and Lemjabbar H (2002) Mechanisms by which gram-positive bacteria and tobacco smoke stimulate mucin induction through the epidermal growth factor receptor (EGFR). *Novartis Found Symp* **248**:171–176.  
 Bazzoni F, Cassatella MA, Rossi F, Ceska M, Dewald B, and Baggiolini M (1991) Phagocytosing neutrophils produce and release high amounts of the neutrophil-activating peptide 1/interleukin 8. *J Exp Med* **173**:771–774.  
 Beckman JS and Koppenol WH (1996) Nitric oxide, superoxide, and peroxynitrite: the good, the bad and the ugly. *Am J Physiol* **271**:C1432–C1437.  
 Beeh KM, Beier J, Kornmann O, and Buhl R (2003a) Sputum matrix metalloproteinase-9, tissue inhibitor of metalloproteinase-1 and their molar ratio in patients with chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis and healthy subjects. *Respir Med* **97**:634–639.  
 Beeh KM, Beier J, Kornmann O, Meier C, Taeumer T, and Buhl R (2003b) A single nasal allergen challenge increases induced sputum inflammatory markers in non-asthmatic subjects with seasonal allergic rhinitis: correlation with plasma interleukin-5. *Clin Exp Allergy* **33**:475–482.  
 Beeh KM, Kornmann O, Buhl R, Culpiitt SV, Giembycz MA, and Barnes PJ (2003c) Neutrophil chemotactic activity of sputum from patients with COPD: role of interleukin 8 and leukotriene B4. *Chest* **123**:1240–1247.  
 Belaouaj A, McCarthy R, Baumann M, Gao Z, Ley TJ, Abraham SN, and Shapiro SD (1998) Mice lacking neutrophil elastase reveal impaired host defense against gram negative bacterial sepsis. *Nat Med* **4**:615–618.  
 Belvisi MG and Bottomley KM (2003) The role of matrix metalloproteinases (MMPs) in the pathophysiology of chronic obstructive pulmonary disease (COPD): a therapeutic role for inhibitors of MMPs? *Inflamm Res* **52**:95–100.  
 Betsuyaku T, Nishimura M, Takeyabu K, Tanino M, Miyamoto K, and Kawakami Y (2000) Decline in FEV<sub>1</sub> in community-based older volunteers with higher levels of neutrophil elastase in bronchoalveolar lavage fluid. *Respiration* **67**:261–267.  
 Betsuyaku T, Nishimura M, Takeyabu K, Tanino M, Venge P, Xu S, and Kawakami Y (1999) Neutrophil granule proteins in bronchoalveolar lavage fluid from subjects with subclinical emphysema. *Am J Respir Crit Care Med* **159**:1985–1991.  
 Bhowmik A, Seemungal TA, Sapsford RJ, and Wedzicha JA (2000) Relation of

- sputum inflammatory markers to symptoms and lung function changes in COPD exacerbations. *Thorax* **55**:114–120.
- Biernacki WA, Kharitonov SA, and Barnes PJ (2003) Increased leukotriene B<sub>4</sub> and 8-isoprostane in exhaled breath condensate of patients with exacerbations of COPD. *Thorax* **58**:294–298.
- Bocchino V, Bertorelli G, Berstrand CP, Ponath PD, Newman W, Franco C, Marruchella A, Merlini S, Del Donno M, Zhuo X, et al. (2002) Eotaxin and CCR3 are up-regulated in exacerbations of chronic bronchitis. *Allergy* **57**:17–22.
- Borchers MT, Carty MP, and Leikauf GD (1999) Regulation of human airway mucins by acrolein and inflammatory mediators. *Am J Physiol* **276**:L549–L555.
- Bowler RP and Crapo JD (2002) Oxidative stress in airways: is there a role for extracellular superoxide dismutase? *Am J Respir Crit Care Med* **166**:S38–S43.
- Bowman EP, Campbell JJ, Druey KM, Scheschonka A, Kehrl JH, and Butcher EC (1998) Regulation of chemotactic and proadhesive responses to chemoattractant receptors by RGS (regulator of G-protein signaling) family members. *J Biol Chem* **273**:28040–28048.
- Brightling CE, Monteiro W, Ward R, Parker D, Morgan MD, Wardlaw AJ, and Pavord ID (2000) Sputum eosinophilia and short-term response to prednisolone in chronic obstructive pulmonary disease: a randomised controlled trial. *Lancet* **356**:1480–1485.
- Britton JR, Pavord ID, Richards KA, Knox AJ, Wisniewski AF, Lewis SA, Tattersfield AE, and Weiss ST (1995) Dietary antioxidant vitamin intake and lung function in the general population. *Am J Respir Crit Care Med* **151**:1383–1387.
- Brunelleschi S, Guidotto S, Viano I, Fantozzi R, Pozzi E, Ghio P, and Alberca C (1996) Tachykinin activation of human alveolar macrophages in tobacco smoke and sarcoidosis: a phenotypical and functional study. *Neuropeptides* **30**:456–464.
- Bucchioni E, Kharitonov SA, Allegra L, and Barnes PJ (2003) High levels of interleukin-6 in the exhaled breath condensate of patients with COPD. *Respir Med* **97**:1299–1302.
- Burge PS, Calverley PMA, Jones PW, Spencer S, Anderson JA, and Maslen T (2000) Randomised, double-blind, placebo-controlled study of fluticasone propionate in patients with moderate to severe chronic obstructive pulmonary disease; the ISOLDE trial. *BMJ* **320**:1297–1303.
- Calikoglu M, Sahin G, Unlu A, Ozturk C, Tamer L, Ercan B, Kanik A, and Atik U (2004) Leptin and TNF- $\alpha$  levels in patients with chronic obstructive pulmonary disease and their relationship to nutritional parameters. *Respiration* **71**:45–50.
- Calikoglu M, Unlu A, Tamer L, Ercan B, Bugdayci R, and Atik U (2002) The levels of serum vitamin C, malonyldialdehyde and erythrocyte reduced glutathione in chronic obstructive pulmonary disease and in healthy smokers. *Clin Chem Lab Med* **40**:1028–1031.
- Campbell EJ, Campbell MA, and Owen CA (2000) Bioactive proteinase 3 on the cell surface of human neutrophils: quantification, catalytic activity and susceptibility to inhibition. *J Immunol* **165**:3366–3374.
- Cantin AM, Fells GA, Hubbard RC, and Crystal RG (1990) Antioxidant macromolecules in the epithelial lining fluid of the normal human lower respiratory tract. *J Clin Invest* **86**:962–971.
- Capelli A, Di Stefano A, Gnemmi I, Balbo P, Cerutti CG, Balbi B, Lusuardi M, and Donner CF (1999) Increased MCP-1 and MIP-1 $\beta$  in bronchoalveolar lavage fluid of chronic bronchitis. *Eur Respir J* **14**:160–165.
- Caramori G, Romagnoli M, Casolari P, Bellettato C, Casoni G, Boschetto P, Fan CK, Barnes PJ, Adcock IM, Ciaccia A, et al. (2003) Nuclear localisation of p65 in sputum macrophages but not in sputum neutrophils during COPD exacerbations. *Thorax* **58**:348–351.
- Carp H and Janoff A (1978) Possible mechanisms of emphysema in smokers. In vitro suppression of serum elastase-inhibitory capacity by fresh cigarette smoke and its prevention by antioxidants. *Am Rev Respir Dis* **118**:617–621.
- Carrell RW and Lomas DA (2002)  $\alpha$ 1-Antitrypsin deficiency—a model for conformational diseases. *N Engl J Med* **346**:45–53.
- Carter AB, Monick MM, and Hunninghake GW (1998) Lipopolysaccharide-induced NF- $\kappa$ B activation and cytokine release in human alveolar macrophages is PKC-independent and TK- and PC-PLC- dependent. *Am J Respir Cell Mol Biol* **18**:384–391.
- Casolaro MA, Bernaudin JF, Saltini C, Ferrans VJ, and Crystal RG (1988) Accumulation of Langerhans' cells on the epithelial surface of the lower respiratory tract in normal subjects in association with cigarette smoking. *Am Rev Respir Dis* **137**:406–411.
- Cataldo D, Munaut C, Noel A, Franckene F, Bartsch P, Foidart JM, and Louis R (2000) MMP-2- and MMP-9-linked gelatinolytic activity in the sputum from patients with asthma and chronic obstructive pulmonary disease. *Int Arch Allergy Immunol* **123**:259–267.
- Cawston T, Carrere S, Catterall J, Duggleby R, Elliott S, Shingleton B, and Rowan A (2001) Matrix metalloproteinases and TIMPs: properties and implications for the treatment of chronic obstructive pulmonary disease. *Novartis Found Symp* **234**:205–218.
- Chalmers GW, Macleod KJ, Sriram S, Thomson LJ, McSharry C, Stack BH, and Thomson NC (1999) Sputum endothelin-1 is increased in cystic fibrosis and chronic obstructive pulmonary disease. *Eur Respir J* **13**:1288–1292.
- Chapman HA, Riese RJ, and Shi GP (1997) Emerging roles for cysteine proteases in human biology. *Annu Rev Physiol* **59**:63–88.
- Chodniewicz D and Zhelev DV (2003) Novel pathways of F-actin polymerization in the human neutrophil. *Blood* **102**:2251–2258.
- Choi AM and Alam J (1996) Heme oxygenase-1: function, regulation and implication of a novel stress-inducible protein in oxidant-induced lung injury. *Am J Respir Cell Mol Biol* **15**:9–19.
- Chung KF (2001) Cytokines in chronic obstructive pulmonary disease. *Eur Respir J Suppl* **34**:50s–59s.
- Chung KF and Barnes PJ (1999) Cytokines in asthma. *Thorax* **54**:825–857.
- Churg A, Wang RD, Tai H, Wang X, Xie C, Dai J, Shapiro SD, and Wright JL (2003) Macrophage metalloelastase mediates acute cigarette smoke-induced inflammation via tumor necrosis factor- $\alpha$  release. *Am J Respir Crit Care Med* **167**:1083–1089.
- Churg A, Wang RD, Tai H, Wang X, Xie C, and Wright JL (2004) Tumor necrosis factor- $\alpha$  drives 70% of cigarette smoke-induced emphysema in the mouse. *Am J Respir Crit Care Med* **170**:492–498.
- Clark-Lewis I, Mattioli I, Gong JH, and Loetscher P (2003) Structure-function relationship between the human chemokine receptor CXCR3 and its ligands. *J Biol Chem* **278**:289–295.
- Comhair SA and Erzurum SC (2002) Antioxidant responses to oxidant-mediated lung diseases. *Am J Physiol Lung Cell Mol Physiol* **283**:L246–L255.
- Condliffe AM, Kitchen E, and Chilvers ER (1998) Neutrophil priming: pathophysiological consequences and underlying mechanisms. *Clin Sci (Lond)* **94**:461–471.
- Corradi M, Majori M, Cacciani GC, Consigli GF, de'Unari E, and Pesci A (1999) Increased exhaled nitric oxide in patients with stable chronic obstructive pulmonary disease. *Thorax* **54**:572–575.
- Corradi M, Montuschi P, Donnelly LE, Pesci A, Kharitonov SA, and Barnes PJ (2001) Increased nitrosothiols in exhaled breath condensate in inflammatory airway diseases. *Am J Respir Crit Care Med* **163**:854–858.
- Corradi M, Rubinstein I, Andreoli R, Manini P, Caglieri A, Poli D, Alinovi R, and Mutti A (2003) Aldehydes in exhaled breath condensate of patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* **167**:1380–1386.
- Cosio MG, Majo J, and Cosio MG (2002) Inflammation of the airways and lung parenchyma in COPD: role of T cells. *Chest* **121** (Suppl):160S–165S.
- Crooks SW, Bayley DL, Hill SL, and Stockley R (2000) Bronchial inflammation in acute bacterial exacerbations of chronic bronchitis: the role of leukotriene B<sub>4</sub>. *Eur Respir J* **15**:274–280.
- Csoma Z, Kharitonov SA, Balint B, Bush A, Wilson NM, and Barnes PJ (2002) Increased leukotrienes in exhaled breath condensate in childhood asthma. *Am J Respir Crit Care Med* **166**:1345–1349.
- Culpitt SV, Nightingale JA, and Barnes PJ (1999) Effect of high dose inhaled steroid on cells, cytokines and proteases in induced sputum in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* **160**:1635–1639.
- Culpitt SV, Rogers DF, Shah P, de Matos C, Russell RE, Donnelly LE, and Barnes PJ (2003) Impaired inhibition by dexamethasone of cytokine release by alveolar macrophages from patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* **167**:24–31.
- Cuzzocrea S, Riley DP, Caputi AP, and Salvemini D (2001) Antioxidant therapy: a new pharmacological approach in shock, inflammation and ischemia/reperfusion injury. *Pharmacol Rev* **53**:135–159.
- DaCosta BS, Major C, Laping NJ, and Roberts AB (2004) SB-505124 is a selective inhibitor of transforming growth factor- $\beta$  type I receptors ALK4, ALK5 and ALK7. *Mol Pharmacol* **65**:744–752.
- Dallas SL, Rosser JL, Mundy GR, and Bonewald LF (2002) Proteolysis of latent transforming growth factor- $\beta$  (TGF- $\beta$ )-binding protein-1 by osteoclasts. A cellular mechanism for release of TGF- $\beta$  from bone matrix. *J Biol Chem* **277**:21352–21360.
- Damiano VV, Tsang A, Kucich U, Abrams WR, Rosenbloom J, Kimbel P, Fallahnejad M, and Weinbaum G (1986) Immunolocalization of elastase in human emphysematous lungs. *J Clin Invest* **78**:482–493.
- Davi G, Basili S, Vieri M, Cipollone F, Santarone S, Alessandri C, Gazzaniga P, Cordova C, and Violi F (1997) Enhanced thromboxane biosynthesis in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* **156**:1794–1799.
- Debigare R, Marquis K, Cote CH, Tremblay RR, Michaud A, Leblanc P, and Maltais F (2003) Catabolic/anabolic balance and muscle wasting in patients with COPD. *Chest* **124**:83–89.
- de Boer WI, Sont JK, van Schadewijk A, Stolk J, van Krieken JH, and Hiemstra PS (2000) Monocyte chemoattractant protein 1, interleukin 8 and chronic airways inflammation in COPD. *J Pathol* **190**:619–626.
- de Boer WI, van Schadewijk A, Sont JK, Sharma HS, Stolk J, Hiemstra PS, and van Krieken JH (1998) Transforming growth factor  $\beta$ 1 and recruitment of macrophages and mast cells in airways in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* **158**:1951–1957.
- DeForge LE, Preston AM, Takeuchi E, Kenney J, Boxer LA, and Remick DG (1993) Regulation of interleukin 8 gene expression by oxidant stress. *J Biol Chem* **268**:25568–25576.
- de Godoy I, Donahoe M, Calhoun WJ, Mancino J, and Rogers RM (1996) Elevated TNF- $\alpha$  production by peripheral blood monocytes of weight-losing COPD patients. *Am J Respir Crit Care Med* **153**:633–637.
- de Jong JW, Belt-Gritter B, Koeter GH, and Postma DS (1997) Peripheral blood lymphocyte cell subsets in subjects with chronic obstructive pulmonary disease: association with smoking, IgE and lung function. *Respir Med* **91**:67–76.
- Dekhuijzen PNR, Aben KHH, Dekker I, Aarts LPHJ, Wielders PLM, van Herwarden CLA, and Bast A (1996) Increased exhalation of hydrogen peroxide in patients with stable and unstable chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* **154**:813–816.
- Demoly P, Simony-Lafontaine J, Chanez P, Pujol JL, Lequeux N, Michel FB, and Bousquet J (1994) Cell proliferation in the bronchial mucosa of asthmatics and chronic bronchitics. *Am J Respir Crit Care Med* **150**:214–217.
- D'Hulst A, Vermeulen KY, and Pauwels RA (2002) Cigarette smoke exposure causes increase in pulmonary dendritic cells. *Am J Respir Crit Care Med* **164**:A604.
- Di Francia M, Barbier D, Mege JL, and Orehek J (1994) Tumor necrosis factor- $\alpha$  levels and weight loss in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* **150**:1453–1455.
- DiMango E, Ratner AJ, Bryan R, Tabibi S, and Prince A (1998) Activation of NF- $\kappa$ B by adherent *Pseudomonas aeruginosa* in normal and cystic fibrosis respiratory epithelial cells. *J Clin Invest* **101**:2598–2605.
- Di Stefano A, Capelli A, Lusuardi M, Balbo P, Vecchio C, Maestrelli P, Mapp CE, Fabbri LM, Donner CF, and Saetta M (1998) Severity of airflow limitation is associated with severity of airway inflammation in smokers. *Am J Respir Crit Care Med* **158**:1277–1285.
- Di Stefano A, Caramori G, Capelli A, Gnemmi I, Ricciardolo F, Oates T, Donner CF, Chung KF, Barnes PJ, and Adcock IM (2004) STAT4 activation in smokers and patients with chronic obstructive pulmonary disease. *Eur Respir J* **24**:78–85.

