Metalloproteinase-1 Induction by Cigarette Smoke*

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The interstitial collagenase matrix metalloproteinase-1 (MMP-1) is up-regulated in the lung during pulmonary emphysema. The mechanisms underlying this aberrant expression are poorly understood. Although cigarette smoking is the predominant cause of emphysema, only 15–20% of smokers develop the disease. To define the signaling pathways activated by smoke and to identify molecules responsible for emphysema-associated MMP-1 expression, we performed several in vitro and in vivo experiments. In this study, we showed that cigarette smoke directly induced MMP-1 mRNA and protein expression and increased the collagenolytic activity of human airway cells. Treatment with various chemical kinase inhibitors revealed that this response was dependent on the extracellular regulated kinase-1/2 (ERK) mitogen activated protein kinase pathway. Cigarette smoke increased phosphorylation of residues Thr-202 and Tyr-204 of ERK in airway lining cells and alveolar macrophages in mice at 10 days and 6 months of exposure. Moreover, analysis of lung tissues from emphysema patients revealed significantly increased ERK activity compared with lungs of control subjects. This ERK activity was evident in airway lining and alveolar cells. The identification of active ERK in the lungs of emphysema patients and the finding that induction of MMP-1 by cigarette smoke in pulmonary epithelial cells is ERK-dependent reveal a molecular mechanism and potential therapeutic target for excessive matrix remodeling in smokers who develop emphysema.

Cigarette smoke exposure is the primary cause of chronic obstructive pulmonary disease, which includes emphysema and chronic bronchitis (1). Cigarette smoking stimulates recruitment of inflammatory cells, cell death, and protease production (2–6). Chronic obstructive pulmonary disease pathology includes small airway inflammation, destruction of airway parenchyma (7), and elevated protease expression (8, 9). However, the intracellular signaling mechanisms activated by tobacco smoke that mediate the protease/antiprotease imbalance and subsequent matrix degradation in the lungs are not well understood.

Increased protease levels are present in lung tissue and lavage fluid from animal models and patients with emphysema (3, 7, 8, 10–12). In patients with deficiency of the elastase inhibitor α1-antitrypsin, the decreased inhibitor concentration leads to increased elastolytic activity and risk for development of emphysema. Studies in mice reveal that knocking out metalloelastase (MMP-12)1 confers resistance to cigarette smoke-induced emphysema (3), although levels of the enzyme are not elevated in human disease (9). Evidence from human tissue and animal models support a role for collagenolytic enzymes in emphysema pathogenesis (9, 10, 13). Elevated MMP-1 levels and decreased pulmonary collagen content are measured in guinea pigs after cigarette smoke exposure (12, 14). Transgenic mice expressing human collagenase (MMP-1)-1 develop emphysematous changes in their lungs (10) caused by months of proteolytic damage to the lung parenchyma (15). Human studies reveal increased collagenolytic activity in bronchoalveolar lavage fluid and lung tissue from patients with chronic obstructive pulmonary disease (8, 16, 17). In addition, a recent report identified MMP-1 mRNA and protein in lung samples from human emphysema patients but not in lung samples from healthy subjects (9). Interestingly, in the human studies, MMP-1 was detected in pneumocytes from patients with emphysema and not from smokers without disease (9). Together, the above studies suggest that MMP-1 is active in the airways and lung tissue of persons with emphysema and that continued MMP-1 expression contributes to tissue breakdown. The current study was performed to elucidate signaling pathways activated by cigarette smoke that mediate the MMP-1 expression associated with emphysema.

EXPERIMENTAL PROCEDURES

Cell Culture and Human Lung Tissue—Human small airway epithelial cells (SAECs) were cultured according to supplier instructions (Columbia Presbyterian Medical Center, New York, NY) (Institutional Review Board protocol no. 9956). These samples were used in a previous study by our laboratory (9). Emphysematous lung tissues (n = 6) were from recipient lungs obtained at transplant or lung volume reduction surgery. Patients had stopped smoking for at least three months. Normal lungs (n = 7) were from accidental death victims or donor lungs harvested for transplant but not used because of recipient complications. All samples were examined for the presence of emphysema, fibrosis, and infection. All emphysema lungs were from persons with a history of smoking. Normal sample N1 was from an active smoker. Assays were performed by operators blinded to the disease status of the samples, which was revealed by the principle investigator only after the experimental data had been collected.

