Mechanisms of cigarette smoke-induced COPD: insights from animal models

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1Department of Pathology, University of British Columbia, Vancouver, British Columbia; and 2Respiratory Division, Royal Victoria Hospital, and Meakins-Christie Laboratories, McGill University, Montreal, Quebec, Canada

Churg A, Cosio M, Wright JL. Mechanisms of cigarette smoke-induced COPD: insights from animal models. Am J Physiol Lung Cell Mol Physiol 294: L612–L631, 2008. First published January 25, 2008; doi:10.1152/ajplung.00390.2007.—Cigarette smoke-induced animal models of chronic obstructive pulmonary disease support the protease-antiprotease hypothesis of emphysema, although which cells and proteases are the crucial actors remains controversial. Inhibition of either serine or metalloproteases produces significant protection against emphysema, but inhibition is invariably accompanied by decreases in the inflammatory response to cigarette smoke, suggesting that these inhibitors do more than just prevent matrix degradation. Direct anti-inflammatory interventions are also effective against the development of emphysema, as are antioxidant strategies; the latter again decrease smoke-induced inflammation. There is increasing evidence for autoimmunity, perhaps directed against matrix components, as a driving force in emphysema. There is intriguing but controversial animal model evidence that failure to repair/failure of lung maintenance also plays a role in the pathogenesis of emphysema. Cigarette smoke produces small airway remodeling in laboratory animals, possibly by direct induction of fibrogenic growth factors in the airway wall, and also produces pulmonary hypertension, at least in part through direct upregulation of vasoactive mediators in the intrapulmonary arteries. Smoke exposure causes goblet cell metaplasia and excess mucus production in the small airways and proximal trachea, but these changes are not good models of either chronic bronchitis or acute exacerbations. Emphysema, small airway remodeling, pulmonary hypertension, and mucus production appear to be at least partially independent processes that may require different therapeutic approaches.

Chronic obstructive pulmonary disease (COPD) is now the fifth leading cause of death worldwide (126). A meta-analysis using data from 28 countries suggests that the prevalence of COPD based on spirometric measurements is 9–10% in adults over age 40. It has been estimated that there are 15–17 million individuals with COPD in the United States alone (62, 160).

By far, the most important risk factor for COPD in the developed world is cigarette smoking; exposures to dusts, fumes, air pollution particles, and, in the developing world, biomass fuels, are also believed to cause COPD (62), but there is much less information available about these etiologies. Genetic predisposition seems to play an important role in the development of COPD, and a variety of genetic polymorphisms related to levels of antiproteases (α1-antitrypsin, the best established and the most clearly important), metalloproteases, proinflammatory and profibrotic cytokines, and various antioxidant enzymes and detoxifying enzymes have been linked to COPD (reviewed in Refs. 43, 81, 147, 178).

This review will concentrate on mechanistic insights from laboratory animal models of COPD caused by cigarette smoke, both because of the overwhelming role of cigarette smoke as a causative agent, and because of a lack of animal models based on dusts, air pollution particles, and biomass fuels. We will emphasize in vivo models and draw on data from tissue culture and explant studies where these appear directly relevant; for reviews that focus more on in vitro experiments, molecular mechanisms, potential targets, and non-smoke-induced COPD models, see Refs. 7, 8, 18, 40, 41, 99, 156, 197. There are plans to cover, in a future review in this journal, the pros and cons of various different animal models of COPD, including cigarette smoke-induced, elastase-induced, and genetic manipulation-induced models, and so these are not addressed here.

Because human COPD really consists of four anatomic lesions (emphysema, small airway remodeling, vascular remodeling with pulmonary hypertension, mucus overproduction and chronic bronchitis) and one functional lesion [acute exacerbation (13, 26)], we have separated our discussion into these broad categories. In some senses, this separation is artificial, since many patients have airflow limitation because of both emphysema and small airway remodeling, and they may have pulmonary hypertension and chronic bronchitis and develop acute exacerbations as well. However, as will become apparent, the mechanisms behind these anatomic lesions may well be different, so separating them is useful in understanding pathogenesis and in devising therapeutic approaches.
Mechanisms Related to the Protease-Antiprotease Hypothesis of Emphysema

Chronic (generally 6 mo or longer) exposure of mice or guinea pigs to cigarette smoke produces lesions that morphologically and physiologically resemble a mild form of centrilobular human emphysema (184) (Fig. 1), and emphysema is the lesion that has received the most study, although the anatomic changes differ from those in humans in a subtle way because these animals do not normally have respiratory bronchioles, the locus of initial destruction in human smoke-induced centrilobular emphysema.

The classic theory of emphysema is the protease antiprotease hypothesis. This hypothesis was formulated from the observation that humans deficient in α1-antitrypsin (A1AT) developed early emphysema, particularly if they smoked (85), and from the experiments of Gross and colleagues (59) showing that instillation of elastolytic enzymes produced emphysema in experimental animals. These observations lead to the idea that smoke evokes an inflammatory cell reaction and that these cells release proteases that overwhelm the antiproteolytic defenses of the lower respiratory tract, leading to matrix destruction and emphysema. Despite a variety of new theories, the protease-antiprotease hypothesis (now expanded to include metallo and cysteine proteases as well as the original serine proteases) remains the generally accepted basis for the destruction of matrix that leads to emphysema, and is the basis of most experimental smoke exposure models, but exactly what cells/proteases are crucial to this process is a complex and controversial issue.

That smoke evokes an inflammatory response in both humans (reviewed in Ref. 165) and animals is clear. Every experimental animal study that has looked at lavage/tissue neutrophils, macrophages, and lymphocytes in guinea pigs, rats, and mouse strains that develop emphysema has found an increase, although the details of cell types, timing, and magnitude of the effect vary (Tables 1, 2, 3), and smoke induces proinflammatory cytokine release from cultured macrophages, epithelial cells, and fibroblasts (90, 195, 198). Most but not all (68, 97) authors report an increase in gene expression/protein in whole lung/bronchoalveolar lavage (BAL) of chemotactic and proinflammatory mediators including TNFα, IL-1β, IL-8, MIP-2, MCP-1, MIP-1α, MIP-1β, MCP-3, KC, PGE2, IL-12, IL-18, RANTES, and IP-10 (28, 30, 32, 61, 78, 83, 92, 98, 166).

Interference with/management of serine proteases. The original formulation of the protease-antiprotease hypothesis postulated the neutrophil, and in particular, neutrophil elastase, as the important effectors in emphysema (76). In recent years, the role of the neutrophil has become controversial; some reports have shown localization of neutrophils in areas of tissue destruction in human emphysema (39) but others have failed to find any correlations between neutrophil numbers in tissue sections and the severity of lung destruction (47, 49).

Table 1 summarizes interventions related to serine proteases. Shapiro et al. (155) showed that mice lacking neutrophil elastase were 59% protected against emphysema, strong evidence for a role for neutrophil elastase (see below). In acute smoke exposure studies, levels of lavage neutrophils correlated with levels of lavage desmosine, a marker of elastin breakdown, and lavage hydroxyproline, a measure of collagen breakdown (30, 44, 186), and administration of anti-neutrophil antibodies before smoke exposure reduced both neutrophils and matrix breakdown (44). In chronic studies, inhaled (128) or injected (30) A1AT or the synthetic serine elastase inhibitor ZD0892 (186) provided partial protection against emphysema (Table 1). Takubo et al. (163) and Cavarra et al. (24) showed that pallid mice, which are naturally deficient in A1AT, developed earlier emphysema than strains with normal A1AT levels.