- Di Stefano A, Caramori G, Capelli A, Lusuardi M, Gnemmi I, Ioli F, Chung KF, Donner CF, Barnes PJ, and Adcock IM (2002) Increased expression of NF- $\kappa$ B in bronchial biopsies from smokers and patients with COPD. *Eur Respir J* **20**:556–563.
- Di Stefano A, Maestrelli P, Roggeri A, Turato G, Calabro S, Potena A, Mapp CE, Ciaccia A, Covacev L, and Fabbri LM (1994) Upregulation of adhesion molecules in the bronchial mucosa of subjects with chronic obstructive bronchitis. *Am J Respir Crit Care Med* **149**:803–810.
- Durant J and Bieth JG (2003) Inhibition of proteinase 3 by  $\alpha$ 1-antitrypsin in vitro predicts very fast inhibition in vivo. *Am J Respir Cell Mol Biol* **29**:57–61.
- Eiserich JP, Hristova M, Cross CE, Jones AD, Freeman BA, Halliwell B, and van der Vliet A (1998) Formation of nitric oxide-derived inflammatory oxidants by myeloperoxidase in neutrophils. *Nature (Lond)* **391**:393–397.
- Fabbri L, Beghe B, Caramori G, Papi A, and Saetta M (1998) Similarities and discrepancies between exacerbations of asthma and chronic obstructive pulmonary disease. *Thorax* **53**:803–808.
- Fabbri LM, Romagnoli M, Corbetta L, Casoni G, Busljetic K, Turato G, Ligabue G, Ciaccia A, Saetta M, and Papi A (2003) Differences in airway inflammation in patients with fixed airflow obstruction due to asthma or chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* **167**:418–424.
- Fadok VA, Bratton DL, Rose DM, Pearson A, Ezekewitz RA, and Henson PM (2000) A receptor for phosphatidylserine-specific clearance of apoptotic cells. *Nature (Lond)* **405**:85–90.
- Faller M, Kessler R, Sapin R, Chaouat A, Ehrhart M, Ducolone A, and Weitzenblum E (1998) Regulation of endothelin-1 at rest and during a short steady-state exercise in 21 COPD patients. *Pulm Pharmacol Ther* **11**:151–157.
- Farshid AA, Sadeghi-Hashjin G, and Ferdowsi HR (2002) Histopathological studies on the effects of peroxytrite on the lungs and trachea of rabbits. *Eur Respir J* **20**:1014–1016.
- Ferrarotti I, Zorzetto M, Beccaria M, Gile LS, Porta R, Ambrosino N, Pignatti PF, Cerveri I, Pozzi E, and Luisetti M (2003) Tumour necrosis factor family genes in a phenotype of COPD associated with emphysema. *Eur Respir J* **21**:444–449.
- Finkelstein R, Fraser RS, Ghezzi H, and Cosio MG (1995) Alveolar inflammation and its relation to emphysema in smokers. *Am J Respir Crit Care Med* **152**:1666–1672.
- Finlay GA, O'Driscoll LR, Russell KJ, D'Arcy EM, Masterson JB, FitzGerald MX, and O'Connor CM (1997) Matrix metalloproteinase expression and production by alveolar macrophages in emphysema. *Am J Respir Crit Care Med* **156**:240–247.
- Fischer B and Voynow J (2000) Neutrophil elastase induces MUC5AC messenger RNA expression by an oxidant-dependent mechanism. *Chest* **117 (Suppl 1)**:317S–320S.
- Fischer BM and Voynow JA (2002) Neutrophil elastase induces MUC5AC gene expression in airway epithelium via a pathway involving reactive oxygen species. *Am J Respir Cell Mol Biol* **26**:447–452.
- Floreani AA, Wyatt TA, Stoner J, Sanderson SD, Thompson EG, Allen-Gipson D, and Heires AJ (2003) Smoke and C5a induce airway epithelial ICAM-1 and cell adhesion. *Am J Respir Cell Mol Biol* **29**:472–482.
- Forman HJ and Torres M (2002) Reactive oxygen species and cell signaling: respiratory burst in macrophage signaling. *Am J Respir Crit Care Med* **166**:S4–S8.
- Fox AJ, Lalloo UG, Belvisi MG, Bernareggi M, Chung KF, and Barnes PJ (1996) Bradykinin-evoked sensitization of airway sensory nerves: a mechanism for ACE-inhibitor cough. *Nat Med* **2**:814–817.
- Francus T, Klein RF, Staiano-Coico L, Becker CG, and Siskind GW (1988) Effects of tobacco glycoprotein (TGP) on the immune system. II. TGP stimulates the proliferation of human T cells and the differentiation of human B cells into Ig secreting cells. *J Immunol* **140**:1823–1829.
- Franklin WA, Veve R, Hirsch FR, Helfrich BA, and Bunn Jr PA (2002) Epidermal growth factor receptor family in lung cancer and premalignancy. *Semin Oncol* **29**:3–14.
- Fujimoto K, Imaizumi T, Yoshida H, Takanashi S, Okumura K, and Satoh K (2001) Interferon- $\gamma$  stimulates fractalkine expression in human bronchial epithelial cells and regulates mononuclear cell adherence. *Am J Respir Cell Mol Biol* **25**:233–238.
- Gaut JP, Byun J, Tran HD, Lauber WM, Carroll JA, Hotchkiss RS, Belaouaj A, and Heinecke JW (2002) Myeloperoxidase produces nitrating oxidants in vivo. *J Clin Invest* **109**:1311–1319.
- Gearing AJ, Beckett P, Christodoulou M, Churchill M, Clements J, Davidson AH, Drummond AH, Galloway WA, Gilbert R, and Gordon JL (1994) Processing of tumour necrosis factor- $\alpha$  precursor by metalloproteinases. *Nature (Lond)* **370**:555–557.
- Geiser T, Dewald B, Ehrenguber MU, Clark-Lewis I, and Baggiolini M (1993) The interleukin-8-related chemotactic cytokines GRO $\alpha$ , GRO $\beta$  and GRO $\gamma$  activate human neutrophil and basophil leukocytes. *J Biol Chem* **268**:15419–15424.
- Germopre PR, Bullock GR, Lambrecht BN, Van De Velde V, Luyten WH, Joos GF, and Pauwels RA (1999) Presence of substance P and neurokinin 1 receptors in human sputum macrophages and U-937 cells. *Eur Respir J* **14**:776–782.
- Giaid A, Yanagisawa M, Langleben D, Michel RP, Levy R, Shenib M, Kimura S, Masaki T, Duguid W, and Stewart DJ (1993) Expression of endothelin-1 in the lungs of patients with pulmonary hypertension. *N Engl J Med* **328**:1732–1739.
- Godoy I, Campana AO, Geraldo RR, Padovani CR, and Paiva SA (2003) Cytokines and dietary energy restriction in stable chronic obstructive pulmonary disease patients. *Eur Respir J* **22**:920–925.
- Gompertz S, O'Brien C, Bayley DL, Hill SL, and Stockley RA (2001) Changes in bronchial inflammation during acute exacerbations of chronic bronchitis. *Eur Respir J* **17**:1112–1119.
- Gorini M, Lupi A, Viglio S, Pamparana F, Cetta G, Iadarola P, Powers JC, and Luisetti M (2001) Inhibition of human neutrophil elastase by erythromycin and flurythromycin, two macrolide antibiotics. *Am J Respir Cell Mol Biol* **25**:492–499.
- Goswami SK, Kivity S, and Marom Z (1990) Erythromycin inhibits respiratory glycoconjugate secretion from human airways in vitro. *Am Rev Respir Dis* **141**:72–78.
- Gotoh Y and Cooper JA (1998) Reactive oxygen species- and dimerization-induced activation of apoptosis signal-regulating kinase 1 in tumor necrosis factor- $\alpha$  signal transduction. *J Biol Chem* **273**:17477–17482.
- Gottlieb DJ, Stone PJ, Sparrow D, Gale ME, Weiss ST, Snider GL, and O'Connor GT (1996) Urinary desmosine excretion in smokers with and without rapid decline of lung function: the Normative Aging Study. *Am J Respir Crit Care Med* **154**:1290–1295.
- Grandjean EM, Berthet P, Ruffmann R, and Leuenberger P (2000) Efficacy of oral long-term N-acetylcysteine in chronic bronchopulmonary disease: a meta-analysis of published double-blind, placebo-controlled clinical trials. *Clin Ther* **22**:209–221.
- Gutierrez-Ramos JC, Lloyd C, Kapsenberg ML, Gonzalo JA, and Coyle AJ (2000) Non-redundant functional groups of chemokines operate in a coordinate manner during the inflammatory response in the lung. *Immunol Rev* **177**:31–42.
- Haddad EB, Fox AJ, Rouseil J, Burgess G, McIntyre P, Barnes PJ, and Chung KF (2000) Post-transcriptional regulation of bradykinin B<sub>1</sub> and B<sub>2</sub> receptor gene expression in human lung fibroblasts by tumor necrosis factor- $\alpha$ : modulation by dexamethasone. *Mol Pharmacol* **57**:1123–1131.
- Haddad J (2004) Redox and oxidant-mediated regulation of apoptosis signaling pathways: immuno-pharmaco-redox conception of oxidative siege versus cell death commitment. *Int Immunopharmacol* **4**:475–493.
- Hageman GJ, Larik I, Pennings HJ, Haenen GR, Wouters EF, and Bast A (2003) Systemic poly(ADP-ribose) polymerase-1 activation, chronic inflammation and oxidative stress in COPD patients. *Free Radic Biol Med* **35**:140–148.
- Hansel TT, Kharitonov SA, Donnelly LE, Erin EM, Currie MG, Moore WM, Manning PT, Recker DP, and Barnes PJ (2003) A selective inhibitor of inducible nitric oxide synthase inhibits exhaled breath nitric oxide in healthy volunteers and asthmatics. *FASEB J* **17**:1298–1300.
- Hardaker EL, Bacon AM, Carlson K, Roshak AK, Foley JJ, Schmidt DB, Buckley PT, Comegys M, Panettieri Jr RA, Sarau HM, et al. (2004) Regulation of TNF- $\alpha$  and IFN- $\gamma$  induced CXCL10 expression: participation of the airway smooth muscle in the pulmonary inflammatory response in chronic obstructive pulmonary disease. *FASEB J* **18**:191–193.
- Hashimoto S, Kobayashi A, Kooguchi K, Kitamura Y, Onodera H, and Nakajima H (2000) Upregulation of two death pathways of perforin/granzyme and FasL/Fas in septic acute respiratory distress syndrome. *Am J Respir Crit Care Med* **161**:237–243.
- Hautamaki RD, Kobayashi DK, Senior RM, and Shapiro SD (1997) Requirement for macrophage metalloelastase for cigarette smoke-induced emphysema in mice. *Science (Wash DC)* **277**:2002–2004.
- Hay DWP and Sarau HM (2001) Interleukin-8 receptor antagonists in pulmonary diseases. *Curr Opin Pharmacol* **1**:242–247.
- Hellermann GR, Nagy SB, Kong X, Lockey RF, and Mohapatra SS (2002) Mechanism of cigarette smoke condensate-induced acute inflammatory response in human bronchial epithelial cells. *Respir Res* **3**:22–28.
- Henricks PA and Nijkamp FP (2001) Reactive oxygen species as mediators in asthma. *Pulm Pharmacol Ther* **14**:409–420.
- Higham MA, Pride NB, Alikhan A, and Morrell NW (2000) Tumour necrosis factor- $\alpha$  gene promoter polymorphism in chronic obstructive pulmonary disease. *Eur Respir J* **15**:281–284.
- Hill AT, Bayley D, and Stockley RA (1999) The interrelationship of sputum inflammatory markers in patients with chronic bronchitis. *Am J Respir Crit Care Med* **160**:893–898.
- Hill AT, Campbell EJ, Hill SL, Bayley DL, and Stockley RA (2000) Association between airway bacterial load and markers of airway inflammation in patients with stable chronic bronchitis. *Am J Med* **109**:288–295.
- Hirano K, Sakamoto T, Uchida Y, Morishima Y, Masuyama K, Ishii Y, Nomura A, Ohtsuka M, and Sekizawa K (2001) Tissue inhibitor of metalloproteinases-2 gene polymorphisms in chronic obstructive pulmonary disease. *Eur Respir J* **18**:748–752.
- Hodge SJ, Hodge GL, Reynolds PN, Scicchitano R, and Holmes M (2003) Increased production of TGF- $\beta$  and apoptosis of T lymphocytes isolated from peripheral blood in COPD. *Am J Physiol Lung Cell Mol Physiol* **285**:L492–L499.
- Hogg JC, Utokaparch S, Yamada Y, Elliott WM, Buzatu L, and Cherniack RM (2004) The nature of small airway obstruction in chronic pulmonary obstructive disease. *N Engl J Med* **350**:2645–2653.
- Hogg JC and Walker BA (1995) Polymorphonuclear leucocyte traffic in lung inflammation. *Thorax* **50**:819–820.
- Hokuto I, Perl AK, and Whitsett JA (2003) Prenatal, but not postnatal, inhibition of fibroblast growth factor receptor signaling causes emphysema. *J Biol Chem* **278**:415–421.
- Holt PG and Stumbles PA (2000) Regulation of immunologic homeostasis in peripheral tissues by dendritic cells: the respiratory tract as a paradigm. *J Allergy Clin Immunol* **105**:421–429.
- Horiguchi T, Tachikawa S, Kondo R, Shiga M, Hirose M, and Fukumoto K (2002) Study on the usefulness of seratrostat in the treatment of chronic pulmonary emphysema. *Arzneim-Forsch* **52**:764–768.
- Huang Q, Liu D, Majewski P, Schulte LC, Korn JM, Young RA, Lander ES, and Hacohen N (2001) The plasticity of dendritic cell responses to pathogens and their components. *Science (Wash DC)* **294**:870–875.
- Huang SL, Su CH, and Chang SC (1997) Tumor necrosis factor- $\alpha$  gene polymorphism in chronic bronchitis. *Am J Respir Crit Care Med* **156**:1436–1439.
- Hulsman AR, Raatgep R, Saxena PR, Kerrebijn KF, and de Jongste JC (1994) Bradykinin-induced contraction of human peripheral airways mediated by both bradykinin B<sub>2</sub> and thromboxane receptors. *Am J Respir Crit Care Med* **150**:1012–1018.
- Hutchison KA, Matic G, Meshinchi S, Bresnick EH, and Pratt WB (1991) Redox manipulation of DNA binding activity and BuGR epitope reactivity of the glucocorticoid receptor. *J Biol Chem* **266**:10505–10509.
- Huynh ML, Fadok VA, and Henson PM (2002) Phosphatidylserine-dependent ingestion of apoptotic cells promotes TGF- $\beta$  secretion and the resolution of inflammation. *J Clin Invest* **109**:41–50.
- Ichinose M, Sugiura H, Yamagata S, Koarai A, and Shirato K (2000) Increase in