1 The abbreviations used are: MMP, matrix metalloproteinase; IL, interleukin; CSE, cigarette smoke extract; SAEC, small airway epithelial cell; ERK, extracellular signal-regulated kinase; p-ERK, phospho-ERK.
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**Reagents**—Unless specified, all reagents were from Calbiochem. Stock solutions of IL-1α and IL-1β were diluted in small airway growth medium to 10 ng/ml. Kinase inhibitors were diluted in small airway growth medium (0.1% medium to 10 ng/ml). Kinase inhibitors were diluted in small airway growth medium to a final concentration of 2% or 5%, and added to cells 1 h before treatment with interleukins and CSE.

**Preparation of Cigarette Smoke Extract**—Cigarette smoke extract (CSE) was prepared using a modified protocol (18). Briefly, a Barnant vacuum pump operating at constant airflow was used to draw the smoke of one unfiltered 2R1 reference cigarette (University of Kentucky) through 25 ml of Dabecco’s phosphate-buffered saline. This solution (100% CSE) was adjusted to pH 7.4, filtered, added to small airway growth medium to a final concentration of 2% or 5%, and added to cells immediately.

**Northern Blotting**—Ten micrograms of total SAEC RNA were separated on a 1.2% agarose-formaldehyde gel and transferred to nylon (Hybond-N; Amersham Biosciences). Human MMP-1, MMP-13, TIMP-1, and glyceraldehyde-3-phosphate dehydrogenase cDNA probes were labeled with [α-32P]ATP using Ready-to-Go DNA Labeling Beads (Amersham Biosciences). In some experiments, membranes were hybridized with a 28S oligonucleotide probe labeled with [γ-32P]ATP to control for loading.

**Immunoblot Analyses**—SAEC monolayers were scraped in cold phosphate-buffered saline and resuspended in 100 μl of protein lysis buffer (50 mM Tris, pH 8.0, 150 mM NaCl, 1% Triton X-100, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 2 μg/ml pepstatin A, 1 mM Na3VO4, 1 mM phenylmethylsulfonyl fluoride), centrifuged, and 20 μg of supernatant were separated on 12% SDS-polyacrylamide gels and transferred to nitrocellulose membranes (Bio-Rad). Rabbit polyclonal antibodies against nonphosphorylated ERK and the Thr-202 and Tyr-204 phosphorylated form of ERK (amersham; University of Kentucky) were detected with enhanced chemiluminescence reagents (Amersham Biosciences). In some experiments, membranes were hybridized with a 28S oligonucleotide probe labeled with [γ-32P]ATP to control for loading.

**MMP-1 Protein and Activity Assays**—MMP-1 expression was determined in SAEC culture media using the Biotrak Human MMP-1 enzyme-linked immunosorbent assay System (Amersham Biosciences), which measures MMP-1-mediated cleavage of fluorescently labeled collagen type I (19). Multiple comparisons were made using analysis of variance with Dunnett’s multiple comparison post tests using Prism software (GraphPad Software, San Diego, CA). All other statistics were performed using two-tailed Student’s t test in Excel. p < 0.05 was considered significant.

**ERK Activity Assays**—SAECs were lysed in mitogen activated protein kinase lysis buffer (10 mM Tris pH 7.4, 2 mM EGTA pH 8.0, 150 mM NaCl, 2 mM DTT, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 1 mM Na3VO4, 1 mM phenylmethylsulfonyl fluoride). Human lung was homogenized in mitogen activated protein kinase assay lysis buffer using a Polytron tissue homogenizer, centrifuged at 25,000 × g for 20 min, and ERK activity in supernatants was determined using the Biotrak p42/p44 mitogen-activated protein kinase enzyme assay system (Amersham Biosciences). Specific p-ERK activity is expressed in picomoles of γ-32P transferred per minute from ATP to a specific p-ERK/1/2 substrate.

**SDS-PAGE Gelatin Zymography**—Equal amounts of protein from SAEC or A549 media (50 μg) were separated by electrophoresis through a 10% polyacrylamide gel containing 0.1% gelatin. The gels were stained with Coomassie Blue and destained in methanol:acetic acid:water (50:10:85). Gelatinolytic zones were quantified using gel scanning densitometry (Alpha Innotech). Weighted multiple comparisons were made using analysis of variance with Dunnett’s multiple comparison post tests using Prism software (GraphPad Software, San Diego, CA). All other statistics were performed using two-tailed Student’s t test in Excel. p < 0.05 was considered significant.

**Results**—Northern blot analysis of MMP-1 and TIMP-1 in SAECs pre-treated for 1 h with various kinase inhibitors before 24 h treatment with 5% CSE or IL-1α, TIMP, tissue inhibitor of matrix metalloproteinases, was recorded. Northern blot analysis of MMP-1 and TIMP-1 in SAECs pre-treated for 1 h with various kinase inhibitors before 24 h treatment with 5% CSE or IL-1α, TIMP, tissue inhibitor of matrix metalloproteinases was recorded. " unless specified, all reagents were from Calbiochem.