While these reports support a role of the neutrophil/neutrophil elastase in the genesis of emphysema, they produced the surprising result that all interventions decreased the inflammatory response and A1AT suppressed smoke-induced elevations of TNFα as well (30). There is extensive evidence from alveolar epithelial cell and alveolar macrophage cultures as well as whole mouse and human lung tissue that smoke directly evokes an inflammatory response by activating NF-κB (112,
and possibly the aryl hydrocarbon receptor (167) and Toll-like receptor-4, at least early on (98). Recent data also suggest that smoke inactivates histone deacetylases, leading to prolonged (and non-steroid-sensitive) inflammation (9, 74, 112). TNFα production is driven by NF-κB and TNFα is generally presumed to be a driver of inflammatory cell influx in smokers. Thus, a priori, one would expect continuing cigarette smoke exposure to generate a continuing inflammatory response, and serine protease inhibitors should provide protection against emphysema without decreasing inflammation; suppression of the inflammatory response thus implies additional anti-inflammatory mechanisms are at work (see below).

### Interference with metalloproteases

In the last 15 years, there has been an increasing interest in metalloproteases (MMP) as mediators of emphysema. This has stemmed in part from the recognition that a number of metalloproteases, including MMP-9 and MMP-12 (124), can degrade elastin; in part from reports of increased levels of MMPs including MMP-1, -2, -9, -14 (50, 72, 120, 150), and in some studies, MMP-12 (42, 60, 72, 111, 182), in BAL fluid, alveolar macrophage supernatant, or whole lung tissue from smokers with emphysema compared with those without; and in particular, from the report (63) that mice with a targeted deletion of MMP-12 (MMP-12−/−) failed to develop emphysema after cigarette smoke exposure. Cigarette smoke causes increased whole lung or alveolar macrophage levels of MMP-2, -9, -12, -13, and -14 in mice (32) and MMP-1 in guinea pigs (151). Table 1 lists experimental studies using genetically targeted mice or MMP inhibitors in smoke exposure models. Several broad conclusions stand out. First, MMP inhibition or deletion can significantly or even totally abrogate the development of emphysema, indicating a clear role for MMPs in this process. In fact, with broad

<table>
<thead>
<tr>
<th>Report (Ref. no.)</th>
<th>Species/Strain</th>
<th>Intervention</th>
<th>Inflammatory Response</th>
<th>% Protection Against Emphysema</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wright (186)</td>
<td>Guinea pig</td>
<td>ZD0892 (serine protease inhibitor)</td>
<td>Decreased</td>
<td>45%</td>
<td>Decreased BAL desmosine, hydroxyproline; treatment for last 2 mo of smoke exposure did not protect against emphysema. Decreased BAL desmosine, hydroxyproline; treatment roughly doubled serum A1AT levels and suppressed smoke-mediated increases in serum TNFα.</td>
</tr>
<tr>
<td>Churg (30)</td>
<td>C57Bl/6 mouse</td>
<td>A1AT injected (prolastin)</td>
<td>Decreased</td>
<td>67%</td>
<td>MMP-12 and neutrophil elastase degrade each other’s inhibitors</td>
</tr>
<tr>
<td>Shapiro (155)</td>
<td>C57Bl/6 mouse</td>
<td>Neutrophil elastase knockout</td>
<td>Decreased neutrophils, macrophages</td>
<td>59%</td>
<td>MMP-12 and neutrophil elastase degrade each other’s inhibitors</td>
</tr>
<tr>
<td>Pemberton (128)</td>
<td>Mouse</td>
<td>A1AT inhaled (recombinant human A1AT)</td>
<td>Decreased</td>
<td>72%</td>
<td>Highest dose increased BAL PMN and gave less protection against emphysema</td>
</tr>
<tr>
<td>Takubo (163)</td>
<td>Pallid mouse</td>
<td>A1AT deficient</td>
<td>Only CD4+ cells significantly increased in tissue</td>
<td>Early onset emphysema</td>
<td>Pan-lobular emphysema</td>
</tr>
<tr>
<td>Cavarra (24)</td>
<td>Pallid mouse</td>
<td>A1AT deficient</td>
<td>Not reported</td>
<td>Early onset emphysema</td>
<td>Pan-lobular emphysema</td>
</tr>
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### Metalloprotease and Cysteine Protease Inhibition or Manipulation

<table>
<thead>
<tr>
<th>Report (Ref. no.)</th>
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<th>Intervention</th>
<th>Inflammatory Response</th>
<th>% Protection Against Emphysema</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hautamaki (63)</td>
<td>129 mouse</td>
<td>MMP-12 knockout</td>
<td>Decreased macrophages but not BAL neutrophils</td>
<td>100%</td>
<td>MMP-12 degrades A1AT and neutrophil elastase degrades TIMP-1</td>
</tr>
<tr>
<td>Shapiro (155)</td>
<td>C57Bl/6 mouse</td>
<td>MMP-9 knockout</td>
<td>Not reported</td>
<td>0%</td>
<td>90% = average value for 2 highest doses</td>
</tr>
<tr>
<td>Mahadeva (99)</td>
<td>Mouse</td>
<td>Broad spectrum MMP inhibitor GM6001</td>
<td>Decreased</td>
<td>90%</td>
<td>Started after 3 mo of smoke exposure; no increase in mean air space size between 3 and 6 mo</td>
</tr>
<tr>
<td>Pemberton (127)</td>
<td>Mouse</td>
<td>Broad spectrum MMP inhibitor RS113456</td>
<td>Not reported</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>Martin (105)</td>
<td>Mouse</td>
<td>Broad spectrum MMP inhibitor RS132908</td>
<td>Not reported</td>
<td>75%</td>
<td></td>
</tr>
<tr>
<td>Selman (152)</td>
<td>Guinea pig</td>
<td>Broad spectrum MMP inhibitor CP471474</td>
<td>Decreased</td>
<td>30%</td>
<td>Measured as alveolar area at 4 mo; decreased MMP-9 levels at 4 mo</td>
</tr>
<tr>
<td>Churg (34)</td>
<td>Guinea pig</td>
<td>MMP-9/-12 inhibitor AZ11557272</td>
<td>Decreased</td>
<td>68%</td>
<td>Decreased BAL desmosine; strong correlation BAL desmosine with mean air space size; inhibitor also prevents small airway remodeling</td>
</tr>
<tr>
<td>Kang (78)</td>
<td>Mouse (C57)</td>
<td>IL-18Rα knockout</td>
<td>Decreased</td>
<td>51%</td>
<td>Decreased apoptosis; decreased cytokines; decreased cathepsin B, S</td>
</tr>
</tbody>
</table>

All studies use 6 mo of smoke exposure, except as noted.
spectrum MMP inhibitors, it is sometimes possible to achieve greater protection against emphysema than with serine elastase inhibitors. Second, the choice of which MMP to target is crucial. Mice lacking MMP-12 are completely protected against emphysema (63, 155), whereas mice lacking MMP-9 show no protection at all (99). This latter observation is of particular interest, since it has been suggested from studies of cultured alveolar human macrophages that MMP-9 is the major mediator of emphysema in humans (143, 144), and some have denied a role for MMP-12 in humans (72). Selman et al. (152) found little protection with a broad spectrum MMP inhibitor in guinea pigs, but we (34) found >70% protection in guinea pigs given a combined MMP-9/-12 inhibitor, AZ11557272, indicating that MMPs are not just confined to murine emphysema models, and lending support to the idea that one or both of these MMPs may be central players in humans. Animals exposed to smoke and AZ11557272 had 70% protection against decreases in airflow compared with animals exposed to smoke alone, thus showing that prevention of anatomic changes in animal models confers a corresponding physiological benefit.