- reactive nitrogen species production in chronic obstructive pulmonary disease airways. *Am J Respir Crit Care Med* **160**:701–706.
- Ihn H (2002) Pathogenesis of fibrosis: role of TGF- $\beta$  and CTGF. *Curr Opin Rheumatol* **14**:681–685.
- Imai K, Dalal SS, Chen ES, Downey R, Schulman LL, Ginsburg M, and D'Armiento J (2001) Human collagenase (matrix metalloproteinase-1) expression in the lungs of patients with emphysema. *Am J Respir Crit Care Med* **163**:786–791.
- Imaizumi T, Albertine KH, Jicha DL, McIntyre TM, Prescott SM, and Zimmerman GA (1997) Human endothelial cells synthesize ENA-78: relationship to IL-8 and to signaling of PMN adhesion. *Am J Respir Cell Mol Biol* **17**:181–192.
- Ishii T, Matsuse T, Teramoto S, Matsui H, Hosoi T, Fukuchi Y, and Ouchi Y (2000a) Association between  $\alpha$ 1-antichymotrypsin polymorphism and susceptibility to chronic obstructive pulmonary disease. *Eur J Clin Invest* **30**:543–548.
- Ishii T, Matsuse T, Teramoto S, Matsui H, Miyao M, Hosoi T, Takahashi H, Fukuchi Y, and Ouchi Y (2000b) Neither IL-1 $\beta$ , IL-1 receptor antagonist, nor TNF- $\alpha$  polymorphisms are associated with susceptibility to COPD. *Respir Med* **94**:847–851.
- Ishikawa T, Kume H, Kondo M, Ito Y, Yamaki K, and Shimokata K (2003) Inhibitory effects of interferon- $\gamma$  on the heterologous desensitization of  $\beta$ -adrenoceptors by transforming growth factor- $\beta$ 1 in tracheal smooth muscle. *Clin Exp Allergy* **33**:808–815.
- Ito K, Barnes PJ, and Adcock IM (2000) Glucocorticoid receptor recruitment of histone deacetylase 2 inhibits IL-1 $\beta$ -induced histone H<sub>4</sub> acetylation on lysines 8 and 12. *Mol Cell Biol* **20**:6891–6903.
- Ito K, Lim S, Caramori G, Chung KF, Barnes PJ, and Adcock IM (2001a) Cigarette smoking reduces histone deacetylase 2 expression, enhances cytokine expression and inhibits glucocorticoid actions in alveolar macrophages. *FASEB J* **15**:1100–1102.
- Ito K, Tomita T, Barnes PJ, and Adcock IM (2004) Oxidative stress reduces histone deacetylase (HDAC)2 activity and enhances IL-8 gene expression: role of tyrosine nitration. *Biochem Biophys Res Commun* **315**:240–245.
- Ito K, Watanabe S, Kharitonov S, Hanazawa T, Adcock IM, and Barnes PJ (2001b) Histone deacetylase activity and gene expression in COPD patients. *Eur Respir J* **18**:316S.
- Janssen-Heininger YM, Poynter ME, and Baeuerle PA (2000) Recent advances towards understanding redox mechanisms in the activation of nuclear factor  $\kappa$ B. *Free Radic Biol Med* **28**:1317–1327.
- Janssen LJ (2001) Isoprostanates: an overview and putative roles in pulmonary pathophysiology. *Am J Physiol Lung Cell Mol Physiol* **280**:L1067–L1082.
- Jazrawi E, Cosio BG, Barnes PJ, and Adcock IM (2003) Inhibition of IKK $\alpha$  and JNK differentially regulates GM-CSF and IL-8 release in epithelial cells and alveolar macrophages. *Am J Respir Crit Care Med* **167**:A798.
- Jiang YJ, Lu B, Choy PC, and Hatch GM (2003) Regulation of cytosolic phospholipase A<sub>2</sub>, cyclooxygenase-1 and -2 expression by PMA, TNF $\alpha$ , LPS and M-CSF in human monocytes and macrophages. *Mol Cell Biochem* **246**:31–38.
- Jin FY, Nathan C, Radzioch D, and Ding A (1997) Secretory leukocyte protease inhibitor: a macrophage product induced by and antagonistic to bacterial lipopolysaccharide. *Cell* **88**:417–426.
- Johnston SL, Papi A, Bates PJ, Mastronarde JG, Monick MM, and Hunninghake GW (1998) Low grade rhinovirus infection induces a prolonged release of IL-8 in pulmonary epithelium. *J Immunol* **160**:6172–6181.
- Jones CE and Chan K (2002) Interleukin-17 stimulates the expression of interleukin-8, growth-related oncogene- $\alpha$  and granulocyte-colony-stimulating factor by human airway epithelial cells. *Am J Respir Cell Mol Biol* **26**:748–753.
- Jones KL, Bryan TW, Jinkins PA, Simpson KL, Grisham MB, Owens MW, Milligan SA, Markewitz BA, and Robbins RA (1998) Superoxide released from neutrophils causes a reduction in nitric oxide gas. *Am J Physiol* **275**:L1120–L1126.
- Joos GF and Pauwels RA (2001) Tachykinin receptor antagonists: potential in airways diseases. *Curr Opin Pharmacol* **1**:235–241.
- Joos L, He JQ, Shepherdson MB, Connert JE, Anthonisen NR, Pare PD, and Sandford AJ (2002) The role of matrix metalloproteinase polymorphisms in the rate of decline in lung function. *Hum Mol Genet* **11**:569–576.
- Kanazawa H, Asai K, Hirata K, and Yoshikawa J (2003a) Possible effects of vascular endothelial growth factor in the pathogenesis of chronic obstructive pulmonary disease. *Am J Med* **114**:354–358.
- Kanazawa H, Hirata K, and Yoshikawa J (2003b) Imbalance between vascular endothelial growth factor and endostatin in emphysema. *Eur Respir J* **22**:609–612.
- Kanazawa H, Shiraishi S, Hirata K, and Yoshikawa J (2003c) Imbalance between levels of nitrogen oxides and peroxynitrite inhibitory activity in chronic obstructive pulmonary disease. *Thorax* **58**:106–109.
- Kang MJ, Oh YM, Lee JC, Kim DG, Park MJ, Lee MG, Hyun IG, Han SK, Shim YS, and Jung KS (2003) Lung matrix metalloproteinase-9 correlates with cigarette smoking and obstruction of airflow. *J Korean Med Sci* **18**:821–827.
- Kasahara Y, Tudor RM, Taraseviciene-Stewart L, Le Cras TD, Abman S, Hirth PK, Waltenberger J, and Voelkel NF (2000) Inhibition of VEGF receptors causes lung cell apoptosis and emphysema. *J Clin Invest* **106**:1311–1319.
- Kawikova I, Barnes PJ, Takahashi T, Tadjkarimi S, Yacoub MH, and Belvisi MG (1996) 8-epi-Prostaglandin F<sub>2 $\alpha$</sub> , a novel non-cyclooxygenase derived prostaglandin, is a potent constrictor of guinea-pig and human airways. *Am J Respir Crit Care Med* **153**:590–596.
- Keatings VM and Barnes PJ (1997) Granulocyte activation markers in induced sputum: comparison between chronic obstructive pulmonary disease, asthma and normal subjects. *Am J Respir Crit Care Med* **155**:449–453.
- Keatings VM, Cave SJ, Henry MJ, Morgan K, O'Connor CM, FitzGerald MX, and Kalsheker N (2000) A polymorphism in the tumor necrosis factor- $\alpha$  gene promoter region may predispose to a poor prognosis in COPD. *Chest* **118**:971–975.
- Keatings VM, Collins PD, Scott DM, and Barnes PJ (1996) Differences in interleukin-8 and tumor necrosis factor- $\alpha$  in induced sputum from patients with chronic obstructive pulmonary disease or asthma. *Am J Respir Crit Care Med* **153**:530–534.
- Keatings VM, Jatakanon A, Worsdell YM, and Barnes PJ (1997) Effects of inhaled and oral glucocorticoids on inflammatory indices in asthma and COPD. *Am J Respir Crit Care Med* **155**:542–548.
- Kenyon KW and Nappi JM (2003) Bosentan for the treatment of pulmonary arterial hypertension. *Ann Pharmacother* **37**:1055–1062.
- Kharitonov SA and Barnes PJ (2001) Exhaled markers of pulmonary disease. *Am J Respir Crit Care Med* **163**:1693–1772.
- Killingsworth CR, Shore SA, Alessandrini F, Dey RD, and Paulauskis JD (1997) Rat alveolar macrophages express preprotachykinin gene-I mRNA- encoding tachykinins. *Am J Physiol* **273**:L1073–L1081.
- Kim WD, Kim WS, Koh Y, Lee SD, Lim CM, Kim DS, and Cho YJ (2002) Abnormal peripheral blood T-lymphocyte subsets in a subgroup of patients with COPD. *Chest* **122**:437–444.
- Kokturk N, Tatlicioğlu T, Memis L, Akyurek N, and Akyol G (2003) Expression of transforming growth factor  $\beta$ 1 in bronchial biopsies in asthma and COPD. *J Asthma* **40**:887–893.
- Kranenburg AR, de Boer WI, van Krieken JH, Mooi WJ, Walters JE, Saxena PR, Sterk PJ, and Sharma HS (2002) Enhanced expression of fibroblast growth factors and receptor FGFR-1 during vascular remodeling in chronic obstructive pulmonary disease. *Am J Respir Cell Mol Biol* **27**:517–525.
- Kroeger KM, Steer JH, Joyce DA, and Abraham LJ (2000) Effects of stimulus and cell type on the expression of the -308 tumor necrosis factor promoter polymorphism. *Cytokine* **12**:110–119.
- Kucukcayan M, Van Krugten M, Pennings HJ, Huizinga TW, Buurman WA, Dentener MA, and Wouters EF (2002) Tumor necrosis factor- $\alpha$  +489G/A gene polymorphism is associated with chronic obstructive pulmonary disease. *Respir Res* **3**:29.
- Kusano K, Miyaura C, Inada M, Tamura T, Ito A, Nagase H, Kamoi K, and Suda T (1998) Regulation of matrix metalloproteinases (MMP-2, -3, -9 and -13) by interleukin-1 and interleukin-6 in mouse calvaria: association of MMP induction with bone resorption. *Endocrinology* **139**:1338–1345.
- Kwon OJ, Au BT, Collins PD, Adcock IM, Mak JC, Robbins RA, Baraniuk JM, Chung KF, and Barnes PJ (1994) Tumor necrosis factor-induced interleukin 8 expression in cultured human epithelial cells. *Am J Physiol* **11**:L398–L405.
- Lacoste JY, Bousquet J, and Chanez P (1993) Eosinophilic and neutrophilic inflammation in asthma, chronic bronchitis and chronic obstructive pulmonary disease. *J Allergy Clin Immunol* **92**:537–548.
- Lacraz S, Nicod LP, Chicheportiche R, Welgus HG, and Dayer JM (1995) IL-10 inhibits metalloproteinase and stimulates TIMP-1 production in human mononuclear phagocytes. *J Clin Invest* **96**:2304–2310.
- Langen RC, Korn SH, and Wouters EF (2003) ROS in the local and systemic pathogenesis of COPD. *Free Radic Biol Med* **35**:226–235.
- Langen RC, Schols AM, Kelders MC, Wouters EF, and Janssen-Heininger YM (2001) Inflammatory cytokines inhibit myogenic differentiation through activation of nuclear factor- $\kappa$ B. *FASEB J* **15**:1169–1180.
- Lanone S, Zheng T, Zhu Z, Liu W, Lee CG, Ma B, Chen Q, Homer RJ, Wang J, Rabach LA, et al. (2002) Overlapping and enzyme-specific contributions of matrix metalloproteinases-9 and -12 in IL-13-induced inflammation and remodeling. *J Clin Invest* **110**:463–474.
- Le Barillec K, Si-Tahar M, Balloy V, and Chignard M (1999) Proteolysis of monocyte CD14 by human leukocyte elastase inhibits lipopolysaccharide-mediated cell activation. *J Clin Invest* **103**:1039–1046.
- Leckie MJ, Jenkins GR, Khan J, Smith SJ, Walker C, Barnes PJ, and Hansel TT (2003) Sputum T lymphocytes in asthma, COPD and healthy subjects have the phenotype of activated intraepithelial T cells (CD69+ CD103+). *Thorax* **58**:23–29.
- Lee E, Robertson T, Smith J, and Kilfeather S (2000) Leukotriene receptor antagonists and synthesis inhibitors reverse survival in eosinophils of asthmatic individuals. *Am J Respir Crit Care Med* **161**:1881–1886.
- Lee LY, Kwong K, Lin YS, and Gu Q (2002) Hypersensitivity of bronchopulmonary C-fibers induced by airway mucosal inflammation: cellular mechanisms. *Pulm Pharmacol Ther* **15**:199–204.
- Lei Y-H, Barnes PJ, and Rogers DF (1996) Involvement of hydroxyl radicals in neurogenic airway plasma exudation and bronchoconstriction in guinea pigs in vivo. *Br J Pharmacol* **117**:449–454.
- Lim S, Groneberg D, Fischer A, Oates T, Caramori G, Mattos W, Adcock I, Barnes PJ, and Chung KF (2000a) Expression of heme oxygenase isoenzymes 1 and 2 in normal and asthmatic airways. Effect of inhaled corticosteroids. *Am J Respir Crit Care Med* **162**:1912–1918.
- Lim S, Roche N, Oliver BG, Mattos W, Barnes PJ, and Fan CK (2000b) Balance of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 from alveolar macrophages in cigarette smokers. Regulation by interleukin-10. *Am J Respir Crit Care Med* **162**:1355–1360.
- Linnevers C, Smeekens SP, and Bromme D (1997) Human cathepsin W, a putative cysteine protease predominantly expressed in CD8<sup>+</sup> T-lymphocytes. *FEBS Lett* **405**:253–259.
- Liu H, Lazarus SC, Caughey GH, and Fahy JV (1999) Neutrophil elastase and elastase-rich cystic fibrosis sputum degranulate human eosinophils in vitro. *Am J Physiol* **276**:L28–L34.
- Lloyd CM, Delaney T, Nguyen T, Tian J, Martinez A, Coyle AJ, and Gutierrez-Ramos JC (2000) CC chemokine receptor (CCR)3/eotaxin is followed by CCR4/monocyte-derived chemokine in mediating pulmonary T helper lymphocyte type 2 recruitment after serial antigen challenge in vivo. *J Exp Med* **191**:265–274.
- Lomas DA and Mahadeva R (2002)  $\alpha$ 1-Antitrypsin polymerization and the serpinopathies: pathobiology and prospects for therapy. *J Clin Invest* **110**:1585–1590.
- Lopez AD and Murray CC (1998) The global burden of disease, 1990–2020. *Nat Med* **4**:1241–1243.
- Lucey EC, Keane J, Kuang PP, Snider GL, and Goldstein RH (2002) Severity of elastase-induced emphysema is decreased in tumor necrosis factor- $\alpha$  and interleukin-1 $\beta$  receptor-deficient mice. *Lab Invest* **82**:79–85.
- Luisetti M, Sturani C, Sella D, Madonini E, Galavotti V, Bruno G, Peona V, Kucich U, Dagnino G, Rosenbloom J, et al. (1996) MR889, a neutrophil elastase inhibitor,