**Fig. 1.** (A) Northern blot of SAEC RNA after 24-h exposure to CSE alone or with IL-1α or IL-1β. Culture media from SAECs treated for 24 h as in A were assayed in an MMP-1-specific enzyme-linked immunosorbent assay to measure MMP-1 protein (solid bars) or in a fluorescence-based MMP-1 collagenase activity assay (striped bars). * p < 0.05, † p = 0.01 compared with control (analysis of variance with Dunnett’s test). Histogram data are means ± S.D. of at least 3 experiments. C, gelatin zymograph of media from SAECs treated for 24 h as indicated.

**Fig. 2.** A. Northern blot analysis of MMP-1 and TIMP-1 in SAECs pre-treated for 1 h with various kinase inhibitors before 24 h treatment with 5% CSE or IL-1α, TIMP, tissue inhibitor of matrix metalloproteinases. B, Northern blot of RNA from SAECs treated with increasing doses of inhibitors 1 h before treatment with 5% CSE. C, conditioned media was collected from SAECs left untreated (open bars) or pre-treated with 40 μM PD98059 (solid bars) before exposure to 5% CSE, † p < 0.05 1-h pretreatment with the non-flavone ERK inhibitor UO126 (10 μM) before treatment with 5% CSE, † p = 0.02. All data are means ± S.D. Herb A, herbimycin A; Calph C, calphostin C.
Values are means treated SAECs (assigned a value of 1.0) normalized to the ERK activity of untreated SAECs. Controls included lung sections incubated with normal goat serum instead of antibody, which demonstrated no reactivity (data not shown). Mice were sacrificed by an overdose of isoflurane. The right lung was homogenized in protein lysis buffer and 100 g of protein was analyzed by immunoblotting. The 17692 blot using a p-ERK antibody (Santa Cruz) demonstrated that p-ERK expression increased in a dose-dependent manner in 10% CSE. Western blot of p-ERK after treatment with increasing doses of H2O2. C, gelatin zymography of media from SAECs treated as in A and D, ERK activity in SAECs left untreated (○) or treated for various times with 5% CSE (●). SAEC lysates were used in a specific ERK (p44/42) activity assay. E, lysates from SAECs treated with 5% CSE for the indicated times were analyzed by Western blot using a p-ERK antibody. F, after 24 h of treatment with 5% CSE, SAEC lysates were prepared and used in the ERK activity assay. * p = 0.02. Values are normalized to the ERK activity of untreated SAECs (assigned a value of 1.0). Values are means ± S.D. SB, SB203580 p38 inhibitor; PD, PD98059 ERK inhibitor.

Animal Experiments—To determine the in vivo effects of smoke exposure, 8-week-old C57BL/CBA mice were exposed to cigarette smoke in a specially designed smoking chamber (Teague Enterprises, Davis, CA). Mice were exposed daily to 6 h of mainstream (active) and sidestream (passive) smoke from 2RAF research cigarettes for 5 days/week for various times. Levels of carboxyhemoglobin in the blood did not exceed 10% (data not shown). Control mice were exposed to room air. Animals were provided food and water ad libitum. Mice were sacrificed by an overdose of isoflurane. The right lung was homogenized in protein lysis buffer and 100 g of protein was analyzed by immunoblotting. The left lung was pressure-perfused at 25 cm 2 H2O with 10% buffered formalin for 20 min, dissected, and stored in formalin for 24 h before paraffin embedding. Experiments were approved by the Institute for Animal Care and Use Committee of Columbia University.

Immunohistochemistry of Murine Lung—Four-micrometer sections of paraffin-embedded lungs from control and smoke-exposed mice were analyzed for ERK activity. Sections were deparaffinized and rehydrated, and antigen retrieval was performed by microwaving slides for 12 min in 10 mM sodium citrate, pH 6.0. Endogenous peroxidase activity was quenched with 3% H2O2, and sections were blocked for 1 h in 10% normal goat serum. Sections were incubated with primary antibodies (1:200 rabbit ERK, Santa Cruz) overnight at 4°C, followed by incubation with a horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (Jackson). Sections were then incubated for 30 min with Vectastain ABC kit (Vector Laboratories, Burlingame, CA), and 3,3′-diaminobenzidine substrate precipitation was performed (Zymed Laboratories Inc., San Francisco, California). Slides were counterstained with hematoxylin. Controls included lung sections incubated with normal goat serum instead of antibody, which demonstrated no reactivity (data not shown).