However, as is true of serine proteases, the exact role of MMPs in the pathogenesis of emphysema is unclear, particularly since neutrophils-serine proteases and MMPs appear to interact, and MMP inhibition reduces smoke-induced neutrophil and macrophage influx (29, 34, 128, 155). In an acute smoke model using MMP-12−/− mice, we (29) showed that MMP-12 was required for smoke-induced neutrophil influx and neutrophils were required for matrix breakdown. This latter idea was also supported by the finding that the broad spectrum MMP inhibitor RS113456 acutely inhibited neutrophil influx (1, 69, 129, 153, 154). Houghton et al. (68) observed that the lavage fluid of wild-type mice contained elastin fragments that were chemotactants for monocytes and that this chemotactant activity was absent from the lavage of MMP-12−/− mice. They suggested that matrix breakdown in emphysema is driven by both neutrophil elastase and MMP-12, with the latter the major player because of the relatively large numbers of monocytes that migrate into the lung after smoke exposure. In practice, MMP-12 may well play both a signaling and a direct matrix destructive role.

More recently, Maeno et al. (97) have proposed that this whole process of smoke-driven matrix destruction is initiated by CD8+ lymphocyte-mediated production of IFN-γ/IP-10, with resulting neutrophil and macrophage infiltration and inflammation.

Additional evidence for interactions of neutrophils and macrophages comes from Shapiro et al. (155) who showed that neutrophil elastase activates pro-MMP-12 and destroys tissue inhibitor of metalloprotease (TIMP)-1; conversely, MMP-12 degrades A1AT. Thus neutrophil elastase and MMP-12 cooperate to increase each other’s proteolytic potential.

There appear to be a variety of mechanisms by which serine and metalloprotease inhibitors exert anti-inflammatory effects. We (186) found that the serine elastase inhibitor ZD0892 inhibited acute smoke-mediated increases in gene expression of the neutrophil/macrophage chemoattractants MIP-2 and MCP-1 in mice. We also observed that alveolar macrophages from MMP-12−/− mice did not increase TNFα secretion after smoke exposure, apparently because MMP-12 functions as a form of TNFα converting enzyme that liberates active TNFα, (Figs. 2 and 3) and lack of TNFα appeared to be one reason for a lack of neutrophil infiltration in the lungs of MMP-12−/− animals (31). As well, A1AT suppresses smoke-mediated increases in TNFα release by inhibiting the serine proteases thrombin and plasmin that leak into the air spaces after smoke exposure (31, 35); thrombin and plasmin in turn activate proteinase-activated receptor-1 (PAR-1), thereby causing MMP-12 and TNFα release (32, 133) (Fig. 2). This whole set of observations would remove MMP-12 from a matrix destructive role to a signaling role in the context of cigarette smoke exposure, and signaling roles are now recognized as major functions of MMPs (125).

An alternate explanation for decreases in inflammatory cell influx is that inhibition of matrix destruction by either neutrophil or macrophage-derived proteases decreases the generation of chemotactic matrix fragments (1, 69, 129, 153, 154). Houghton et al. (68) observed that the lavage fluid of wild-type mice contained elastin fragments that were chemotactants for monocytes and that this chemotactic activity was absent from the lavage of MMP-12−/− mice. They suggested that matrix breakdown in emphysema is driven by both neutrophil elastase and MMP-12, with the latter the major player because of the relatively large numbers of monocytes that migrate into the lung after smoke exposure. In practice, MMP-12 may well play both a signaling and a direct matrix destructive role.

More recently, Maeno et al. (97) have proposed that this whole process of smoke-driven matrix destruction is initiated by CD8+ lymphocyte-mediated production of IFN-γ/IP-10, with resulting neutrophil and macrophage infiltration and inflammation.
creases in MMP-12 production (Fig. 3). Of note, these data are similar to findings reported in human lungs with emphysema by Grumelli et al. (60).

Cantor et al. (20, 21) showed that inhaled hyaluronan (HA) can bind to elastin fibers in vivo and in vitro and prevents elastolysis by elastases. HA reduced air space enlargement caused by porcine pancreatic elastase (20), provided 100% protection against emphysema in smoke-exposed DBA/2J mice, and reduced smoke-mediated increases in lavage desmosine. These findings support matrix attack as a fundamental feature of smoke-induced emphysema.

Cysteine proteases. Kang et al. (78) showed that smoke induces production of the cysteine proteases cathepsins B and S in mice via a mechanism involving IL-18, and, probably, interferon-γ. These proteases can degrade matrix components and thus might play a role in emphysema. The same report found greater levels of cathepsins B and S in alveolar macrophages from cigarette smokers compared with controls. IL-18Ra−/− mice were significantly protected against emphysema and had decreases in smoke-induced lavage neutrophils and macrophages as well as a variety of chemokines and cathepsins, along with decreased levels of MMP-12. These data thus present the same mix of possible effectors seen with studies inhibiting/deleting serine and metalloproteases.

Anti-inflammatory interventions. If the protease-antiprotease hypothesis (i.e., that the smoke-driven inflammatory response leads to proteolytic matrix destruction) is correct, one could inhibit smoke-mediated inflammatory responses instead of proteases, and a number of studies have directly or indirectly targeted the production and/or signaling of proinflammatory molecules (Table 2).

TNFα is consistently elevated in human smokers (10, 165). In mice, knockout of TNFα receptors 1 and 2 greatly reduced inflammatory infiltrates, emphysema, levels of some MMPs, and gene expression of proinflammatory cytokines (28, 32, 46) after smoke exposure; TNFα receptor 2 appeared to be the major mediator of the inflammatory response (46). Conversely, inducible overexpression of TNFα produced an increase in neutrophils, parenchymal B cell nodules, MMP-12, cathepsin K, and emphysema in the absence of smoke (176). There was an extremely strong correlation ($R = 0.89, P \lt 0.0001$) between serum TNFα levels and air space size after 6 mo of smoke exposure in guinea pigs (34). While these reports imply an important role for TNFα, two studies in humans using TNFα antagonists failed to show a benefit (10, 134, 172), suggesting that translation of anti-inflammatory therapies from animal models to humans is not straightforward.