in patients with chronic obstructive pulmonary disease: a double-blind, randomized, placebo-controlled clinical trial. *Eur Respir J* **9**:1482–1486.

Lukacs NW (2001) Role of chemokines in the pathogenesis of asthma. *Nat Rev Immunol* **1**:108–116.

Lung Health Study Research Group (2000) Effect of inhaled triamcinolone on the decline in pulmonary function in chronic obstructive pulmonary disease. *N Engl J Med* **343**:1902–1909.

MacNee W (2000) Oxidants/antioxidants and COPD. *Chest* **117** (Suppl 1):303S–317S.

MacNee W (2001) Oxidative stress and lung inflammation in airways disease. *Eur J Pharmacol* **429**:195–207.

MacNee W, Wiggs B, Belzberg AS, and Hogg JC (1989) The effect of cigarette smoking on neutrophil kinetics in human lungs. *N Engl J Med* **321**:924–928.

Maestrelli P, Paska A, Saetta M, Turato G, Nowicki Y, Monti S, Formichi B, Miniati M, and Fabbri LM (2003) Decreased haem oxygenase-1 and increased inducible nitric oxide synthase in the lung of severe COPD patients. *Eur Respir J* **21**:971–976.

Mahadeva R and Lomas DA (1998) Genetics and respiratory disease. 2.  $\alpha$ 1-Antitrypsin deficiency, cirrhosis and emphysema. *Thorax* **53**:501–505.

Majo J, Ghezzi H, and Cosio MG (2001b) Lymphocyte population and apoptosis in the lungs of smokers and their relation to emphysema. *Eur Respir J* **17**:946–953.

Majori M, Corradi M, Caminati A, Cacciani G, Bertacco S, and Pesci A (1999) Predominant TH1 cytokine pattern in peripheral blood from subjects with chronic obstructive pulmonary disease. *J Allergy Clin Immunol* **103**:458–462.

Mak JC, Roussel J, Haddad EB, and Barnes PJ (2000) Transforming growth factor- $\beta$ 1 inhibits  $\beta_2$ -adrenoceptor gene transcription. *Naunyn Schmiedeberg Arch Pharmacol* **362**:520–525.

Mao JT, Tashkin DP, Belloni PN, Baileyhealy I, Baratelli F, and Roth MD (2003) All-trans retinoic acid modulates the balance of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 in patients with emphysema. *Chest* **124**:1724–1732.

Mapp CE, Miotto D, Braccioni F, Saetta M, Turato G, Maestrelli P, Krause JE, Karpitskiy V, Boyd N, Geppetti P, et al. (2000) The distribution of neurokinin-1 and neurokinin-2 receptors in human central airways. *Am J Respir Crit Care Med* **161**:207–215.

Marc MM, Korosec P, Kosnik M, Kern I, Flezar M, Suskovic S, and Sorli J (2004) Complement factors C3a, C4a and C5a in chronic obstructive pulmonary disease and asthma. *Am J Respir Cell Mol Biol* **31**:216–219.

Maziak W, Loukides S, Culpitt S, Sullivan P, Kharitonov SA, and Barnes PJ (1998) Exhaled nitric oxide in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* **157**:998–1002.

Meja KK, Barnes PJ, and Gienbycz MA (1997) Characterization of the prostanoid receptor(s) on human blood monocytes at which prostaglandin  $E_2$  inhibits lipopolysaccharide-induced tumour necrosis factor- $\alpha$ . *Br J Pharmacol* **122**:149–157.

Meshi B, Vitalis TZ, Ionescu D, Elliott WM, Liu C, Wang XD, Hayashi S, and Hogg JC (2002) Emphysematous lung destruction by cigarette smoke. The effects of latent adenoviral infection on the lung inflammatory response. *Am J Respir Cell Mol Biol* **26**:52–57.

Miller AL, Strieter RM, Gruber AD, Ho SB, and Lukacs NW (2003) CXCR2 regulates respiratory syncytial virus-induced airway hyperreactivity and mucus overproduction. *J Immunol* **170**:3348–3356.

Minematsu N, Nakamura H, Tateno H, Nakajima T, and Yamaguchi K (2001) Genetic polymorphism in matrix metalloproteinase-9 and pulmonary emphysema. *Biochem Biophys Res Commun* **289**:116–119.

Mio T, Romberger DJ, Thompson AB, Robbins RA, Heires A, and Rennard SI (1997) Cigarette smoke induces interleukin-8 release from human bronchial epithelial cells. *Am J Respir Crit Care Med* **155**:1770–1776.

Miotto D, Ruggieri MP, Boschetto P, Cavallesco G, Papi A, Bononi I, Piola C, Murer B, Fabbri LM, and Mapp CE (2003) Interleukin-13 and -4 expression in the central airways of smokers with chronic bronchitis. *Eur Respir J* **22**:602–608.