Immunofluorescence Staining of Human Lung—Human lung tissues fixed in formalin were processed in a Leica TP1020 tissue processor and embedded in paraffin, and 4-μm sections were cut onto glass slides. After removal of paraffin by xylene, sections were rehydrated, and nonspecific protein binding was blocked with 10% normal goat serum in phosphate-buffered saline. A primary p-ERK antibody (Cell Signaling) was used at 1:800 overnight at 4°C, followed by incubation with a rhodamine-conjugated anti-rabbit secondary antibody for 1 h at room temperature. Nuclear chromatin was detected with Hoechst dye. Slides were washed with phosphate-buffered saline, coverslipped using FluorSave aqueous mounting media (Calbiochem), and viewed with a Nikon Eclipse E400 microscope. Images of p-ERK staining were merged onto the Hoechst images using Adobe Photoshop.

RESULTS

CSE Increases MMP-1 in Airway Epithelial Cells—Previous studies by our laboratory (9) and others (8) have demonstrated MMP-1 expression in lung epithelial cells of patients with emphysema. Therefore, human SAECs were treated with CSE to determine whether cigarette smoke can directly induce MMP-1 expression in these cells. SAECs were treated with IL-1α or IL-1β, because IL-1 has been shown to induce MMP-1 expression in fibroblasts and synovial cells (20). Northern analysis revealed that 24-h exposure to CSE alone induced a significant dose-dependent increase in MMP-1 (Fig. 1A). Interestingly, treatment with 5% CSE in combination with either IL-1α or IL-1β resulted in MMP-1 expression greater than treatment with CSE alone, even though treatment with either interleukin alone had no effect. The MMP inhibitor TIMP-1 did not change with any treatment (Fig. 1A). Thus, CSE increases the MMP-1/TIMP-1 ratio in SAECs.

We next assessed MMP-1 protein in SAEC culture media by enzyme-linked immunosorbent assay (Fig. 1B, solid bars). Before treatment, SAECs demonstrated MMP-1 protein expression (5.9 ± 1.6 ng/ml), which increased after 24-h CSE exposure (8.4 ± 1.9 ng/ml for 2% CSE, p > 0.05 and 15.6 ± 5.0 ng/ml for 5% CSE, p < 0.05). Treatment with the inflammatory cytokine IL-1α did not increase MMP-1 protein (11.1 ± 4.9 ng/ml, p > 0.05). CSE in combination with IL-1α induced MMP-1 expression (14.8 ± 5.5 ng/ml, p < 0.05). Treatment with IL-1β did not change MMP-1 expression (7.1 ± 3.3 ng/ml, p > 0.05). Exposure to IL-1β plus 5% CSE significantly induced MMP-1 protein levels (16.0 ± 7.6 ng/ml, p < 0.05). This induction, however, was not greater than that with CSE alone (p = 0.79). CSE Increases Collagenolytic but Not Gelatinolytic Activity of SAECs—The SAEC culture media was analyzed for MMP-1...
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ERK Inhibitors Reduce MMP-1 Induction in SAECs—To define the intracellular signaling mechanisms mediating airway MMP-1 induction by cigarette smoke and interleukins, SAECs were pretreated with specific protein kinase inhibitors before exposure to CSE or IL-1α. We chose to inhibit ERK, c-src tyrosine kinase, and protein kinase C pathways because of their involvement in MMP expression (21–23). Northern blot analysis showed that 24-h treatment with 5% CSE alone increased MMP-1 mRNA in SAECs (Fig. 2A). SAECs pre-incubated for 1 h with 40 μM PD98059 (a selective inhibitor of mitogen-activated protein kinase kinase-1/2, the kinase upstream of ERK-1/2) showed reduced baseline MMP-1 mRNA expression (Fig. 2A, compare lanes 1 and 5). In addition, PD98059 reduced CSE-mediated increases in MMP-1 (Fig. 2A, compare lanes 2 and 6) and IL-1α-mediated increases in MMP-1 (compare lanes 3 and 7). PD98059 also reduced MMP-1 induction by CSE and IL-1α in combination (Fig. 2A, compare lanes 4 and 8). The src inhibitor herbimycin A (1 μM) and protein kinase C inhibitor calphostin C (0.1 μM) had no effect on MMP-1 induction CSE or IL-1α (Fig. 2A). The inhibitors had no effect on TIMP-1. These data show that c-src and protein kinase C are not involved in MMP-1 induction by cigarette smoke or IL-1α in human airway epithelial cells in vitro. It is noteworthy that these data support a specific role for ERK in the SAEC response to cigarette smoke.