Using a different anti-inflammatory approach, Thatcher et al. (166) prevented neutrophil influx by administration of SCH-N, a CXCR2 inhibitor, to mice, with an ~50% reduction in parenchymal cells after a 3-day smoke exposure. SCH-N did not decrease smoke-mediated increases in TNFα, IL-6, or PGE2, and levels of the neutrophil chemoattractant CXCR2 ligands KC and MIP-2 were considerably elevated. Maes et al. (98) showed that, in mice, Toll-like receptor-4 plays a role in the early but not the chronic inflammatory response to cigarette smoke.

Phosphodiesterase-4 degrades the anti-inflammatory nucleotide cyclic 3′,5′-adenosine monophosphate. In C57Bl/6 mice exposed to smoke for 7 mo, Rolflumilast, a phosphodiesterase-4 inhibitor, provided 100% protection against smoke-induced increases in air space size, reversed smoke-induced loss of lung desmosine, reduced neutrophil and especially macrophage influx, and more than doubled levels of the anti-inflammatory cytokine, IL-10 (106). One of the effects of Rolflumilast is to decrease macrophage production of TNFα, and in a human trial, 4 wk of Rolflumilast decreased blood/sputum TNFα, IL-8, and neutrophil elastase, and improved FEV1 (58).

IFNγ appears to drive many proinflammatory cytokines via CCR5 (92), IFNγ−/− or CCR5−/− mice exposed to smoke for 6 mo were completely protected against emphysema and showed decreased inflammatory cells, apoptosis, levels of MIP-1α, MIP-1β, and RANTES (16, 92). Protection may have been mediated through decreased MMP-12, since transgenic mice overexpressing IFNγ increased MMP-12 production (92).

Administration of the 3-hydroxy-3-methyl-glutaryl-coenzyme-A reductase inhibitor, simvastatin (87), to rats completely abolished smoke-induced emphysema and prevented peribronchial and perivascular accumulation of lymphocytes. This report is difficult to interpret: the authors reported a remarkable degree of air space enlargement (80% increase in mean air space size) after a relatively short exposure period (16 wk). Furthermore, statins have been reported to increase macrophage production of MMP-12 (4), which should make emphysema worse. However, there are reports of beneficial effects of statins in patients with COPD (102, 159) and reports
that statins enhance clearance of apoptotic cells (114), so this type of compound may be worth further investigation.

In aggregate, both interventions directed against proteases (serine, cysteine, or metalloproteases) and interventions directed against the development of an inflammatory influx provide significant protection against emphysema in animal models and thus support the protease-antiprotease hypothesis, although the mechanism(s) involved are complex and not entirely clear.

**Effects of Smoke on Different Mouse Strains: Evidence for a Genetic Propensity to Emphysema**

As noted above, susceptibility to COPD likely results from multiple genetic and environmental effects. Cavarra et al. (24) showed that mouse strains with differing antioxidant/antiprotease capacity reacted differently to cigarette smoke (see *Mechanisms Related to Oxidative Stress in Emphysema*). Guerassimov et al. (61) exposed five different strains of mice to smoke in an attempt to define genetically susceptible and resistant varieties. The strains were chosen based on differences in the MHC haplotype, a major determinant of the inflammatory response in mice. After 6 mo of smoking, NZW/Lac/J mice had no increase in mean air space size (Lm), whereas AJ, SJL, C57BL/6, and AKR mice had 17.9%, 23.8%, 13.2%, and 38% increases, respectively. Because, as noted by Henson and Vandivier (64), it is remarkably easy to induce alveolar enlargement with a very wide variety of manipulations in the mouse, Guerassimov et al. (61) defined emphysema as an increase in Lm along with an increase in lung compliance (Fig. 4). By these criteria, only the AKR strain had significant emphysema, whereas AJ, SJL, and C57BL6 strains appeared to be mildly susceptible to smoke and NZW were resistant.

Hoshino and Cosio (unpublished data) then investigated the genetic response to cigarette smoking in resistant (NZW) and susceptible (AKR) strains utilizing expression microarrays. There were striking constitutive differences between the two strains, mainly in the expression of genes that encode for proteins with immune function. The NZW mice had higher constitutive expression of genes that inhibit differentiation and proliferation of T and B cells and protect against apoptosis and T cell activation (Ii203, CD72, C4, Kila-1, 8, and 13), along with higher levels of several antioxidant genes that were not prominently expressed in AKR mice. In contrast, the constitutive inflammatory genes expressed in AKR mice were proin-

### Table 2. Effects of manipulation of the immune/inflammatory response in chronic smoke exposure studies

<table>
<thead>
<tr>
<th>Report (Ref. no.)</th>
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<th>Inflammatory Response</th>
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<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Churg (32)</td>
<td>Mouse (C57Bl/6)</td>
<td>TNFα R1 R2−/−</td>
<td>Decreased BAL macrophages, neutrophils</td>
<td>71%</td>
<td>Increased MMP-2, −9, −12, −13, −14 with smoke exposure; knockout mice: decreased MMP-12, −13, −14; decreased gene expression of TNFα, MCP-1, MIP-2</td>
</tr>
<tr>
<td>D’hulst (46)</td>
<td>Mouse (C57)</td>
<td>TNFα R1−/−</td>
<td>Not affected</td>
<td>None</td>
<td>Decreased CD4+ and CD8+ cells</td>
</tr>
<tr>
<td>D’hui (46)</td>
<td>Mouse (C57)</td>
<td>TNFα R2−/−</td>
<td>Decreased BAL neutrophils, macrophages, lymphocytes</td>
<td>100%</td>
<td>4-mo smoke exposure; also decreased inflammatory mediators</td>
</tr>
<tr>
<td>Martorana (106)</td>
<td>Mouse (C57)</td>
<td>PDE4 inhibitor Rofumilast</td>
<td>Decreased</td>
<td>100%</td>
<td>7-mo smoke exposure; increased IL-10</td>
</tr>
<tr>
<td>Lee (87)</td>
<td>Rat (Sprague-Dawley)</td>
<td>Simvastatin</td>
<td>Decreased peribronchial lymphoid aggregates</td>
<td>100%</td>
<td>Putative protection of elastin fibers; possible technical problems with study</td>
</tr>
<tr>
<td>Cantor (21)</td>
<td>Mouse (DBA/2)</td>
<td>Hyaluron aerosol</td>
<td>Not reported</td>
<td>100%</td>
<td>No protection against small airway remodeling; decreased MMP-12, BAL PMN, T lymphocytes, macrophages</td>
</tr>
<tr>
<td>Bracke (13)</td>
<td>Mouse (C57Bl/6)</td>
<td>CCR6−/−</td>
<td>Decreased</td>
<td>67%</td>
<td>Decreased apoptosis; MMP-12 probably involved</td>
</tr>
<tr>
<td>Ma (92)</td>
<td>Mouse (C57Bl/6)</td>
<td>IFNγ−/−</td>
<td>Not reported</td>
<td>100%</td>
<td>Decreased cytokines, apoptosis</td>
</tr>
<tr>
<td>Maeno (97)</td>
<td>Mouse (C57Bl/6)</td>
<td>CD8−/−</td>
<td>Decreased</td>
<td>100%</td>
<td>Decreased PMN, macrophages, dendritic cells, lymphocytes; no protection against small airway remodeling</td>
</tr>
<tr>
<td>D’d’hui (45)</td>
<td>SCID mouse</td>
<td>CD4−/−</td>
<td>Not increased</td>
<td>None</td>
<td>No T or B lymphocytes; no lymphoid follicles</td>
</tr>
<tr>
<td>Maes (98)</td>
<td>Mouse (C3H/HeJ)</td>
<td>TLR4−/−</td>
<td>Increased BAL PMN macrophages at 26 wk</td>
<td>None</td>
<td>Decreased BAL lymphocytes and dendritic cells at 26 wk</td>
</tr>
<tr>
<td>van der Deen (172)</td>
<td>Mouse (FVB)</td>
<td>Multi-drug resistance protein−/−</td>
<td>Decreased inflammation and inflammatory mediators</td>
<td>100%</td>
<td>No emphysema produced in wild type or knockout</td>
</tr>
</tbody>
</table>