Mirzadegan T, Diehl F, Ebi B, Bhakta S, Polsky I, McCarley D, Mulkins M, Weatherhead GS, Lapiere JM, Dankwardt J, et al. (2000) Identification of the binding site for a novel class of CCR2b chemokine receptor antagonists: binding to a common chemokine receptor motif within the helical bundle. *J Biol Chem* **275**:25562–25571.

Montuschi P and Barnes PJ (2002a) Analysis of exhaled breath condensate for monitoring airway inflammation. *Trends Pharmacol Sci* **23**:232–237.

Montuschi P and Barnes PJ (2002b) Exhaled leukotrienes and prostaglandins in asthma. *J Allergy Clin Immunol* **109**:615–620.

Montuschi P, Collins JV, Ciabattini G, Lazzeri N, Corradi M, Kharitonov SA, and Barnes PJ (2000) Exhaled 8-isoprostane as an in vivo biomarker of lung oxidative stress in patients with COPD and healthy smokers. *Am J Respir Crit Care Med* **162**:1175–1177.

Montuschi P, Kharitonov SA, and Barnes PJ (2001) Exhaled carbon monoxide and nitric oxide in COPD. *Chest* **120**:496–501.

Montuschi P, Kharitonov SA, Ciabattini G, and Barnes PJ (2003) Exhaled leukotrienes and prostaglandins in COPD. *Thorax* **58**:585–588.

Morris DG, Huang X, Kaminski N, Wang Y, Shapiro SD, Dolganov G, Glick A, and Sheppard D (2003) Loss of integrin  $\alpha$ v  $\beta$ 6-mediated TGF- $\beta$  activation causes MMP12-dependent emphysema. *Nature (Lond)* **422**:169–173.

Morrison D, Strieter RM, Donnelly SC, Burdick MD, Kunkel SL, and MacNee W (1998) Neutrophil chemokines in bronchoalveolar lavage fluid and leukocyte-conditioned medium from nonsmokers and smokers. *Eur Respir J* **12**:1067–1072.

Morrow JD (2000) The isoprostanes: their quantification as an index of oxidant stress status in vivo. *Drug Metab Rev* **32**:377–385.

Mostafa ME, Chollet-Martin S, Oudghiri M, Laquay N, Jacob MP, Michel JB, and Feldman LJ (2001) Effects of interleukin-10 on monocyte/endothelial cell adhesion and MMP-9/TIMP-1 secretion. *Cardiovasc Res* **49**:882–890.

Mukaida N (2003) Pathophysiological roles of interleukin-8/CXCL8 in pulmonary diseases. *Am J Physiol* **284**:L566–L577.

Murray CJL, Lopez AD, Mathers CD, and Stein C (2001) The Global Burden of Disease 2000 Project. Global programme on evidence for health policy discussion, paper #36. World Health Organization, Geneva, Switzerland, <http://www3.who.int/whois/burden/papers/discussion%20paper%2036%20revised.doc>.

Nabeyrat E, Jones GE, Fenwick PS, Barnes PJ, and Donnelly LE (2003) Mitogen-activated protein kinases mediate peroxynitrite-induced cell death in human bronchial epithelial cells. *Am J Physiol* **284**:L1112–L1120.

Nadel JA (1990) Decreased neutral endopeptidases: possible role in inflammatory diseases of airways. *Lung* **168**:123–127.

Nadel JA (2000) Role of neutrophil elastase in hypersecretion during COPD exacerbations and proposed therapies. *Chest* **117** (Suppl 2):386S–389S.

Nadel JA and Burchell PR (2001) The role of epidermal growth factor in mucus production. *Curr Opin Pharmacol* **1**:254–258.

Nagaki M, Shimura S, Irokawa T, Sasaki T, Oshiro T, Nara M, Kakuta Y, and Shirato K (1996) Bradykinin regulation of airway submucosal gland secretion: role of bradykinin receptor subtype. *Am J Physiol* **270**:L907–L913.

Nagase H and Brew K (2003) Designing TIMP (tissue inhibitor of metalloproteinases) variants that are selective metalloproteinase inhibitors. *Biochem Soc Symp* **70**:201–212.

Nakamura H, Yoshimura K, Jaffe HA, and Crystal RG (1991a) Interleukin-8 gene expression in human bronchial epithelial cells. *J Biol Chem* **266**:19611–19617.

Nakamura H, Yoshimura K, Jaffe HA, and Crystal RG (1991b) Transcriptional regulation of interleukin-8 gene expression by tumor necrosis factor- $\alpha$  in bronchial epithelial cells. *Am Rev Respir Dis* **143**:A201.

Nakamura H, Yoshimura K, McElvaney NG, and Crystal RG (1992) Neutrophil elastase in respiratory epithelial lining fluid of individuals with cystic fibrosis induces interleukin-8 gene expression in a human bronchial epithelial cell line. *J Clin Invest* **89**:1478–1484.

Newton R, Hart L, Chung KF, and Barnes PJ (2000) Ceramide induction of COX-2 and PGE<sub>2</sub> in pulmonary A549 cells does not involve activation of NF- $\kappa$ B. *Biochem Biophys Res Commun* **277**:675–679.

Newton R, Kuitert LM, Bergmann M, Adcock IM, and Barnes PJ (1997) Evidence for involvement of NF- $\kappa$ B in the transcriptional control of COX-2 gene expression by IL-1 $\beta$ . *Biochem Biophys Res Commun* **237**:28–32.

Nichol G, Nix A, Barnes PJ, and Chung KF (1990) Prostaglandin F<sub>2 $\alpha$</sub>  enhancement of capsaicin induced cough in man: modulation by  $\beta_2$ -adrenergic and anticholinergic drugs. *Thorax* **45**:694–698.

Nocker RE, Schoonbrood DF, Van de Graaf EA, Hack CE, Lutter R, Jansen HM, and Out TA (1996) Interleukin-8 in airway inflammation in patients with asthma and chronic obstructive pulmonary disease. *Int Arch Allergy Immunol* **109**:183–191.

Noguera A, Batle S, Miralles C, Iglesias J, Busquets X, MacNee W, and Agusti AG (2001) Enhanced neutrophil response in chronic obstructive pulmonary disease. *Thorax* **56**:432–437.

Nossaman BD, Dabisch PA, Liles JT, Baber SR, Champion HC, Kaye AD, Feng CJ, Anwar M, Bivalacqua TJ, Santiago JA, et al. (2004) Peroxynitrite does not impair pulmonary and systemic vascular responses. *J Appl Physiol* **96**:455–462.

Nowak D, Kasielski M, Antczak A, Pietras T, and Bialasiewicz P (1999) Increased content of thiobarbituric acid-reactive substances and hydrogen peroxide in the expired breath condensate of patients with stable chronic obstructive pulmonary disease: no significant effect of cigarette smoking. *Respir Med* **93**:389–396.

O'Shaughnessy TC, Ansari TW, Barnes NC, and Jeffery PK (1997) Inflammation in bronchial biopsies of subjects with chronic bronchitis: inverse relationship of CD8<sup>+</sup> T lymphocytes with FEV<sub>1</sub>. *Am J Respir Crit Care Med* **155**:852–857.

Ogawa E, Elliott WM, Hughes F, Eichholtz TJ, Hogg JC, and Hayashi S (2004) Latent adenoviral infection induces production of growth factors relevant to airway remodeling in COPD. *Am J Physiol* **286**:L189–L197.

Ogura M and Kitamura M (1998) Oxidant stress incites spreading of macrophages via extracellular signal-regulated kinases and p38 mitogen-activated protein kinase. *J Immunol* **161**:3569–3574.

Ohbayashi H (2002) Neutrophil elastase inhibitors as treatment for COPD. *Expert Opin Invest Drugs* **11**:965–980.

Ohnishi K, Takagi M, Kurokawa Y, Satomi S, and Kontinen YT (1998) Matrix metalloproteinase-mediated extracellular matrix protein degradation in human pulmonary emphysema. *Lab Invest* **78**:1077–1087.

Ohru T, Sekizawa K, Yamauchi K, Ohkawara Y, Nakazawa H, Aikawa T, Sasaki H, and Takishima T (1991) Chemical oxidant potentiates electrically and acetylcholine-induced contraction in rat trachea: possible involvement of cholinesterase inhibition. *J Pharmacol Exp Ther* **259**:371–376.

Okamoto K, Tanaka H, Ogawa H, Makino Y, Eguchi H, Hayashi S, Yoshikawa N, Poellinger L, Umesono K, and Makino I (1999) Redox-dependent regulation of nuclear import of the glucocorticoid receptor. *J Biol Chem* **274**:10363–10371.

Okazawa A, Kawikova I, Cui ZH, Skoogh BE, and Lotvall J (1997) 8-Epi-PGF<sub>2 $\alpha$</sub>  induces airflow obstruction and airway plasma exudation in vivo. *Am J Respir Crit Care Med* **155**:436–441.

Olson TS and Ley K (2002) Chemokines and chemokine receptors in leukocyte trafficking. *Am J Physiol Regul Integr Comp Physiol* **283**:R7–R28.

Owen CA, Campbell MA, Boukedes SS, and Campbell EJ (1997) Cytokines regulate membrane-bound leukocyte elastase on neutrophils: a novel mechanism for effector activity. *Am J Physiol* **272**:L385–L393.

Owen CA, Hu Z, Barrick B, and Shapiro SD (2003) Inducible expression of tissue inhibitor of metalloproteinases-resistant matrix metalloproteinase-9 on the cell surface of neutrophils. *Am J Respir Cell Mol Biol* **29**:283–294.

Owen CA, Hu Z, Lopez-Otin C, and Shapiro SD (2004) Membrane-bound matrix metalloproteinase-8 on activated polymorphonuclear cells is a potent, tissue inhibitor of metalloproteinase-resistant collagenase and serpinase. *J Immunol* **172**:7791–7803.

Padovan E, Spagnoli GC, Ferrantini M, and Heberer M (2002) IFN- $\alpha$ 2a induces IP-10/CXCL10 and MIG/CXCL9 production in monocyte-derived dendritic cells and enhances their capacity to attract and stimulate CD8<sup>+</sup> effector T cells. *J Leukoc Biol* **71**:669–676.

Palladino MA, Bahjat FR, Theodorakis EA, and Moldaver LL (2003) Anti-TNF- $\alpha$  therapies: the next generation. *Nat Rev Drug Discov* **2**:736–746.

