Transcriptional regulation of pulmonary elastin gene expression in elastase-induced injury

Celeste B. Rich, Isabel Carreras, Edgar C. Lucey, Julie A. Jaworski, Jo Ann Buczek-Thomas, Matthew A. Nugent, Phillip Stone, and Judith Ann Foster

Department of Biochemistry, Boston University School of Medicine, Boston, Massachusetts 02118

Submitted 24 January 2003; accepted in final form 31 March 2003

Rich, Celeste B., Isabel Carreras, Edgar C. Lucey, Julie A. Jaworski, Jo Ann Buczek-Thomas, Matthew A. Nugent, Phillip Stone, and Judith Ann Foster. Transcriptional regulation of pulmonary elastin gene expression in elastase-induced injury. Am J Physiol Lung Cell Mol Physiol 285: L354–L362, 2003.—Previously we have shown that treatment of confluent, pulmonary fibroblast cultures with elastase results in upregulation of elastin mRNA and protein levels. In the present study we focused on determining the level at which elastin expression is upregulated after elastase exposure. We examined as models for this investigation elastin gene expression in primary pulmonary fibroblast cells during the transition from subconfluent to confluent cultures and in confluent, matrix-laden cultures treated briefly with elastase. In addition, we extended our studies to mice that were given an intratracheal dose of elastase; the effects on lung elastin mRNA and elastin promoter activity levels were measured and compared with results from in vitro cell models. The results demonstrate that upregulation of elastin gene expression during the transition of subconfluent to confluent cultures and after elastase injury is associated with an increase in the level of transcription both in vitro and in vivo. Furthermore, intratracheal administration of elastase to transgenic mice illustrates that the increased levels of elastin mRNA are accompanied by increased activity of the elastin gene promoter in cells spatially positioned near major sites of tissue injury.

pulmonary fibroblasts; chronic obstructive pulmonary disease; elastic fibers; promoter; transgenic mice

ELASTIN IS AN EXTRACELLULAR PROTEIN whose intrinsic ability to passively expand and contract under gas and liquid pressure gradients renders it an important element in maintaining proper pulmonary function. In chronic obstructive pulmonary diseases (COPD) such as emphysema, the loss of elastin from the alveolar walls appears to be a major event in precipitating the clinical pathology (38). Initially this loss of elastin was thought to result from elastolytic enzymes secreted by macrophages and/or neutrophils that have migrated into the lungs after prolonged physical or chemical insult (1, 36, 38). More recently, Kasahara et al. (22) have shown that chronic administration of a VEGF receptor blocker to rats resulted in a lung pathology similar to emphysema. These authors further show that administration of the receptor blocker results in alveolar septal cell apoptosis, specifically endothelial cells, and suggest that resultant release of caspases may inactivate protease inhibitors and/or activate matrix protease activity. In either case, elastin is lost from the alveolar septa, resulting in air space enlargement.

A number of experimental models have been developed for the study of biochemical events accompanying elastolytic injury to lung tissue and elastogenic cells. Intratracheal administration of elastolytic enzymes to rodents shows that increased elastin synthesis followed the proteolytic injury (39). Furthermore, in situ hybridization of lung sections obtained from elastase-treated animals demonstrates an increase in elastin mRNA levels, especially in areas adjacent to the sites of tissue damage (26). Our research group has developed an in vitro model for elastase injury where matrix-laden pulmonary fibroblast cell cultures were briefly exposed to elastase, and the response to elastase-released products was examined (14). The results of these studies showed that elastase-treated cell cultures exhibit an increase in elastin mRNA levels with a concomitant increase in soluble elastin synthesis. Interestingly, we found that products released by elastase treatment are inhibitory to elastin gene expression in mock-treated fibroblast cultures. Overall the behavior of the cell model is consistent with data from the in vivo models and further provides a rationale for the spatially confined upregulation of elastin expression in cells adjacent to elastase injury.