To rule out the possibility that the inhibition of MMP-1 was caused by nonspecific action of PD98059 at the high dose used (24), we performed dose response studies. In addition, we extended our studies to include SB203580, which selectively blocks the p38 pathway (Fig. 2B). Increasing doses of SB203580 did not inhibit MMP-1 induction by cigarette smoke. In contrast, low doses of PD98059 reduced the ability of CSE to induce MMP-1 mRNA (Fig. 2B, compare lane 2 with lanes 7–10). The effect of PD98059 was dose dependent. Herbimycin A and calphostin C did not prevent induction of MMP-1 by CSE.

Next, MMP-1 protein levels were examined after inhibition of ERK with PD98059 (Fig. 2C). No significant reduction in baseline MMP-1 protein (Control, open bar) was observed in culture media from SAECs treated for 1 h with 40 μM PD98059 (Control, solid bar). Treatment for 24 h with 5% CSE induced MMP-1 protein levels (5% CSE open bar, 142.7% ± 3.3% of control, p < 0.01). This increase was completely blocked by PD98059 (5% CSE, solid bar; p < 0.05, PD98059 + 5% CSE significantly lower than 5% CSE alone). Because MMP-1 protein levels measured in the media from SAECs treated with PD98059 in combination 5% CSE were the same as that of untreated control, this result suggests that there is a requirement for ERK in MMP-1 induction by cigarette smoke. In addition, 1-h pretreatment of SAECs with a structurally distinct ERK inhibitor (UO126; 20 μM) completely blocked induction of MMP-1 activity by 5% CSE (Fig. 2D, 48.7 ± 3.1% of 5% CSE alone, p = 0.02).

Hydrogen Peroxide Activates MMP-1 in SAECs—Northern blot analysis revealed that 24-h treatment with 200 μM H2O2 increased MMP-1 mRNA expression (Fig. 3A). This induction did not occur through p38 or ERK cascades because pretreatment with 20 μM SB203580 or 40 μM PD98059 did not prevent MMP-1 induction. H2O2 did not increase MMP-1, another interstitial collagenase (Fig. 3A), nor did it increase ERK phosphorylation, as determined by Western blotting (Fig. 3B). Thus, although CSE and H2O2 each increases MMP-1 expression in SAECs, CSE requires ERK signaling, whereas H2O2 does not. In particular, these data show that the intracellular signals mediating MMP-1 expression during CSE exposure are specific to cigarette smoke and are not a reaction to an increased oxidant burden. H2O2 did not increase MMP-2 or MMP-9 activity, and pretreatment with SB203580 or PD98059 had no effect on these activities (Fig. 3C). These H2O2 studies demonstrate that cultured human small airway epithelial cells respond to CSE and H2O2 by increasing expression of MMP-1 but not MMP-2 nor MMP-9.

CSE Activates ERK in SAECs—We examined the ability of CSE to activate ERK directly. CSE progressively increased...
p-ERK activity (Fig. 3D). SAECs expressed low p-ERK activity at baseline (0.4 ± 0.05 pmol of phosphate/min). After addition of fresh media, there was a rapid increase in p-ERK activity for both control and CSE-treated cells at 15 min. At 30 min, SAECs treated with 5% CSE demonstrated p-ERK activity that was significantly higher than control (2.1 ± 0.3 pmol/min, p = 0.02) and continued to rise during the 60-min treatment period (2.5 ± 0.08 pmol/min, p = 0.001). In addition, increased expression of p-ERK was detected by Western blot at 30 min after 5% CSE treatment and was still present at 60 min (Fig. 3E). Increased p-ERK was also observed in A549 alveolar epithelial cells treated with CSE (data not shown). CSE did not induce phosphorylation of p38 or stress activated protein kinase/Jun terminal kinase (data not shown). A study of the longer-term effects of smoke on ERK signaling revealed significantly increased ERK activity after 24-h treatment with 5% CSE, compared with untreated SAECs (Fig. 3F, 1.9 ± 0.3-fold above control, p = 0.016). These results demonstrate that ERK is directly activated by CSE in human airway cells in vitro.