- Panina-Bordignon P and D'Ambrosio D (2003) Chemokines and their receptors in asthma and chronic obstructive pulmonary disease. *Curr Opin Pulm Med* **9**:104–110.
- Panzner P, Lafitte JJ, Tscopoulos A, Hamid Q, and Tulic MK (2003) Marked up-regulation of T lymphocytes and expression of interleukin-9 in bronchial biopsies from patients with chronic bronchitis with obstruction. *Chest* **124**:1909–1915.
- Papi A, Romagnoli M, Baraldo S, Braccioni F, Guzzinati I, Saetta M, Ciaccia A, and Fabbri LM (2000) Partial reversibility of airflow limitation and increased exhaled NO and sputum eosinophilia in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* **162**:1773–1777.
- Paredi P, Kharitonov SA, and Barnes PJ (2002) Analysis of expired air for oxidation products. *Am J Respir Crit Care Med* **166**:S31–S37.
- Paredi P, Kharitonov SA, Leak D, Ward S, Cramer D, and Barnes PJ (2000) Exhaled ethane, a marker of lipid peroxidation, is elevated in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* **162**:369–373.
- Parker LC, Whyte MK, Vogel SN, Dower SK, and Sabroe I (2004) Toll-like receptor (TLR)2 and TLR4 agonists regulate CCR expression in human monocytes. *J Immunol* **172**:4977–4986.
- Patel IS, Seemungal TA, Wilks M, Lloyd-Owen SJ, Donaldson GC, and Wedzicha JA (2002) Relationship between bacterial colonisation and the frequency, character and severity of COPD exacerbations. *Thorax* **57**:759–764.
- Pauwels RA, Lofdahl CG, Laitinen LA, Schouten JP, Postma DS, Pride NB, and Ohlsson SV (1999) Long-term treatment with inhaled budesonide in persons with mild chronic obstructive pulmonary disease who continue smoking. *N Engl J Med* **340**:1948–1953.
- Pavord ID and Tattersfield AE (1995) Bronchoprotective role for endogenous prostaglandin E<sub>2</sub>. *Lancet* **344**:436–438.
- Peleman RA, Ryttila PH, Kips JC, Joos GF, and Pauwels RA (1999) The cellular composition of induced sputum in chronic obstructive pulmonary disease. *Eur Respir J* **13**:839–843.
- Pesci A, Balbi B, Majori M, Cacciani G, Bertacco S, Alciato P, and Donner CF (1998) Inflammatory cells and mediators in bronchial lavage of patients with chronic obstructive pulmonary disease. *Eur Respir J* **12**:380–386.
- Petersen CA and Adler KB (2002) Airways inflammation and COPD: epithelial-neutrophil interactions. *Chest* **121** (Suppl):142S–150S.
- Phillips JE, Hey JA, and Corboz MR (2003) Tachykinin NK<sub>3</sub> and NK<sub>1</sub> receptor activation elicits secretion from porcine airway submucosal glands. *Br J Pharmacol* **138**:254–260.
- Pilette C, Ouadrhiri Y, Godding V, Vaerman JP, and Sibille Y (2001) Lung mucosal immunity: immunoglobulin-A revisited. *Eur Respir J* **18**:571–588.
- Pinamonti S, Leis M, Barbieri A, Leoni D, Muzzoli M, Sostero S, Chicca MC, Carrieri A, Ravenna F, Fabbri LM, et al. (1998) Detection of xanthine oxidase activity products by EPR and HPLC in bronchoalveolar lavage fluid from patients with chronic obstructive pulmonary disease. *Free Radic Biol Med* **25**:771–779.
- Pitsiou G, Kyriazis G, Hatzizisi O, Argyropoulou P, Mavrofridis E, and Patakas D (2002) Tumor necrosis factor- $\alpha$  serum levels, weight loss and tissue oxygenation in chronic obstructive pulmonary disease. *Respir Med* **96**:594–598.
- Poole PJ and Black PN (2001) Oral mucolytic drugs for exacerbations of chronic obstructive pulmonary disease: systematic review. *BMJ* **322**:1271–1274.
- Pratico D, Basili S, Vieri M, Cordova C, Violi F, and Fitzgerald GA (1998) Chronic obstructive pulmonary disease is associated with an increase in urinary levels of isoprostane F<sub>2a</sub>-III, an index of oxidant stress. *Am J Respir Crit Care Med* **158**:1709–1714.
- Prause O, Laan M, Lotvall J, and Linden A (2003) Pharmacological modulation of interleukin-17-induced GCP-2-, GRO- $\alpha$ - and interleukin-8 release in human bronchial epithelial cells. *Eur J Pharmacol* **462**:193–198.
- Prieto A, Reyes E, Bernstein ED, Martinez B, Monserrat J, Izquierdo JL, Collot L, de Lucas P, Alvarez-Sala R, Alvarez-Sala JL, et al. (2001) Defective natural killer and phagocytic activities in chronic obstructive pulmonary disease are restored by glycosphosphopeptid (immunoferon). *Am J Respir Crit Care Med* **163**:1578–1583.
- Profita M, Chiappara G, Mirabella F, Di Giorgi R, Chimenti L, Costanzo G, Riccobono L, Bellia V, Bousquet J, and Vignola AM (2003) Effect of cilomilast (Ariflo) on TNF- $\alpha$ , IL-8 and GM-CSF release by airway cells of patients with COPD. *Thorax* **58**:573–579.
- Proudfoot AE (2002) Chemokine receptors: multifaceted therapeutic targets. *Nat Rev Immunol* **2**:106–115.
- Pryor WA and Stone K (1993) Oxidants in cigarette smoke. Radicals, hydrogen peroxide, peroxynitrate and peroxynitrite. *Ann NY Acad Sci* **686**:12–27.
- Pulleyn LJ, Newton R, Adcock IM, and Barnes PJ (2001) TGF  $\beta$ 1 allele association with asthma severity. *Hum Genet* **109**:623–627.
- Punturieri A, Filippov S, Allen E, Caras I, Murray R, Reddy V, and Weiss SJ (2000) Regulation of elastolytic cysteine proteinase activity in normal and cathepsin K-deficient human macrophages. *J Exp Med* **192**:789–800.
- Qiu Y, Zhu J, Bandi V, Atmar R, Hattotuwa K, Guntupalli K, and Jeffery P (2003) Biopsy neutrophilia, neutrophil chemokine and receptor gene expression in severe exacerbations of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* **168**:968–975.
- Rabinovich RA, Figueras M, Ardite E, Carbo N, Troosters T, Fillella X, Barbera JA, Fernandez-Checa JC, Argiles JM, and Roca J (2003) Increased tumour necrosis factor- $\alpha$  plasma levels during moderate-intensity exercise in COPD patients. *Eur Respir J* **21**:789–794.
- Rabinowitz MH, Andrews RC, Becherer JD, Bickett DM, Bubacz DG, Conway JG, Cowan DJ, Gaul M, Glennon K, Lambert MH, et al. (2001) Design of selective and soluble inhibitors of tumor necrosis factor- $\alpha$  converting enzyme (TACE). *J Med Chem* **44**:4252–4267.
- Rahman I (2003) Oxidative stress, chromatin remodeling and gene transcription in inflammation and chronic lung diseases. *J Biochem Mol Biol* **36**:95–109.
- Rahman I, Morrison D, Donaldson K, and MacNee W (1996) Systemic oxidative stress in asthma, COPD and smokers. *Am J Respir Crit Care Med* **154**:1055–1060.
- Rahman I, van Schadewijk AA, Crowther AJ, Hiemstra PS, Stolk J, MacNee W, and de Boer WI (2002) 4-Hydroxy-2-nonenal, a specific lipid peroxidation product, is elevated in lungs of patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* **166**:490–495.
- Rao NV, Marshall BC, Gray BH, and Hoidal JR (1993) Interaction of secretory leukocyte protease inhibitor with proteinase-3. *Am J Respir Cell Mol Biol* **8**:612–616.
- Rao NV, Wehner NG, Marshall BC, Gray WR, Gray BH, and Hoidal JR (1991) Characterization of proteinase-3 (PR-3), a neutrophil serine proteinase. Structural and functional properties. *J Biol Chem* **266**:9540–9548.
- Reader JR, Hyde DM, Schelegle ES, Aldrich MC, Stoddard AM, McLane MP, Levitt RC, and Tepper JS (2003) Interleukin-9 induces mucous cell metaplasia independent of inflammation. *Am J Respir Cell Mol Biol* **28**:664–672.
- Reddy VY, Zhang QY, and Weiss SJ (1995) Pericellular mobilization of the tissue-destructive cysteine proteinases, cathepsins B, L and S, by human monocyte-derived macrophages. *Proc Natl Acad Sci USA* **92**:3849–3853.
- Reibman J, Hsu Y, Chen LC, Bleck B, and Gordon T (2003) Airway epithelial cells release MIP-3 $\alpha$ /CCL20 in response to cytokines and ambient particulate matter. *Am J Respir Cell Mol Biol* **28**:648–654.
- Repine JE, Bast A, and Lankhorst I (1997) Oxidative stress in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* **156**:341–357.
- Retamales I, Elliott WM, Meshi B, Coxson HO, Pare PD, Sciurba FC, Rogers RM, Hayashi S, and Hogg JC (2001) Amplification of inflammation in emphysema and its association with latent adenoviral infection. *Am J Respir Crit Care Med* **164**:469–473.
- Rhoden KJ and Barnes PJ (1989) Effect of oxygen-derived free radicals on responses of guinea-pig tracheal smooth muscle in vitro. *Br J Pharmacol* **98**:325–330.
- Richards GA, Theron AJ, Van der Merwe CA, and Anderson R (1989) Spirometric abnormalities in young smokers correlate with increased chemiluminescence responses of activated blood phagocytes. *Am Rev Respir Dis* **139**:181–187.
- Rogers DF, Aursudkij B, and Barnes PJ (1989) Effects of tachykinins on mucus secretion on human bronchi in vitro. *Eur J Pharmacol* **174**:283–286.
- Roland M, Bhowmik A, Sapsford RJ, Seemungal TA, Jeffries DJ, Warner TD, and Wedzicha JA (2001) Sputum and plasma endothelin-1 levels in exacerbations of chronic obstructive pulmonary disease. *Thorax* **56**:30–35.
- Rooney CP, Taggart C, Coakley R, McElvaney NG, and O'Neill SJ (2001) Anti-proteinase 3 antibody activation of neutrophils can be inhibited by  $\alpha$ 1-antitrypsin. *Am J Respir Cell Mol Biol* **24**:747–754.
- Rose Jr CE, Sung SS, and Fu SM (2003) Significant involvement of CCL2 (MCP-1) in inflammatory disorders of the lung. *Microcirculation* **10**:273–288.
- Rossi D and Zlotnik A (2000) The biology of chemokines and their receptors. *Annu Rev Immunol* **18**:217–242.
- Rubinstein I, Kumar B, and Schriever C (2004) Long-term montelukast therapy in moderate to severe COPD—a preliminary observation. *Respir Med* **98**:134–138.
- Rupp J, Kothe H, Mueller A, Maass M, and Dalhoff K (2003) Imbalanced secretion of IL-1 $\beta$  and IL-1RA in *Chlamydia pneumoniae*-infected mononuclear cells from COPD patients. *Eur Respir J* **22**:274–279.
- Russell RE, Culpitt SV, DeMatos C, Donnelly L, Smith M, Wiggins J, and Barnes PJ (2002a) Release and activity of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 by alveolar macrophages from patients with chronic obstructive pulmonary disease. *Am J Respir Cell Mol Biol* **26**:602–609.
- Russell RE, Thorley A, Culpitt SV, Dodd S, Donnelly LE, Demattos C, Fitzgerald M, and Barnes PJ (2002b) Alveolar macrophage-mediated elastolysis: roles of matrix metalloproteinases, cysteine and serine proteases. *Am J Physiol Lung Cell Mol Physiol* **283**:L867–L873.
- Rusznak C, Devalia JL, Sapsford RJ, and Davies RJ (1996) Ozone-induced mediator release from human bronchial epithelial cells in vitro and the influence of nedocromil sodium. *Eur Respir J* **9**:2298–2305.
- Rusznak C, Mills PR, Devalia JL, Sapsford RJ, Davies RJ, and Lozewicz S (2000) Effect of cigarette smoke on the permeability and IL-1 $\beta$  and sICAM-1 release from cultured human bronchial epithelial cells of never-smokers, smokers and patients with chronic obstructive pulmonary disease. *Am J Respir Cell Mol Biol* **23**:530–536.
- Rutgers SR, Van der Mark TW, Coers W, Moshage H, Timens W, Kauffman HF, Koeter GH, and Postma DS (1999) Markers of nitric oxide metabolism in sputum and exhaled air are not increased in chronic obstructive pulmonary disease [see comments]. *Thorax* **54**:576–580.
- Saetta M, Baraldo S, Corbino L, Turato G, Braccioni F, Rea F, Cavalleco G, Tropeano G, Mapp CE, Maestrelli P, et al. (1999) CD8+ve cells in the lungs of smokers with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* **160**:711–717.
- Saetta M, Distefano A, Maestrelli P, Graziella T, Rugieri MP, Roggeri A, Calcagni P, Mapp CE, Ciaccia A, and Fabbri LM (1994) Airway eosinophilia in chronic bronchitis during exacerbations. *Am J Respir Crit Care Med* **150**:1646–1652.
- Saetta M, Di Stefano A, Maestrelli P, Turato G, Mapp CE, Pieno M, Zanguochi G, Del Prete G, and Fabbri LM (1996) Airway eosinophilia and expression of interleukin-5 protein in asthma and in exacerbations of chronic bronchitis. *Clin Exp Allergy* **26**:766–774.
- Saetta M, Di Stefano A, Turato G, Facchini FM, Corbino L, Mapp CE, Maestrelli P, Ciaccia A, and Fabbri LM (1998) CD8+ T-lymphocytes in peripheral airways of smokers with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* **157**:822–826.
- Saetta M, Mariani M, Panina-Bordignon P, Turato G, Buonsanti C, Baraldo S, Bellettato CM, Papi A, Corbetta L, Zuin R, et al. (2002) Increased expression of the chemokine receptor CXCR3 and its ligand CXCL10 in peripheral airways of smokers with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* **165**:1404–1409.
- Saetta M, Turato G, Maestrelli P, Mapp CE, and Fabbri LM (2001) Cellular and structural bases of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* **163**:1304–1309.
- Sakao S, Tatsumi K, Hashimoto T, Igari H, Shino Y, Shirasawa H, and Kuriyama T (2003) Vascular endothelial growth factor and the risk of smoking-related COPD. *Chest* **124**:323–327.