Studies of various cytokines and growth factors associated with elastase treatment have provided insight into signal pathways and molecular mechanisms leading to the downregulation of elastin expression in pulmonary fibroblast cultures (2, 9, 11, 23, 34). However, very little information is available on the mechanisms underlying the reinitiation of elastin gene expression after elastase exposure. As in any efficient repair response, the ability to confine the process to a defined area of damaged tissue/matrix involves stimulation of a specific set of cells spatially positioned or recruited to synthesize matrix components. We hypothesize that this repair process involves the transcriptional control...
of elastin gene expression similar to the regulation operative in the orchestrated patterning of elastin synthesis during lung development (3, 19, 42). Consequently, the goal of the present study was to gain insight into the mechanisms underlying the increase of elastin gene expression after elastase treatment.

MATERIALS AND METHODS

Isolation and treatment of cell cultures. Neonatal rat pulmonary fibroblast cells were isolated from lungs of 3-day-old Sprague-Dawley rats and seeded in second passage as previously described (33). Confluent cell cultures were plated at 2 × 10^6/cm^2 in 75-cm^2 flasks and maintained for 2–3 wk in 5% fetal bovine serum/Dulbecco’s modified Eagle’s medium (FBS/DMEM), and medium was changed twice weekly. Neonatal mouse lung fibroblasts were isolated from 6-day-old FVB mice carrying an elastin gene promoter reporter transgene (see Generation, characterization, and elastase treatment of transgenic mice carrying the elastin promoter transgene for description). Cells were isolated as described above for rat lung fibroblasts except that the mouse fibroblasts were allowed to adhere to the flask for 3 h rather than 2 h used for rat fibroblasts (34). Confluent, matrix-laden fibroblast cultures were washed twice with Puck’s saline and once with 44 mM sodium bicarbonate solution (pH 7.4). Five milliliters of 5 μg/ml of porcine pancreatic elastase (Elastin Products, Owensville, MO) were added, and the flask was incubated at 37°C for 5 min. Two conditions were used to study the effect of elastase. In one, the elastase solution was removed, and 20 ml of 5% FBS/DMEM were added, and in the other, elastase-released products were retained, and 15 ml of complete medium were added such that 5% FBS/DMEM was attained in a 20-ml volume. The cells were incubated at 37°C for 1–4 days. Control cells were treated with 44 mM sodium bicarbonate solution without elastase. To make certain that the added elastase was inhibited after removal, 48 h before the experiment we examined two flasks from each treatment condition every 24 h by phase microscopy to ensure that no cell lifting or further matrix damage occurred (14).

Quantitation of basic fibroblast growth factor. We measured basic fibroblast growth factor (bFGF) concentrations in the elastase supernatants using the human FGF basic Quantikine immunoassay kit from R & D Systems (Minneapolis, MN).

Isolation and analysis of RNA. Total RNA was isolated and analyzed by Northern blotting as described previously by Wolfe et al. (44). The rat actin cDNA was a gift of Dr. B. Nadal-Ginard at the University of North Carolina. The rat tropoelastin cDNA is described in Rich and Foster (33). Mouse histone H3.2 plasmid was provided by Dr. W. F. Marzluff at the University of North Carolina in Chapel Hill, NC.

Isolation and quantitation of insoluble elastin. After extraction of cell layers with guanidine thiocyanate (see Isolation and analysis of RNA), the resultant insoluble residue was washed with deionized water and incubated in 0.1 N NaOH at 95°C for 45 min (25). The hot alkali residue was hydrolyzed in 6 N HCl for 24 h at 110°C. Amino acid analysis (Beckman model 6300 with System Gold software; Palo Alto, CA) was used to confirm the characteristic amino acid composition of the elastin, typically consisting of >80% nonpolar amino acids and the characteristic amino acids, desmosine and isodesmosine (40). Elastin present (in micrograms) was calculated by multiplying the sum (in nanomoles) of all amino acid residues present by 85, the average residue mass.

Transcription run-on analysis. Intact nuclei were isolated according to the method of Dean et al. (9). The transcription assay was performed in the presence of [α-^32P]UTP for 20 min at 30°C essentially as previously described (44). We applied 10 μg of pBluescript DNA and chimeric pBluescript DNA containing cDNAs for elastin, actin, and histone in duplicate onto nitrocellulose (Schleicher and Schuell, Keene, NH) using a slot-blot apparatus. Prehybridized filters were hybridized with the resultant transcription run-on solutions at 65°C for 72 h and then membranes were washed and exposed to an X-ray film for visualization and quantitation of the signal on a laser densitometer (Molecular Dynamics, Sunnyvale, CA).