**Activation of ERK in Murine Lung by Cigarette Smoke**—Because smoke exposure in vivo affects a variety of cell types in the lung, and because cell culture studies cannot mimic the complexity of animal physiology, we performed in vivo studies to examine the signaling pathways activated in murine lung after exposure to cigarette smoke. Western blotting of normal mouse lung homogenates showed increased p-ERK at 10 days

**Fig. 5. ERK in human emphysema.** A, lung homogenates from patients with emphysema (solid bars, n = 6) and from healthy persons (open bars, n = 7) were used in an ERK (p44/42) activity assay. *, p = 0.016, right. Data are means ± S.D., normalized to the average kinase activity in normal tissue (assigned a value of 1.0). B, lung homogenates from four emphysema patients (E1 to E4) and four persons without emphysema (N1 to N4) were used in Western blotting for p-ERK. C and D, fluorescence immunohistochemistry using a p-ERK antibody on lung tissue sections from a person without emphysema (C) and from a patient with emphysema (D). Cytoplasmic/perinuclear staining of p-ERK is detected in alveolar and airway lining cells of the emphysematous lung and not in normal lung (40× magnification).
of smoke exposure compared with lungs from control (unexposed) mice (Fig. 4A). Immunohistochemistry of lung tissue sections revealed nominal p-ERK staining in airway lining cells and red blood cells in control mice, with no staining in alveolar cells (Figs. 4, B and C). Robust expression of p-ERK is observed in airway lining cells, as well as in macrophages in the alveolar spaces at 10 days of smoke exposure (Figs. 4, D and E). Strong airway and alveolar p-ERK staining was still present at 6 months of exposure (Fig. 4, F and G). No staining was detected in slides incubated with pre-immune serum instead of antibody (data not shown). These data demonstrate that cigarette smoke activates p-ERK in resident airway epithelial cells, alveolar cells, and alveolar macrophages in vivo.

Emphysematous Human Lungs Express p-ERK Activity—Because our in vitro and animal studies revealed directly increased p-ERK activity with smoke exposure, we obtained human lung samples from patients with emphysema to examine whether changes in p-ERK were associated with disease. A previous study by our laboratory (9) showed that emphysematous lungs express MMP-1 whereas normal adult lungs do not. To determine whether this MMP-1 expression correlated with elevated mitogen-activated protein kinase (MAPK) activity, we analyzed these same human samples for p-ERK activity. Initial experiments were performed in the absence of the specific p42/p44 (ERK) substrate to determine endogenous protein phosphorylation in normal and emphysematous lungs. No difference was detected (Fig. 5A, left). However, when specific p-ERK activity was examined, significantly higher levels were detected in lung homogenates from patients with emphysema, compared with healthy control subjects (Fig. 5A, right: 1.9 ± 0.2-fold increase above normal lung, p = 0.02). We next assessed levels of ERK in eight human lung samples. Western blot analysis revealed that emphysematous lungs expressed higher p-ERK levels than normal lungs (Fig. 5B). No differences in p38 were detected (data not shown). These results reveal a potential role for p-ERK in emphysema pathogenesis. Interestingly, p-ERK expression in normal lung was lower than emphysematous lung, regardless of smoking status. For example, lung tissue from an active smoker without emphysema (sample N1) showed very low levels of p-ERK activity, compared with patients with emphysema. Thus, it seems that p-ERK activity is specific to the presence of emphysema in human tissue, not a marker of smoking status.

As a result of elevated p-ERK in lung homogenates, we performed fluorescence immunohistochemistry on human lung sections to identify the site of p-ERK expression. We prepared lung tissue sections from a patient with advanced emphysema and from a person without emphysema. Modest staining is observed in the normal sample (Fig. 5C). However, increased p-ERK is detected in the cytoplasm of cells lining the airways as well as in alveolar cells in the emphysema sample (Fig. 5D). Additional studies should clarify the specific cell types involved.

DISCUSSION

The data in this study provide evidence for activation of ERK and MMP-1 in the lungs of patients with emphysema. Several reports have documented MMP expression in the airways of tobacco smokers (3, 7, 8, 10–12). In the present study, the large increase in collagenase activity after treatment with CSE in combination with either IL-1α or IL-1β may result from induction of MMP activators. We know that cigarette smoke and interleukins increase protease production and reduce antiprotease activity in inflammatory lung cells (25–28). The demonstration that SAEs produce proteases is significant because studies have shown that pathological changes in the small airways are linked to changes in airway resistance in emphysema (29, 30).

Three major consequences of cigarette smoke-induced lung injury are chronic mucus hypersecretion, airway inflammation and narrowing, and destruction of alveolar septi (31–35). It has been shown that mitogen-activated protein kinase activation alters mucin production and inflammation in the lung (36). The present study links this same kinase pathway to increased MMP-1 expression seen in emphysema and alveolar destruction (10). The MAP kinase pathway is also implicated in apoptosis (37–39), a cell death mechanism associated with emphysema (14, 40, 41). Together, these studies suggest a central role for ERK in cigarette smoke-induced MMP-1 activity in lung epithelia and in multiple emphysema-associated pathologies.