- Sakao S, Tatsumi K, Igari H, Watanabe R, Shino Y, Shirasawa H, and Kuriyama T (2002) Association of tumor necrosis factor- $\alpha$  gene promoter polymorphism with low attenuation areas on high-resolution CT in patients with COPD. *Chest* **122**: 416–420.
- Sallenne JM (2000) The role of secretory leukocyte proteinase inhibitor and elafin (elastase-specific inhibitor/skin-derived antileukoprotease) as alarm antiproteases in inflammatory lung disease. *Respir Res* **1**:87–92.
- Sallusto F and Lanzavecchia A (2000) Understanding dendritic cell and T-lymphocyte traffic through the analysis of chemokine receptor expression. *Immunol Rev* **177**:134–140.
- Santos S, Peinado VI, Ramirez J, Morales-Blanchir J, Bastos R, Roca J, Rodriguez-Roisin R, and Barbera JA (2003) Enhanced expression of vascular endothelial growth factor in pulmonary arteries of smokers and patients with moderate chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* **167**:1250–1256.
- Schulz C, Kratzel K, Wolf K, Schroll S, Kohler M, and Pfeifer M (2004) Activation of bronchial epithelial cells in smokers without airway obstruction and patients with COPD. *Chest* **125**:1706–1713.
- Schulz C, Wolf K, Harth M, Kratzel K, Kunz-Schughart L, and Pfeifer M (2003) Expression and release of interleukin-8 by human bronchial epithelial cells from patients with chronic obstructive pulmonary disease, smokers and never-smokers. *Respiration* **70**:254–261.
- Schunemann HJ, Freudenheim JL, and Grant BJ (2001) Epidemiologic evidence linking antioxidant vitamins to pulmonary function and airway obstruction. *Epidemiol Rev* **23**:248–267.
- Seggev JS, Thornton Jr WH, and Edes TE (1991) Serum leukotriene B<sub>4</sub> levels in patients with obstructive pulmonary disease. *Chest* **99**:289–291.
- Selman M, Cisneros-Lira J, Gaxiola M, Ramirez R, Kudlacz EM, Mitchell PG, and Pardo A (2003) Matrix metalloproteinases inhibition attenuates tobacco smoke-induced emphysema in guinea pigs. *Chest* **123**:1633–1641.
- Senior RM, Tegner H, Kuhn K, Ohlsson K, Starcher BC, and Pierce JA (1977) The induction of pulmonary emphysema with human leukocyte elastase. *Am Rev Respir Dis* **116**:469–475.
- Shamsuddin M, Chen E, Anderson J, and Smith LJ (1997) Regulation of leukotriene and platelet-activating factor synthesis in human alveolar macrophages. *J Lab Clin Med* **130**:615–626.
- Shao MX, Nakanaga T, and Nadel JA (2004) Cigarette smoke induces MUC5AC mucin overproduction via tumor necrosis factor- $\alpha$  converting enzyme in human airway epithelial (NCI-H292) cells. *Am J Physiol* **287**:L420–L427.
- Shapiro SD (1999) The macrophage in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* **160**:S29–S32.
- Shapiro SD (2002) Neutrophil elastase: path clearer, pathogen killer, or just pathologic? *Am J Respir Cell Mol Biol* **26**:266–268.
- Shapiro SD, Goldstein NM, Houghton AM, Kobayashi DK, Kelley D, and Belaouaj A (2003) Neutrophil elastase contributes to cigarette smoke-induced emphysema in mice. *Am J Pathol* **163**:2329–2335.
- Shapiro SD and Senior RM (1999) Matrix metalloproteinases. Matrix degradation and more. *Am J Respir Cell Mol Biol* **20**:1100–1102.
- Shim JJ, Dabbagh K, Ueki IF, Dao-Pick T, Burgel PR, Takeyama K, Tam DC, and Nadel JA (2001) IL-13 induces mucin production by stimulating epidermal growth factor receptors and by activating neutrophils. *Am J Physiol* **280**:L134–L140.
- Shimbara A, Christodouloupolos P, Soussi-Gounni A, Olivenstein R, Nakamura Y, Levitt RC, Nicolaides NC, Holroyd KJ, Tscipoulos A, Lafitte JJ, et al. (2000) IL-9 and its receptor in allergic and nonallergic lung disease: increased expression in asthma. *J Allergy Clin Immunol* **105**:108–115.
- Shindo K, Koide K, and Fukumura M (1998) Platelet-activating factor increases leukotriene B<sub>4</sub> release in stimulated alveolar macrophages from asthmatic patients. *Eur Respir J* **11**:1098–1104.
- Sies H and Masumoto H (1997) Ebselen as a glutathione peroxidase mimic and as a scavenger of peroxynitrite. *Adv Pharmacol* **38**:229–246.
- Silbaugh SA, Stengel PW, Cockerham SL, Froelich LL, Bendele AM, Spaeth SM, Sofia MJ, Sawyer JS, and Jackson WT (2000) Pharmacologic actions of the second generation leukotriene B<sub>4</sub> receptor antagonist LY29311: in vivo pulmonary studies. *Naunyn Schmiedebergs Arch Pharmacol* **361**:397–404.
- Silverman ES, Palmer LJ, Subramanian V, Hallock A, Mathew S, Vallone J, Faffe DS, Shikanai T, Raby BA, Weiss ST, et al. (2004) Transforming growth factor- $\beta$ 1 promoter polymorphism C-509T is associated with asthma. *Am J Respir Crit Care Med* **169**:214–219.
- Soler N, Ewig S, Torres A, Filella X, Gonzalez J, and Zaubet A (1999) Airway inflammation and bronchial microbial patterns in patients with stable chronic obstructive pulmonary disease. *Eur Respir J* **14**:1015–1022.
- Soler P, Moreau A, Basset F, and Hance AJ (1989) Cigarette smoking-induced changes in the number and differentiated state of pulmonary dendritic cells/Langerhans cells. *Am Rev Respir Dis* **139**:1112–1117.
- Sommerhoff CP, Nadel JA, Basbaum CB, and Caughey GH (1990) Neutrophil elastase and cathepsin G stimulate secretion from cultured bovine airway gland serous cells. *J Clin Invest* **85**:682–689.
- Song W, Zhao J, and Li Z (2001) Interleukin-6 in bronchoalveolar lavage fluid from patients with COPD. *Chin Med J (Engl)* **114**:1140–1142.
- Sparrow D, Glynn RJ, Cohen M, and Weiss ST (1984) The relationship of the peripheral leukocyte count and cigarette smoking to pulmonary function among adult men. *Chest* **86**:383–386.
- Spicuzza L, Barnes PJ, Di Maria GU, and Belvisi MG (2001) Effect of 8-iso-prostaglandin F<sub>2 $\alpha$</sub>  on acetylcholine release from parasympathetic nerves in guinea pig airways. *Eur J Pharmacol* **416**:231–234.
- Spicuzza L, Giembycz MA, Barnes PJ, and Belvisi MG (1998) Prostaglandin E<sub>2</sub> suppression of acetylcholine release from parasympathetic nerves innervating guinea-pig trachea by interacting with prostanoid receptors of the EP<sub>3</sub>-subtype. *Br J Pharmacol* **123**:1246–1252.
- Spiropoulos K, Trakada G, Nikolaou E, Prodromakis E, Efremidis G, Pouli A, and Koniavitou A (2003) Endothelin-1 levels in the pathophysiology of chronic obstructive pulmonary disease and bronchial asthma. *Respir Med* **97**:983–989.
- Spruit MA, Gosselink R, Troosters T, Kasran A, Gayan-Ramirez G, Bogaerts P, Bouillon R, and Decramer M (2003) Muscle force during an acute exacerbation in hospitalised patients with COPD and its relationship with CXCL8 and IGF-I. *Thorax* **58**:752–756.
- Stamenkovic I (2003) Extracellular matrix remodelling: the role of matrix metalloproteinases. *J Pathol* **200**:448–464.
- Stanescu D, Sanna A, Veriter C, Kostianev S, Callagni PG, Fabbri LM, and Maestrelli P (1996) Airways obstruction, chronic expectoration and rapid decline in FEV<sub>1</sub> in smokers are associated with increased levels of sputum neutrophils. *Thorax* **51**:267–271.
- Stockley RA (2001) Proteases and antiproteases. *Novartis Found Symp* **234**:189–199.
- Stone R, Barnes PJ, and Fuller RW (1992) Contrasting effects of prostaglandins E<sub>2</sub> and F<sub>2 $\alpha$</sub>  on sensitivity of the human cough reflex. *J Appl Physiol* **73**:649–653.
- Strunk RC, Eidlend DM, and Mason RJ (1988) Pulmonary alveolar type II epithelial cells synthesize and secrete proteins of the classical and alternative complement pathways. *J Clin Invest* **81**:1419–1426.
- Sugiura H, Ichinose M, Tomaki M, Ogawa H, Koarai A, Kitamura T, Komaki Y, Akita T, Nishino H, Okamoto S, et al. (2004) Quantitative assessment of protein-bound tyrosine nitration in airway secretions from patients with inflammatory airway disease. *Free Radic Res* **38**:49–57.
- Sunyer J (2001) Urban air pollution and chronic obstructive pulmonary disease: a review. *Eur Respir J* **17**:1024–1033.
- Taggart C, Cervantes-Laurean D, Kim G, McElvaney NG, Wehr N, Moss J, and Levine RL (2000) Oxidation of either methionine 351 or methionine 358 in  $\alpha$ 1-antitrypsin causes loss of anti-neutrophil elastase activity. *J Biol Chem* **275**: 27258–27265.
- Taggart CC, Greene CM, McElvaney NG, and O'Neill S (2002) Secretory leucoprotease inhibitor prevents lipopolysaccharide-induced I $\kappa$ B $\alpha$  degradation without affecting phosphorylation or ubiquitination. *J Biol Chem* **277**:33648–33653.
- Taggart CC, Lowe GJ, Greene CM, Mulgrew AT, O'Neill SJ, Levine RL, and McElvaney NG (2001) Cathepsin B, L, and S cleave and inactivate secretory leucoprotease inhibitor. *J Biol Chem* **276**:33345–33352.
- Taha R, Olivenstein R, Utsumi T, Ernst P, Barnes PJ, Rodger IW, and Gaid A (2000) Prostaglandin H synthase 2 expression in airway cells from patients with asthma and chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* **161**:636–640.
- Takanashi S, Hasegawa Y, Kanehira Y, Yamamoto K, Fujimoto K, Satoh K, and Okamura K (1999) Interleukin-10 level in sputum is reduced in bronchial asthma, COPD and in smokers. *Eur Respir J* **14**:309–314.
- Takeyabu K, Betsuyaku T, Nishimura M, Yoshioka A, Tanino M, Miyamoto K, and Kawakami Y (1998) Cysteine proteinases and cystatin C in bronchoalveolar lavage fluid from subjects with subclinical emphysema. *Eur Respir J* **12**:1033–1039.
- Takeyama K, Agusti C, Ueki I, Lausier J, Cardell LO, and Nadel JA (1998) Neutrophil-dependent goblet cell degranulation: role of membrane-bound elastase and adhesion molecules. *Am J Physiol* **275**:L294–L302.
- Takeyama K, Dabbagh K, Jeong SJ, Dao-Pick T, Ueki IF, and Nadel JA (2000) Oxidative stress causes mucin synthesis via transactivation of epidermal growth factor receptor: role of neutrophils. *J Immunol* **164**:1546–1552.
- Takeyama K, Dabbagh K, Lee HM, Agusti C, Lausier JA, Ueki IF, Grattan KM, and Nadel JA (1999) Epidermal growth factor system regulates mucin production in airways. *Proc Natl Acad Sci USA* **96**:3081–3086.
- Takeyama K, Jung B, Shim JJ, Burgel PR, Dao-Pick T, Ueki IF, Protin U, Kroschel P, and Nadel JA (2001) Activation of epidermal growth factor receptors is responsible for mucin synthesis induced by cigarette smoke. *Am J Physiol* **280**:L165–L172.
- Takizawa H, Tanaka M, Takami K, Ohtoshi T, Ito K, Satoh M, Okada Y, Yamasawa F, Nakahara K, and Umeda A (2001) Increased expression of transforming growth factor- $\beta$ 1 in small airway epithelium from tobacco smokers and patients with chronic obstructive pulmonary disease (COPD). *Am J Respir Crit Care Med* **163**: 1476–1483.
- Takubo Y, Guerassimov A, Ghezzi H, Triantafillopoulos A, Bates JH, Hoidal JR, and Cosio MG (2002)  $\alpha$ 1-Antitrypsin determines the pattern of emphysema and function in tobacco smoke-exposed mice: parallels with human disease. *Am J Respir Crit Care Med* **166**:1596–1603.
- Tamaoki J, Chiyotani A, Kobayashi S, Noritaka T, Kanemura T, and Takizawa T (1992) Effect of indomethacin on bronchorrhea with chronic bronchitis, diffuse panbronchiolitis or bronchiectasis. *Am Rev Respir Dis* **145**:548–552.
- Tamaoki J, Isono K, Takeyama K, Tagaya E, Nakata J, and Nagai A (2004) Ultrafine carbon black particles stimulate proliferation of human airway epithelium via EGF receptor-mediated signaling pathway. *Am J Physiol*, in press.
- Tanino M, Betsuyaku T, Takeyabu K, Tanino Y, Yamaguchi E, Miyamoto K, and Nishimura M (2002) Increased levels of interleukin-8 in BAL fluid from smokers susceptible to pulmonary emphysema. *Thorax* **57**:405–411.
- Tazi A, Moreau J, Bergeron A, Dominique S, Hance AJ, and Soler P (1999) Evidence that Langerhans cells in adult pulmonary Langerhans cell histiocytosis are mature dendritic cells: importance of the cytokine microenvironment. *J Immunol* **163**:3511–3515.
- Tazi A, Soler P, and Hance AJ (2000) Adult pulmonary Langerhans' cell histiocytosis. *Thorax* **55**:405–416.
- Terashima T, Wiggs B, English D, Hogg JC, and van Eeden SF (1997) Phagocytosis of small carbon particles (PM<sub>10</sub>) by alveolar macrophages stimulates the release of polymorphonuclear leukocytes from bone marrow. *Am J Respir Crit Care Med* **155**:1441–1447.
- Tokuyama K, Kuo H-P, Rohde JAL, Barnes PJ, and Rogers DF (1990) Neural control of goblet cell secretion in guinea pig airways. *Am J Physiol* **259**:L108–L115.
- Tomaki M, Ichinose M, Miura M, Hirayama Y, Yamauchi H, Nakajima N, and Shirato K (1995) Elevated substance P content in induced sputum from patients