Administration of elastase to mice. Female FVB mice from Taconic Farms were anesthetized by methoxyflurane inhalation and given an intratracheal instillation of 0.1 ml of physiological saline or porcine pancreatic elastase (10 μg in 0.1 ml of saline solution). Treatment of the mice with elastase was approved by Boston University School of Medicine Institutional Animal Care and Use Committee (Protocol number: 02-166). The mice were anesthetized with pentobarbital sodium 4 days after treatment and exsanguinated by cutting the abdominal aorta. The sternum was cut, and the diaphragm was removed to expose the lungs. We inflated the lungs until they were visibly taut (maximum volume) with freshly prepared paraformaldehyde through a tracheal cannula, using previously published methods (26, 27). The maximum volume was maintained for at least 2 min before the trachea was tied off to maintain inflation. The lungs, which harden rapidly with paraformaldehyde, were excised and placed in cold fixative. Two transverse slabs of tissue were cut from the left lung and one from the right caudal lobe. The same locations were sampled in all mice. The tissue was embedded in paraffin and 4-μm serial sections were cut, individually handled and numbered, and transferred to slides.

Generation, characterization, and elastase treatment of transgenic mice carrying the elastin promoter transgene. A 4.13-kb DNA fragment containing the human elastin promoter sequence from −2,260 to +2 driving expression of the chloramphenicol acetyl transferase (CAT) was used as a transgene (Fig. 1). The 4.13-kb DNA fragment was excised from a human elastin gene promoter/CAT reporter plasmid previously described (44). We generated transgenic mice at the Boston University Transgenic Core Facility by microinjecting the 4.13-kb DNA fragment into fertilized pronuclei eggs from FVB mice (Charles River Laboratories, Wilmington, MA). Microinjected eggs were implanted into foster recipient female mice of CD1 strain (Charles River Laboratories). Genomic DNA extracted from the tip of the mice tail was used for detection and analysis of the transgene. Screen-
ing for the transgene was performed by PCR using primers within the CAT coding region (5’TTTGAGGCTTTCCATG- CAGTTGCTCACC3’ and 5’ATGTGATAGAAACTGCGG- GAAATCGTCTG3’). To evaluate the transgene copy number, we digested genomic DNA with the restriction enzyme HaeII, and serial dilutions of the HaeII DNA fragment of the transgene were analyzed by Southern blot hybridization as described in Sambrook et al. (35). The HaeII DNA fragment of the transgene radiolabeled by random priming (Hoffmann-La Roche) with [α-32P]dCTP, and [α-32P]dGTP (New England Nuclear Products, Boston, MA) was used as a probe in the hybridization. Hybridized blots were exposed to X-ray film and analyzed by laser densitometry. To evaluate CAT expression in transgenic mice, we obtained protein extracts from tissues and cells homogenized in 0.25 M Tris-HCl, pH 7.5, using a Brinkman polytron and centrifuged at 10,000 g for 15 min at 4°C. We analyzed 100 μg of protein for CAT activity using [14C]chloramphenicol as a substrate as described by Donoghue et al. (12). The acetylated and nonacetylated forms of radioactive chloramphenicol were separated by thin-layer chromatography, and CAT activity was determined by the radioactivity corresponding to the acetylated forms as measured by laser densitometry (Molecular Dynamics, Sunnyvale, CA).

Elastase was instilled into the lungs of 3- to 5-mo-old transgenic mice carrying the 2.2-kb human elastin gene promoter/CAT reporter transgene as described above. Lungs were isolated at specified times after administration of elastase or saline. Total RNA and protein were extracted as previously described (19) and processed for Northern blots and CAT assays as described above.