Thousands of chemicals exist in tobacco and tobacco smoke (42). Because most particulate matter is filtered out by the airways, volatile components of the gas phase of smoke are believed to be responsible for changes in enzymatic activity, such as oxidative inhibition of α1-antitrypsin (43). Of these, nicotine, acrolein, and acetaldehyde seem to be key short-acting pharmacological agents (44). Interestingly, acrolein stimulates release of inflammatory cytokines and can alter cellular repair processes by impairing fibroblast function (45). Previous evidence of a role for ERK in matrix remodeling has also been shown using human fibroblasts, in which ERK was associated with inhibition of collagen type I expression (46). Additional studies will identify transcription factors involved in the airway response to cigarette smoke.

The present study shows that MMP-1 induction by cigarette smoke in epithelial cells of the human airway is ERK-dependent, revealing a novel mechanism for matrix remodeling in human emphysema. Our previous studies identified a causal relationship between MMP-1 production and emphysema (9, 10). The present findings suggest that emphysema patients may represent a subset of persons predisposed to long-term ERK signaling in the lung, which may render them susceptible to increased collagen destruction by MMP-1. Our human data suggest that a switch in ERK activity from transient to constitutive may be critical in emphysema progression. This hypothesis is supported by our observation that even though the persons with emphysema have abstained from smoking for months, their pulmonary p-ERK activity is significantly higher than that of active smokers without the disease. In addition, these data demonstrate that cigarette smoke activates MMP-1 in the absence of a complete inflammatory program, as shown by our epithelial culture system, providing further support for chronic obstructive pulmonary disease research efforts targeted at not only proteolytic enzymes, but also the internal signaling pathways of cells of the lung (47, 48).

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REFERENCES

1. Crozat, T. L., Weimann, G. G., Senior, R. M., Wise, R., Crapo, J. D., and Buist, S. (2003) Am. J. Respir. Crit. Care Med. 167, 1142–1149.
2. Carp, H., and Janoff, A. (1978) Am. Rev. Respir. Dis. 118, 617–621.
3. Hatazaki, R., Kobayashi, D., Senior, R., and Shapira, S. (1997) Science 277, 2002–2004.
4. Kuchner, W., D’Alessandria, A., Wong, H., and Blanc, P. (1996) Eur. Respir. J. 9, 1989–1994.
5. Sperati, M. (2002) Nat. Rev. Immunol. 2, 372–377.
6. Wright, J., and Churg, A. (1990) Am. Rev. Respir. Dis. 142, 1422–1428.
7. Jeffery, P. (2001) Am. J. Respir. Crit. Care Med. 164, S28–S38.
8. Segura-Valdez, L., Parco, A., Gaszola, M., Ubal, B., Becerril, C., and Selman, M. (2000) Chest 117, 684–694.
9. Inai, K., Dalal, S., Chen, E., Downey, R., Schulman, L., Ginsburg, M., and D’Armiento, J. (2001) Am. J. Respir. Crit. Care Med. 163, 766–791.
10. D’Armiento, J., Dalal, S., Okada, Y., Berg, R., and Chada, K. (1992) Cell 71, 955–961.
11. Reusch, C., Peto, R., Tinker, C., and Speizer, F. (1976) The Natural History of Chronic Bronchitis and Emphysema, pp 82–85, Oxford University Press, Oxford, UK.
12. Wright, J., and Churg, A. (1995) Am. J. Respir. Crit. Care Med. 152, L17–L20.
13. Shiozawa, T., Okada, Y., Furuyu, B., Schilt, J., Jaenich, R., and D’Armiento, J. (2003) Exp. Lung Res. 29, 1–15.
14. Selman, M., Montano, M., Ramos, C., Vanda, B., Becerril, C., Delgado, J.,
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Santeres, R., Barrios, R., and Pardo, A. (1996) Am. J. Physiol. 271, L734–L743

15. Foronyi, R., Okada, Y., Cole, R., and D’Armiento, J. (2003) Am. J. Physiol. 284, L727–L737

16. Finlay, G. A., O’Driscoll, L., Russell, K. J., D’Arcy, E. M., Masterson, J. B., Fitzgerald, M. X., and O’Connor, C. (1997) Am. J. Respir. Crit. Care Med. 156, 240–247

17. Ohnishi, K., Takagi, M., Kurokawa, Y., Satomi, S., and Konttinen, Y. (1998) Lab. Investig. 78, 1077–1087

18. Laurent, P., Janoff, A., and Kagan, H. (1983) Am. Rev. Respir. Dis.

19. Brenneisen, P., Wlaschek, M., Schwamborn, E., Schneider, L., Ma, W., Sies

20. Fini, M. E., Cook, J. R., Mohan, R., and Brinckerhoff, C. E. (1998) in Metalloproteinases (Mecham, R. P., and Parks, W. C., eds), pp. 299–356, Academic Press, San Diego