All studies use 6 mo of smoke exposure, except as noted.
Inflammatory, and several were related to adaptive immunity. With smoke exposure there was a striking difference in gene response: the NZW strain demonstrated decreased expression of 77 of the 82 genes that were significantly changed in response to smoke, and 44% of the genes attenuated by smoke exposure influence immune and/or inflammatory processes including immunoglobulins, complement, chemokines, cytokines, and other proinflammatory factors that enhance the functions of neutrophils, macrophages, and T and B cells. NZW mice also upregulated antioxidant genes. In contrast, AKR mice showed increased expression of 52 of the 57 genes that were changed by smoke exposure, and 25% of the genes increased after smoking had functions related to the immune response; 13% were proapoptotic (Fig. 5).

In another study aimed at investigating the potentiation of inflammation by cigarette smoke, Reynolds et al. (137) investigated the expression of Egr-1 (early growth response gene 1) in NZW and AKR mice. Egr-1 gene induces IL-1β and TNFα, cytokines that contribute to the recruitment of inflammatory cells after smoke exposure. Egr-1 expression was marginally detected by immunochemistry in the lungs of nonsmoking mice, but increased markedly in the susceptible AKR strain after smoke exposure. However, Egr-1 was only minimally induced in the lungs of the resistant NZW.

These studies suggest that resistant animals do not increase or actively decrease the proinflammatory response to smoke while increasing the antioxidant response, thus preventing matrix breakdown and a potential acquired immune response to matrix fragments. In contrast, inflammation in the susceptible strain is progressive, probably due to ongoing stimulation of innate and adaptive immunity by the expression of genes involved in antigen presentation and T and B cell activation. These findings support the possibility of an autoimmune process triggered by smoke exposure as a factor important in the maintenance of the inflammation and the development of emphysema.

Evidence for Acquired Immunity in Animal Models of Smoke-Induced Emphysema

An “abnormal inflammatory response” is an important component in the definition of COPD (131). Besides neutrophils and alveolar macrophages, both CD4+ (T helper) and CD8+ (cytotoxic) T cells are increased in the airways and lung parenchyma of patients with COPD with a predominance of CD8+ T cells, and Finkelstein et al. (49) showed that in human lungs, there is a correlation between the number of T lymphocytes/mm³ of lungs and the extent of emphysema. This infiltration with T cells, seen in smokers who develop COPD, but not in normal smokers, represents an activation of the adaptive immunity that presumably follows from the initial and then sustained innate immune response characterized by increased numbers of macrophages and neutrophils.

The T cells found in patients with COPD are fully activated (60, 146), expressing a large array of Th1 chemokines and cytokines. This inflammatory process most likely also involves
the migration of dendritic cells, since it has been recently reported that T cells in humans with emphysema are being presented with antigens derived from the breakdown of elastin (88). These findings support a role for autoimmunity in the pathogenesis of COPD.

Guerrassimov et al. (61) investigated the inflammatory response to long-term cigarette smoking in the mice with different susceptibilities to emphysema described in effects of smoke on different mouse strains: evidence for a genetic propensity to emphysema. To approximate human studies, they used morphometric methods to quantitate the percentage of immunostained inflammatory cells in the alveolar walls. After 6 mo of smoking, only the susceptible AKR strain had a florid cellular inflammatory infiltrate comprising CD4+, CD8+, and γδ T cells, along with macrophages and neutrophils. Neither the resistant nor the mildly susceptible strains exhibited an inflammatory infiltrate containing T cells other than some γδ cells in the NZW strain (Fig. 6). Similar results were found by Takubo et al. (163) in the Pallid mouse, a strain that also develops marked emphysema after 6 mo of smoking exposure. Analysis of inflammatory chemokines and cytokines in the lungs (Fig. 7) in the three groups with different susceptibilities confirmed that a true Th1 inflammatory response, secondary to T cell activation, had developed in the susceptible AKR mice but was not present in the resistant or mildly susceptible strains. These findings are of interest since they mimic the inflammatory changes that smokers develop, and, as in the human smokers, only the susceptible animals develop the adaptive immune reaction seen in humans.

Mechanisms Related to Oxidative Stress in Emphysema

Cigarette smoke is an extremely concentrated source of reactive oxygen species (ROS) and reactive nitrogen species (130). The inflammatory response to smoke potentially augments oxidative stress, since neutrophils and macrophages release ROS, and those from smokers release even greater amounts (17, 66, 91, 95, 96).

There is considerable biochemical evidence of oxidative stress in cigarette smokers and greater levels in those with COPD (reviewed in Refs. 14, 95, 96). These changes include increased exhaled H2O2 and 8-isoprostane, decreased plasma antioxidants, and increased plasma and tissue levels of oxidized proteins, various lipid peroxidation products such as 4-hydroxynonenal, as well as protein tyrosine residues/3-nitrotyrosine, indicators of attack by reactive nitrogen species. Antioxidant enzyme levels are also altered. Oxidative damage is believed to decrease production of/inactivate some...
histone deacetylases (9, 74), leading to a prolonged inflammatory response.

Evidence of oxidant attack is also seen in animal models. Aoshiba et al. (2) showed that even a 1-h exposure of mice to cigarette smoke resulted in immunochemically detectable 4-hydroxynonenal and 8-hydroxy-2-deoxyguanosine, a marker of oxidative DNA damage, in the airway and alveolar epithelial cells. We (27) found that a 10-min smoke exposure produced lipid peroxidation in rat tracheal explants. Rangasamy et al. (132), using microarrays, showed upregulation of a variety of antioxidant enzymes after smoke exposure of ICR mice. McCusker and Hoidal (109) found increases in superoxide dismutase and catalase expression in alveolar macrophages from smoke-exposed hamsters, whereas Cavarra et al. (25) showed depletion of protein thiols, ascorbic acid, and glutathione, all antioxidant substances, in smoke-exposed C57Bl/6 mice.

Interference with the antioxidant response by deletion of Nrf2 (Nrf2<sup>−/−</sup>) (71, 132), a redox-sensitive transcription factor that upregulates a variety of detoxification and antioxidant genes, caused increased smoke-induced inflammation and earlier and more severe emphysema than in wild-type (ICR or ICR/Balb/c) mice, and Nrf2<sup>−/−</sup> mice had impaired upregulation of antioxidant enzymes (157). As well, Nrf2<sup>−/−</sup> mice were deficient in A1AT and secretory leukoprotease inhibitor (SLPI). These findings link a lack of antioxidant protection to the protease-antiprotease hypothesis (Fig. 3).