- with asthma and patients with chronic bronchitis. *Am J Respir Crit Care Med* **151**:613–617.
- Tomita K, Barnes PJ, and Adcock IM (2003) The effect of oxidative stress on histone acetylation and IL-8 release. *Biochem Biophys Res Commun* **301**:572–577.
- Tomita K, Caramori G, Lim S, Ito K, Hanazawa T, Oates T, Chiselita I, Jazrawi E, Chung KF, Barnes PJ, et al. (2002) Increased p21CIP1/WAF1 and B cell lymphoma leukemia-xL expression and reduced apoptosis in alveolar macrophages from smokers. *Am J Respir Crit Care Med* **166**:724–731.
- Toossi Z, Hirsch CS, Hamilton BD, Knuth CK, Friedlander MA, and Rich EA (1996) Decreased production of TGF- $\beta$  1 by human alveolar macrophages compared with blood monocytes. *J Immunol* **156**:3461–3468.
- Trakada G, Marangos M, and Spiropoulos K (2001) Mechanisms of endothelin-1 elevation in chronic obstructive pulmonary disease patients with nocturnal oxymoglobin desaturation. *Respiration* **68**:134–139.
- Traves SL, Culpitt S, Russell REK, Barnes PJ, and Donnelly LE (2002) Elevated levels of the chemokines GRO- $\alpha$  and MCP-1 in sputum samples from COPD patients. *Thorax* **57**:590–595.
- Traves SL, Smith SJ, Barnes PJ, and Donnelly LE (2004) Specific CXC but not CC chemokines cause elevated monocyte migration in COPD: a role for CXCR2. *J Leukoc Biol* **76**:441–450.
- Trevisani M, Schmidlin F, Tognetto M, Nijkamp FP, Gies JP, Frossard N, Amadesi S, Folkerts G, and Geppetti P (1999) Evidence for in vitro expression of B<sub>1</sub> receptor in the mouse trachea and urinary bladder. *Br J Pharmacol* **126**:1293–1300.
- Tsukagoshi H, Haddad E-B, Barnes PJ, and Chung KF (1995) Bradykinin receptor subtypes in rat lung: effect of interleukin-1 $\beta$ . *J Pharmacol Exp Ther* **273**:1257–1263.
- Tuder RM, Zhen L, Cho CY, Taraseviciene-Stewart L, Kasahara Y, Salvemini D, Voelkel NF, and Flores SC (2003) Oxidative stress and apoptosis interact and cause emphysema due to vascular endothelial growth factor receptor blockade. *Am J Respir Cell Mol Biol* **29**:88–97.
- Turato G, Zuin R, and Saetta M (2001) Pathogenesis and pathology of COPD. *Respiration* **68**:117–128.
- Turk V, Turk B, and Turk D (2001) Lysosomal cysteine proteases: facts and opportunities. *EMBO (Eur Mol Biol Organ) J* **20**:4629–4633.
- Tzanakis N, Chrysafakis G, Tsoumakidou M, Kyriakou D, Tsiligianni J, Bourou D, and Siafakas NM (2004) Induced sputum CD8<sup>+</sup> T-lymphocyte subpopulations in chronic obstructive pulmonary disease. *Respir Med* **98**:57–65.
- Van Den Steen PE, Proost P, Wuyts A, Van Damme J, and Opendakker G (2000) Neutrophil gelatinase B potentiates interleukin-8 tenfold by aminoterminal processing, whereas it degrades CTAP-III, PF-4 and GRO- $\alpha$  and leaves RANTES and MCP-2 intact. *Blood* **96**:2673–2681.
- van der Pouw Kraan TC, Kucukaycan M, Bakker AM, Baggen JM, van der Zee JS, Dentener MA, Wouters EF, and Verweij CL (2002) Chronic obstructive pulmonary disease is associated with the -1055 IL-13 promoter polymorphism. *Genes Immun* **3**:436–439.
- van der Vliet A, Eiserich JP, Shigenaga MK, and Cross CE (1999a) Reactive nitrogen species and tyrosine nitration in the respiratory tract: epiphenomena or a pathobiologic mechanism of disease? *Am J Respir Crit Care Med* **160**:1–9.
- van der Vliet A, O'Neill CA, Cross CE, Koostra JM, Volz WG, Halliwell B, and Louie S (1999b) Determination of low-molecular-mass antioxidant concentrations in human respiratory tract lining fluids. *Am J Physiol* **276**:L289–L296.
- Vandivier RW, Fadok VA, Hoffmann PR, Bratton DL, Penvari C, Brown KK, Brain JD, Accurso FJ, and Henson PM (2002) Elastase-mediated phosphatidylserine receptor cleavage impairs apoptotic cell clearance in cystic fibrosis and bronchiectasis. *J Clin Invest* **109**:661–670.
- Vernooy JH, Kucukaycan M, Jacobs JA, Chavannes NH, Buurman WA, Dentener MA, and Wouters EF (2002) Local and systemic inflammation in patients with chronic obstructive pulmonary disease: soluble tumor necrosis factor receptors are increased in sputum. *Am J Respir Crit Care Med* **166**:1218–1224.
- Vestbo J, Sorensen T, Lange P, Brix A, Torre P, and Viskum K (1999) Long-term effect of inhaled budesonide in mild and moderate chronic obstructive pulmonary disease: a randomised controlled trial. *Lancet* **353**:1819–1823.
- Vogelmeier C, Hubbard RC, Fells GA, Schnebli HP, Thompson RC, Fritz H, and Crystal RG (1991) Anti-neutrophil elastase defense of the normal human respiratory epithelial surface provided by the secretory leukoprotease inhibitor. *J Clin Invest* **87**:482–488.
- Voynow JA, Young LR, Wang Y, Horger T, Rose MC, and Fischer BM (1999) Neutrophil elastase increases MUC5AC mRNA and protein expression in respiratory epithelial cells. *Am J Physiol* **276**:L835–L843.
- Vukmanovic-Stejić M, Vyas B, Gorak-Stolinska P, Noble A, and Kemeny DM (2000) Human Tc1 and Tc2/Tc0 CD8 T-cell clones display distinct cell surface and functional phenotypes. *Blood* **95**:231–240.
- Wagner PD (2003) Vascular endothelial growth factor and the pathogenesis of emphysema. *Am J Med* **114**:413–414.
- Wakeling AE (2002) Epidermal growth factor receptor tyrosine kinase inhibitors. *Curr Opin Pharmacol* **2**:382–387.
- Wallace AM and Sandford AJ (2002) Genetic polymorphisms of matrix metalloproteinases: functional importance in the development of chronic obstructive pulmonary disease? *Am J Pharmacogenomics* **2**:167–175.
- Wang B, Cleary PP, Xu H, and Li JD (2003) Up-regulation of interleukin-8 by novel small cytoplasmic molecules of nontypeable *Haemophilus influenzae* via p38 and extracellular signal-regulated kinase pathways. *Infect Immun* **71**:5523–5530.
- Wang Z, Zheng T, Zhu Z, Homer RJ, Riese RJ, Chapman Jr HA, Shapiro SD, and Elias JA (2000) Interferon  $\gamma$  induction of pulmonary emphysema in the adult murine lung. *J Exp Med* **192**:1587–1600.
- Ward PA (2004) The dark side of C5a in sepsis. *Nat Rev Immunol* **4**:133–142.
- Wark PA (2002) DX-890 (Dyax). *IDrugs* **5**:586–589.
- Wedzicha JA, Seemungal TA, MacCallum PK, Paul EA, Donaldson GC, Bhowmik A, Jeffries DJ, and Meade TW (2000) Acute exacerbations of chronic obstructive pulmonary disease are accompanied by elevations of plasma fibrinogen and serum IL-6 levels. *Thromb Haemostasis* **84**:210–215.
- White JR, Lee JM, Young PR, Hertzberg RP, Jurewicz AJ, Chaikin MA, Widdowson K, Foley JJ, Martin LD, Griswold DE, et al. (1998) Identification of a potent, selective non-peptide CXCR2 antagonist that inhibits interleukin-8-induced neutrophil migration. *J Biol Chem* **273**:10095–10098.
- Williams L, Bradley L, Smith A, and Foxwell B (2004) Signal transducer and activator of transcription 3 is the dominant mediator of the anti-inflammatory effects of IL-10 in human macrophages. *J Immunol* **172**:567–576.
- Witherden IR, Vanden Bon EJ, Goldstraw P, Ratcliffe C, Pastorino U, and Tetley TD (2004) Primary human alveolar type II epithelial cell chemokine release: effects of cigarette smoke and neutrophil elastase. *Am J Respir Cell Mol Biol* **30**:500–509.
- Witko-Sarsat V, Halbwachs-Mecarelli L, Schuster A, Nusbaum P, Ueki I, Canteloup S, Lenoir G, Descamps-Latscha B, and Nadel JA (1999) Proteinase 3, a potent secretagogue in airways, is present in cystic fibrosis sputum. *Am J Respir Cell Mol Biol* **20**:729–736.
- Woolhouse IS, Bayley DL, and Stockley RA (2002) Sputum chemotactic activity in chronic obstructive pulmonary disease: effect of  $\alpha_1$ -antitrypsin deficiency and the role of leukotriene B<sub>4</sub> and interleukin 8. *Thorax* **57**:709–714.
- Wright JL, Farmer SG, and Churg A (2002) Synthetic serine elastase inhibitor reduces cigarette smoke-induced emphysema in guinea pigs. *Am J Respir Crit Care Med* **166**:954–960.
- Wu L, Chau J, Young RP, Pokorny V, Mills GD, Hopkins R, McLean L, and Black PN (2004) Transforming growth factor- $\beta$ 1 genotype and susceptibility to chronic obstructive pulmonary disease. *Thorax* **59**:126–129.
- Xanthoudakis S and Curran T (1996) Redox regulation of AP-1: a link between transcription factor signaling and DNA repair. *Adv Exp Med Biol* **387**:69–75.
- Yamamoto C, Yoneda T, Yoshikawa M, Fu A, Tokuyama T, Tsukaguchi K, and Narita N (1997) Airway inflammation in COPD assessed by sputum levels of interleukin-8. *Chest* **112**:505–510.
- Yang XD, Corvalan JR, Wang P, Roy CM, and Davis CG (1999) Fully human anti-interleukin-8 monoclonal antibodies: potential therapeutics for the treatment of inflammatory disease states. *J Leukoc Biol* **66**:401–410.
- Yokomizo T, Kato K, Terawaki K, Izumi T, and Shimizu T (2000) A second leukotriene B<sub>4</sub> receptor BLT2. A new therapeutic target in inflammation and immunological disorders. *J Exp Med* **192**:421–432.
- Yoshioka A, Betsuyaku T, Nishimura M, Miyamoto K, Kondo T, and Kawakami Y (1995) Excessive neutrophil elastase in bronchoalveolar lavage fluid in subclinical emphysema. *Am J Respir Crit Care Med* **152**:2127–2132.
- Yu Q and Stamenkovic I (2000) Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF- $\beta$  and promotes tumor invasion and angiogenesis. *Genes Dev* **14**:163–176.
- Zeid NA and Muller HK (1995) Tobacco smoke induced lung granulomas and tumors: association with pulmonary Langerhans cells. *Pathology* **27**:247–254.
- Zeidel A, Beilin B, Yardeni I, Mayburd E, Smirnov G, and Bessler H (2002) Immune response in asymptomatic smokers. *Acta Anaesthesiol Scand* **46**:959–964.
- Zhang Y, DeWitt DL, McNeely TB, Wahl SM, and Wahl LM (1997) Secretory leukocyte protease inhibitor suppresses the production of monocyte prostaglandin H synthase-2, prostaglandin E<sub>2</sub> and matrix metalloproteinases. *J Clin Invest* **99**:894–900.
- Zheng T, Zhu Z, Wang Z, Homer RJ, Ma B, Riese Jr RJ, Chapman Jr HA, Shapiro SD, and Elias JA (2000) Inducible targeting of IL-13 to the adult lung causes matrix metalloproteinase- and cathepsin-dependent emphysema. *J Clin Invest* **106**:1081–1093.
- Zhu J, Qiu YS, Majumdar S, Gamble E, Matin D, Turato G, Fabbri LM, Barnes N, Saetta M, and Jeffery PK (2001) Exacerbations of bronchitis: bronchial eosinophilia and gene expression for interleukin-4, interleukin-5 and eosinophil chemoattractants. *Am J Respir Crit Care Med* **164**:109–116.