21. Heffernan, G., Nagy, S., Keng, X., Lockey, R., and Mohapatra, S. (2002) Respir. Res. 3, 22

22. Kashyap, R., Floreani, A., Heires, A., Sanderson, S., and Wyatt, T. (2002) J. Investig. Med.

23. Vincenti, M., White, L., Schron, D., U., and Brinckerhoff, C. (1996) Crit. Rev. Eukaryotic Gene Expression 6, 391–411

24. Davies, S., Reddy, H., Cavano, M., and Cohen, P. (2000) Biochem. J. 351, 95–105

25. Blue, M. L., and Janoff, A. (1978) Am. Rev. Respir. Dis. 117, 317–325

26. Choudhury, B., Wild, J., Alam, R., Kliman, D., Boldugh, I., Bharajiyana, N., Mileski, W., and Sur, S. (2002) J. Immunol.

27. Reunanen, N., Koski, M., Han, J., and Rennard, S. (1998) Am. J. Physiol. 274, L591–L598

28. Reunanen, N., Koski, M., Han, J., and Rennard, S. (1998) Am. J. Physiol. 274, L591–L598

29. Hogg, J. C., Macklem, P. T., and Thurlbeck, W. M. (1968) N. Engl. J. Med. 278, 1355–1360

30. Shaw, R. J., Djukanovic, R., Tashkin, D. P., Millar, A. B., du Bois, R. M., and Orr, P. A. (2002) Respir. Med. 96, 67–80

31. Carter, A., Monick, M., and Hunninghake, G. (1999) Am. J. Respir. Cell Mol. Biol. 20, 751–758

32. Cosio, M., and Guerassimov, A. (1999) Am. J. Respir. Crit. Care Med. 160, S21–S25

33. Saetta, M. (1999) Am. J. Respir. Crit. Care Med. 160, S17–S20

34. Thurlbeck, W. M., and Wright, J. L. (1999) in Chronic Airflow Obstruction (Thurlbeck, W. M., ed.) B. C. Decker Inc., Ontario, Canada

35. Turato, G., Zuin, R., Mininati, M., Baraldo, S., Rea, F., Beghe, B., Monti, S., Formichi, B., Boschetto, P., Harari, S., Papi, A., Maestrelli, P., Fahbri, L., and Saetta, M. (2002) Am. J. Respir. Crit. Care Med. 160, 105–110

36. Takeyama, K., Dabbagh, K., Jeong Shim, J., Doo-Pick, T., Ueki, I., and Nadel, J. (2000) J. Immunol. 164, 1546–1552

37. Kling, D., Lorentzen, H., Trbovich, A., Kimane, T., Donahoe, P., and Schnitzer, J. (2002) Am. J. Physiol. 283, L370–L376

38. Orlow, R., Small, G., and Shi, Y. (2002) J. Biol. Chem. 277, 27864–27871

39. Petrache, I., Chui, M., Otterbein, L., Chin, B., Mantell, L., Horowitz, S., and Chai, A. (1999) Am. J. Physiol. 277, L589–L595

40. Kasahara, Y., Tuder, R., Taraseviciene-Stewart, L., Le Cras, T., Ahman, S., Hirth, P., Waltenberger, J., and Voelkel, N. (2000) J. Clin. Investig. 106, 1311–1319

41. Majo, J., Ghezzo, H., and Cosio, M. (2001) Eur. Respir. J. 17, 946–953

42. US Department of Health, Education and Welfare (1979) Smoking and Health: A Report of the US Surgeon General. US Government Printing Office, Washington, D. C. DHHS publication (PHS) 79-50066

43. Ogushi, F., Hubbard, R., Vogelmeier, C., Fells, G., and Crystal, R. (1991) J. Clin. Investig. 87, 1060–1065

44. Pinkelstein, E., Nardini, M., and Van der Vliet, A. (2001) Am. J. Physiol. 281, L727–L739

45. Carnevali, S., Nakamura, Y., Min, T., Liu, X., Takigawa, K., Romberger, D., Spurzem, J., and Rennard, S. (1998) Am. J. Physiol. 274, L591–L598

46. Reunanen, N., Koski, M., Han, J., and Rennard, S. (1998) Am. J. Physiol. 274, L591–L598

47. Carnevali, S., Nakamura, Y., Min, T., Liu, X., Takigawa, K., Romberger, D., Spurzem, J., and Rennard, S. (1998) Am. J. Physiol. 274, L591–L598

48. Vlahos, C., McDowell, S., and Clerk, A. (2003) Nat. Rev. Drug. Discov. 2, 99–113
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