Conversely, several investigators have acutely increased antioxidant protection (Table 3) with consequent decreases in inflammatory cell influx, protein oxidation, inflammatory mediators, and airway squamous metaplasia after short-term smoke exposure (5, 116, 158). Rubio et al. (142) found that the antioxidant N-acetyl cysteine ameliorated elastase-induced emphysema in rats.

Cavarra et al. (24) reported that ICR mice, which increased antioxidant levels in BAL after smoke exposure, were protected against emphysema, whereas C57Bl/6 and DBA/2, which did not increase antioxidant levels, developed emphysema; of note, levels of antioxidant protection appeared to be more important than BAL levels of elastase inhibitory capacity in determining the severity of emphysema. Foronjy et al. (52) showed that chronically smoke-exposed transgenic CuZnSOD animals had much smaller increases in neutrophil and macrophage infiltration than did wild-type animals, did not show increases in production of a variety of MMPs including MMP-12, were protected against generation of lipid peroxidation products, and were 100% protected against the development of emphysema. There was substantial protection against elastase-induced emphysema as well.

These studies thus support a role for oxidant attack in the genesis of smoke-induced emphysema, but it is noteworthy that the immediate mechanism of attack appears to be through increases in inflammatory cells, and, probably, decreases in antiprotease protection, thus linking oxidant attack to the protease-antiprotease hypothesis.

**Mechanisms Related to Repair of Alveolar Structure in Emphysema**

Retinoids accumulate in the lung during embryogenesis and are essential to alveolar septation; mice lacking retinoid receptors have lower levels of elastin and enlarged alveolar spaces (108). Massaro and Massaro (107) reported that treatment with all-trans retinoic acid reversed the emphysematous changes seen in rats after instillation of elastase, and this was confirmed in rats by Belloni et al. (12). However, reports in other species and with cigarette smoke have been discouraging. March et al. (103) did not find any protective effects of retinoids against smoke-induced emphysema in either A/J or B6C3F1 mice, and Meshi et al. (110) did not observe any benefit in smoke-exposed guinea pigs. A human trial of retinoid therapy did not show any clear improvement in
Mechanisms Related to Failure to Repair/Failure of Lung Maintenance in Emphysema

While there appears to be overwhelming evidence that cigarette smoke-induced damage to the alveolar wall matrix as a result of inflammatory cell-derived protease, and, probably, oxidant, attack (but, again, with oxidant-driven increases in inflammatory cells) is the driving force behind emphysema, one of the curious features of emphysema is that the alveolar wall largely fails to regenerate new matrix. This is in sharp contradistinction to the small airways and the intrapulmonary arteries, where the response to smoke is a marked increase in matrix and/or structural cells (see Mechanisms of Small Airway Remodeling and Mechanisms of Vascular Remodeling and Pulmonary Hypertension), despite the fact that these anatomic compartments are separated by only a few micrometers.

A variety of theories, conveniently grouped as “failure to repair/failure of lung maintenance” have been advanced to account for this phenomenon (reviewed in Refs. 134, 170, 171). Increases in apoptotic cells, sometimes accompanied by decreases in proliferating cells, have been found in severely emphysematous compared with nonemphysematous human lungs (73, 100, 170, 196). Administration of agents that cause endothelial or epithelial cell apoptosis to animals results in rapid air space enlargement, although, unlike smoke-induced emphysema, this process is also rapidly reversible and is not accompanied by an inflammatory response (3, 79). In tissue culture models, smoke exposure interferes with cell proliferation, chemotaxis, and production/remodeling of matrix components by fibroblasts (23, 134) and depresses the production and activity of lysyl oxidase (53, 86), an enzyme crucial to the formation of stable insoluble elastin and collagen. Fibroblasts from emphysematous human lungs proliferate more slowly than those from nonemphysematous lungs (67, 117) and show increased expression of a variety of senescence-associated markers (118, 169).

Only a few animal models have looked at this issue using smoke exposure. Mice lacking senescence-associated marker 30, an anti-aging calcium binding protein, show increased

Table 3. Effects of antioxidant manipulation in acute and chronic smoke exposure studies

<table>
<thead>
<tr>
<th>Report (Ref. no.)</th>
<th>Species/Strain</th>
<th>Intervention</th>
<th>Inflammatory Response</th>
<th>Effect on Emphysema</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nishikawa (116)</td>
<td>Guinea pig</td>
<td>rhSOD</td>
<td>Decreased</td>
<td>Acute study only</td>
<td>Decreased IL-8 gene expression, NF-κB activation</td>
</tr>
<tr>
<td>Smith (158)</td>
<td>Rat</td>
<td>Catalytic antioxidant AEOL 10150</td>
<td>Decreased</td>
<td>Acute study only</td>
<td>Decreased MIP-2, ICAM-1, squamous metaplasia in airways</td>
</tr>
<tr>
<td>Banerjee (5)</td>
<td>Guinea pig</td>
<td>Black tea (antioxidant) in drinking water</td>
<td>Not reported</td>
<td>Acute study only</td>
<td>Decreased oxidation of lung proteins</td>
</tr>
<tr>
<td>Cavarra (24)</td>
<td>ICR, C57, DBA/2 mice</td>
<td>ICR mice have increased antioxidant protection, cf. to C57, DBA/2</td>
<td>Not reported</td>
<td>5% increase in mean air space size in ICR, not significant; C57 and DBA/2 get emphysema</td>
<td>Elastin content decreased in C57 and DBA/2 but not in ICR</td>
</tr>
<tr>
<td>Rangasamy (32)</td>
<td>Mouse (ICR)</td>
<td>Nrf2 knockout</td>
<td>Increased</td>
<td>Earlier and more severe</td>
<td>Decreased A1AT; increased apoptosis</td>
</tr>
<tr>
<td>Iizuka (71)</td>
<td>Mouse (Balb/c)</td>
<td>Nrf2 knockout</td>
<td>Increased</td>
<td>Earlier and more severe</td>
<td>Decreased SLPI</td>
</tr>
<tr>
<td>Foronjy (52)</td>
<td>Mouse</td>
<td>Tg ZnCuSOD</td>
<td>Decreased</td>
<td>100% protection</td>
<td>Protects against smoke-mediated increase in lipid peroxidation</td>
</tr>
</tbody>
</table>
involved in the repair of the parenchyma after long-term smoke exposure. There is not a total absence of repair in emphysema, since human centrilobular emphysema is associated with increased numbers of apoptotic lung structural cells (113). In advanced COPD is unclear (65). In cigarette smoke-exposed animals, peribronchial lymphoid aggregates increase in number (Fig. 10), but in CCR6−/− and CCR5−/− mice, a lack of peribronchial lymphoid aggregates with chronic smoke exposure did not protect against SAR (15, 16).

Mechanisms of Vascular Remodeling and Pulmonary Hypertension

Pulmonary hypertension (PHT) is a relatively common form of cigarette smoke-induced lung disease and develops in ~6% of subjects with COPD, but is present in ~40% of patients with an FEV1 of less than 1 l (6, 19, 93, 94, 180). PHT is an important complication since it is a significant predictor of

Mechanisms of Small Airway Remodeling

Small airway remodeling (increases in bronchiolar wall fibrous tissue, muscle, inflammatory cells, and luminal mucus) (SAR) is now recognized as an important cause of airflow obstruction in cigarette smokers (65, 122). There are only a few reports that have examined smoke-induced SAR in animals. The changes found are subtle and easily missed on casual examination, and some investigators deny that SAR occurs (104). However, careful morphometric studies from several laboratories have confirmed that there is indeed SAR, largely manifest as increases in airway wall collagen, in both guinea pigs (34) and mice (15, 33, 16) after chronic smoke exposure (Fig. 8). Wright and colleagues (192) reported an increase in thick collagen fibers (Fig. 8), an effect that probably increases airway stiffness, in the small airways of guinea pigs exposed to cigarette smoke for 6 mo. They found negative correlations of the amount of thick collagen fibers with peak expiratory flow and FEV0.1/FVC, and positive correlations with airway resistance, thus indicating that smoke-induced airway remodeling in animals is associated with abnormal physiology.

In humans, the usual assumption has been that, since emphysema is driven by inflammatory cells and their proteases, SAR should follow the same pathways (77). In fact, little is known about the pathogenesis of SAR in humans. One piece of evidence against a primary role of inflammation in SAR are reports (15, 16) that mice lacking CCR6 and CCR5 (receptors for various chemoattractant chemokines) had decreased inflammation and decreased emphysema after smoke exposure, but no decrease in SAR compared with wild-type animals. SAR was also prevented in smoke-exposed guinea pigs by an MMP-9/-12 inhibitor, again evidence that mechanisms other than inflammation are important (34).

Using laser capture microdissection of small airways (bronchioles) from the lungs of smoke-exposed C57Bl/6 mice, Churg et al. (33) found that smoke persistently upregulated gene expression of type I procollagen and profibrotic cytokines, particularly those related to TGF-β signaling. With a single acute exposure, elevations in gene expression were seen within 2 h of starting smoke exposure and mostly decreased over 24 h, as opposed to numbers of lavage inflammatory cells that increased slowly over 24 h (44), implying that, at least in the short term, upregulation of the fibrotic response is independent of inflammation.

In rat tracheal explants, an airway model system that is free of smoke-evoked inflammatory cells, a very brief (15-min) exposure to smoke resulted in increases in the same set of genes mentioned above, along with collagen (hydroxyproline) by 24 h, and these increases could be prevented with an inhibitor of TGF-β receptor 1 (Churg, unpublished observations) or a TGF-β competitor, fetuin (179). The smoke-exposed explants released increased amounts of TGF-β1 via an oxidant-driven mechanism (Fig. 9).

In short, the animal models suggest that small airway remodeling is driven by direct smoke induction of fibrogenic growth factors, and the role of inflammatory cells is uncertain; rather, the initial driving force appears to be oxidant-mediated activation of TGF-β. However, since neutrophils and macrophages release oxidants in response to smoke (17, 66, 91, 95, 96), such cells might potentiate TGF-β release and hence potentiate small airway remodeling (Fig. 9).

Both B and T lymphocytes are increased in the small airway walls in human COPD (37, 65, 145, 173). Whether they play a direct role in the pathogenesis of SAR or reflect colonization of the small airways by infectious agents in patients with advanced COPD is unclear (65). In cigarette smoke-exposed animals, peribronchial lymphoid aggregates increase in number (Fig. 10), but in CCR6−/− and CCR5−/− mice, a lack of peribronchial lymphoid aggregates with chronic smoke exposure did not protect against SAR (15, 16).
mortality, and is a major cause of morbidity, in patients with COPD (36, 168, 189).

The mechanism(s) of PHT in smokers is not known. Although it is often stated that PHT arises as a result of loss of vascular bed secondary to emphysematous lung destruction and/or hypoxic vasoconstriction, recent data indicate that this is not true (reviewed in Ref. 75). Guinea pigs exposed to cigarette smoke develop an ~25% increase in mean pulmonary artery pressure (191). Using plastic vascular casts in such animals, we showed that PHT is not associated with significant alveolar capillary destruction (193, 194). Likewise, although there is a correlation between PHT and PO2, PO2 has not been found to be an independent predictor of pulmonary arterial pressure (149).

In guinea pigs, smoke-increased arterial muscularization (Fig. 11) correlates with pulmonary arterial pressure, and interestingly, also correlates with increased mRNA and protein levels of the vasoactive mediators endothelin and VEGF in these vessels (198). Smoke-induced vascular remodeling also appears to be related to matrix reorganization, since in guinea pigs, increases in pulmonary arterial pressure and vascular

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**Fig. 8.** Small airway remodeling in guinea pigs. Photomicrographs of Picrosirius Red-stained small airways (membranous bronchioles) from control (A and B) and 6-mo smoke-exposed (C and D) guinea pigs. Note the distinct increase in airway wall collagen. B and D are taken with polarized light; the bright white birefringence indicates that this is thick fiber collagen, and increases in thick fiber collagen cause airway wall stiffness, leading to increased resistance to airflow (see Ref. 192).

**Fig. 9.** Postulated mechanisms of small airway remodeling. In this model, oxidants in cigarette smoke, and perhaps oxidants released by smoke-evoked inflammatory cells, cause activation of latent TGF-β on the airway epithelial cell surface and also TGF-β bound to matrix, leading to TGF-β signaling through Smads and connective tissue growth factor (CTGF), and increased collagen production. In addition, oxidant attack may activate MMP-9, which in turn can activate latent TGF-β. Modeled from Refs. 33, 34, 179.
muscularization could be reduced by administration of the serine elastase inhibitor ZD0892 (188), and in smoke-exposed mice, there was an early TNF-$\alpha$/H9251-dependent upregulation of MMP-2, -9, -12, and -13 in the small intrapulmonary arteries, with only MMP-12 persisting over a 6-mo period (190). However, an MMP-9/-12 inhibitor did not prevent PHT in guinea pigs (34).

In rats (164), simvastatin reduced pulmonary arterial pressure, perhaps through suppressing inflammation and MMP-9 induction, or perhaps, as in other models of pulmonary hypertension (56), through mechanisms involving increased apoptosis or the Rho-kinase pathway. Figure 12 shows some of the factors that appear to drive PHT in COPD.

**Mechanisms of Goblet Cell Metaplasia, Mucus Hypersecretion, and Exacerbations**

In humans, goblet cell metaplasia is a frequent finding in the large and small airways of cigarette smokers (115, 183), as are increases in the number and size of mucus-producing glands in the large airways. Indeed, the amount of intraluminal mucus in the small airways has been shown to represent a major difference between subjects with significant [GOLD Stage 3 and 4 (48)] COPD compared with subjects with a lesser degree of airflow obstruction [GOLD Stage 1 and 2 (48)] (65), and the excess mucus production that defines chronic bronchitis is also thought to be important in the pathogenesis of acute exacerbations of COPD (reviewed in Ref. 13).

As opposed to humans, bronchial glands are concentrated in the proximal trachea in rats and mice, whereas in guinea pigs, they are more diffusely distributed but relatively sparse beyond the proximal trachea (57, 181). This anatomic distribution means that it is difficult to produce a model of smoke-induced chronic bronchitis in these animals, and for this reason, the literature tends to emphasize instead goblet cell metaplasia. However, goblet cell metaplasia, mucin production, mucin secretion, and bronchial gland hypertrophy are probably independently regulated processes (reviewed in Ref. 140). There are also a variety of types of mucin (140), and these may not correspond exactly between humans and animals. All of these factors make pathological smoke-induced changes related to mucin production somewhat difficult to model in animals.

In the guinea pig model, chronic cigarette smoke exposure induces secretory cell metaplasia of the small non-cartilaginous airway epithelium (185) (Fig. 13), a finding reasonably analogous to that seen in humans, but does not produce the luminal mucous plugs seen in patients with COPD (185). Smoking cessation reduces the degree of metaplasia (187). By contrast, in mice, tobacco smoke has a relatively minor effect, with only a few secretory cells appearing in the small airways (11).

Some studies report significant degrees of goblet cell metaplasia in smoke-exposed rats (84, 138, 139, 177), and this can...
be prevented, or postexposure regression enhanced, with the antioxidant/mucolytic agent N-acetyl cysteine, as well as a variety of nonsteroidal anti-inflammatory agents, and steroids (138, 139). In rats, the gastro-protective agent rebamipide (OPC-12759) decreased TNFα/H9251 production and reduced MUC5AC production, reduced inflammatory cells in BAL, and inhibited smoke-induced goblet cell metaplasia in the trachea (89). Tracheal goblet cell metaplasia in guinea pigs was induced by a 2-wk smoke exposure and could be attenuated by use of a platelet-activating factor antagonist (82). By contrast, a PDE4 inhibitor had no significant effect on the minimal goblet cell metaplasia induced by cigarette smoke in the mouse model (106). These findings suggest that goblet cell metaplasia/mucin production is driven by a variety of mechanisms. Some of these have been elucidated in tissue culture systems (reviewed in Refs. 140, 175).

Investigation of exacerbations of COPD is an area of great interest, as exacerbations are a major (poor) prognostic feature in human smokers and are a major health care burden (174). This phenomenon is complicated by a lack of a universally accepted definition (13, 101), and the triggers of exacerbation are not definitively known; bacterial and viral agents are suggested instigators. A recent human model of virus infection using rhinovirus recapitulated the symptoms and signs of an exacerbation (123). Regardless of etiology, there is evidence of upper and lower respiratory tract inflammation during exacerbations (70).

Since chronic bronchitis is a clinical definition, involving cough and sputum production, it is difficult to establish an animal model as sputum production is difficult to monitor in animals and probably sparse; the lack of diffuse increases in mucus production likewise makes it difficult to make models of acute exacerbations. Potential models of exacerbation include administration of lipopolysaccharide or administration of bacterial or viral agents with or without cigarette smoke. For recent reviews on models of acute exacerbations, the reader is referred to Refs. 54, 80, 1017.

Conclusions

Taken as a whole, the data on proteases and anti-inflammatory agents still support the idea that inflammatory cell-derived proteases are the major mediators of emphysema in animal models. However, which cells/proteases are most important in this process remains uncertain, and there are clearly complex interactions among them (Fig. 3). Antioxidant protection is important as well, and oxidative stress is linked to increases in inflammation and decreases in antiproteolytic protection, thereby connecting oxidative damage to the protease-antiprotease hypothesis (Fig. 3). As opposed to these various initiating factors, failure of the parenchyma to properly repair also appears to play a role in the genesis of emphysema, but the data

Fig. 12. Postulated mechanisms involved in the development of pulmonary hypertension in COPD. Smoke (probably oxidant)-driven increases in vasoconstrictive and vasoconstrictive agents (endothelin and VEGF) and TNFα-driven increases in MMP activation result in vascular remodeling and endothelial dysfunction. The normally vasorelaxant endothelial nitric oxide synthase (eNOS) pathway is disrupted by smoke-generated oxidants and also by TNFα, thus increasing the vasoconstrictive effects and endothelial dysfunction. Vascular remodeling and endothelial dysfunction act in a vicious circle producing pulmonary hypertension. Modeled from Refs. 189, 190, 191.

Fig. 13. Goblet cell metaplasia. Periodic acid Schiff/diastase-stained sections of small airways from control guinea pig (A) and guinea pig exposed to smoke for 6 mo (B). Note the increased numbers of mucin-containing goblet cells in the epithelium of the smoke-exposed animal.
are more controversial and most failure-to-repair models have not used cigarette smoke.

This wealth of data in fact produces the interesting conundrum that there are too many potential actors, and sorting out which processes are fundamental to the development of emphysema and which are epiphenomena is a crucial and difficult problem; in this regard, it appears that MMP-12 is consistently implicated (when looked for) in the murine models and may represent a central checkpoint (Fig. 3). Whether this is true of humans is an important question.

One of the puzzling facts about the role of proteases and oxidative stress has always been why only some smokers, mouse or human, develop emphysema while most are spared. Part of the answer probably lies in the genotypic ability of individuals to mount an inflammatory/antioxidant response. Recent data on the role of T cells and the adaptive immune response suggest a new paradigm that could partially explain this question, namely that COPD (at least emphysema) is a consequence of an autoimmune reaction to antigenic peptides derived from the breakdown of elastin and perhaps other lung components (37, 38, 98, 97, 100). Thus proteases along with reactive oxygen species would be the initial culprits initiating the breakdown of elastic and collagen tissue and producing the necessary antigenic peptides to potentially trigger the autoimmune reaction. However, only genetically predisposed humans, and mice, would develop the adaptive immune response necessary to enhance and maintain the initial inflammatory response to cigarette smoke and eventually produce disease.

The animal data suggest a variety of therapeutic approaches to human emphysema [indeed, thus far animal models have addressed only a few out of many potential targets (40, 41)], but translation to the human setting is not straightforward, as evidenced by the apparent lack of efficacy of anti-TNFα therapy in humans (10, 135, 174) compared with the clear role of TNFα in murine emphysema (32, 176). The reason for this discrepancy is unclear but probably relates in large part to the inability to produce in laboratory animals severe COPD with cigarette smoke; i.e., the smoke-induced animal models are reproducing GOLD Stage 1 and 2 disease, but symptomatic COPD patients have GOLD 3/4 disease, and thus far no model reproduces either the anatomic or functional changes or the smoke-independent progression (136), seen in such patients. This finding implies that the mechanisms driving human early stage and late stage disease may be quite different and that the animal models are limited to relatively early stage disease, but that does not make the animal models useless, since effective early intervention is probably much easier to design than effective late stage intervention.

Studies on SAR and PHT suggest that these processes are at least partially independent of the factors that drive emphysema, although there are clearly areas of overlap, such as the role of oxidants in SAR and serine elastases in vascular remodeling, and the same is true of increased mucus production and goblet cell metaplasia. This situation implies that a therapeutic approach to human COPD may have to target each anatomic compartment and a single therapeutic agent may not be able to prevent all of the separate manifestations.

GRANTS

This work was supported by Canadian Institutes of Health Research Grants 42539 and 81409.


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