In situ hybridization and immunohistochemistry. Sections of tissue were cut from the lung and dehydrated and embedded in Paraplast Plus embedding medium. Serial paraffin sections (4 μm) were cut, individually mounted, and numbered on Superfrost Plus slides for in situ hybridization or staining. We transcribed single-stranded sense and antisense RNA probes from a transcription vector containing a CAT encoding DNA fragment coding elastin using the Riboprobe Gemini System (Promega, Madison, WI). The elastin probe used was a rat tropoelastin cDNA described in Rich and Foster (33). The probe was radiolabeled with [35S]UTP (New England Nuclear). After incubation at 37°C for 2 h, the DNA templates were digested with 1 μg of RQ1 RNase-free DNase (Promega) at 37°C for 15 min. The sense and antisense probes were subjected to alkaline hydrolysis to decrease their average fragment length to 150 bases. The probes were then extracted with phenol-chloroform and precipitated in ethanol. In situ hybridization was performed as previously described (26). Hybridized tissue slides were observed with dark field and phase optics with Leitz NIP Fluotar phase objectives. Paraffin sections, neighboring those used for in situ hybridization, were deparaffinized and stained with Verhoeff’s elastin stain with and without the van Gieson counterstain (Fisher Scientific, Suwanee, GA). On stained tissue slides, the mean linear intercept (an indicator of air space size) was measured by projection microscopy in 20 randomly selected fields from each lung (13).

For immunohistochemical analysis, deparaffinized tissue sections were pretreated for antigen retrieval following the citrate buffer protocol described in Shi et al. (37). Immunohistochemical analyses were conducted according to Grushkin-Lerner and Brinkman (38). The polyclonal sheep anti-CAT-digoxigenin (Hoffmann-La Roche) was diluted 1:10 in 3% bovine serum albumin-phosphate-buffered saline (BSA/PBS), and the secondary antibody anti-digoxigenin-fluorescein (FITC) (Hoffmann-La Roche) was diluted 1:100 in 3% BSA/PBS. Microscopy was performed on a Zeiss Axiosvert 35 microscope with a Hamamatsu digital camera and OpenLab Improvion software.

RESULTS

Elastin mRNA levels increase during the transition from subconfluence to confluence transition, and the increase is largely due to increased transcriptional levels. In a recent publication, we compared responses of subconfluent and confluent pulmonary fibroblast cultures to the addition of bFGF and subsequent effects onmitogen-activated protein kinase (MAPK) signaling and elastin gene expression (7). Our rationale for the comparative analysis of the two different states of fibroblast cultures was that it would provide insight into events expected after elastase treatment where cell/matrix contacts are disrupted and contact inhibition is lost (14). In the present study we pursued the characterization of the subconfluent to confluent transition and compared these results directly to confluent, matrix-laden fibroblast cultures that were treated with elastase. Figure 2A presents a representative Northern blot analysis where elastin and histone mRNAs were examined from fibroblast cells 2–8 days after seeding. A composite analysis of four separate experiments is given in Fig. 2B, where the levels of elastin mRNA are quantitated to verify the consistency of cell behavior. The results demonstrate that elastin mRNA levels began to increase between days 3 and 4, concomitant with a rapid decline in histone mRNA levels. At this stage, the cells are physically in contact with one another. However, the major burst of elastin mRNA occurred between days 5 and 6, followed by a sustained high level of expression. These results suggest that withdrawal from the cell cycle, as measured by histone mRNA levels, is not a de facto trigger for the increase in elastin mRNA levels but that cell-cell and/or cell-matrix contacts may be important for attaining and sustaining high levels of elastin gene expression.

To determine the level at which the major increase in elastin mRNA is controlled, we performed nuclear run-on assays. Results shown in Fig. 2C demonstrate that the level of elastin gene transcription was significant after 8 days of culturing. It should be noted that in three separate experiments the transcriptional level of the elastin gene was very low between days 2 and 4, suggesting that the initial increase in elastin mRNA is controlled at a posttranscriptional level. Alternatively, a low level of elastin gene transcription between days 2 and 4 may be below detection by nuclear run-on analysis. Although the level of histone 3.2 transcription decreased between the samples examined, this decrease was not comparable with that found for the mRNA levels (see Fig. 2A). This finding is consistent with the report that histone 3.2 mRNA is regulated primarily at the posttranscriptional level (16).

Overall these results show that elastin mRNA levels in primary cultures of pulmonary fibroblasts are increased after cells withdraw from the cell cycle and that this increase is controlled largely at the transcriptional level. Because the major burst of elastin mRNA occurs several days after cell proliferation ceases, fac-
tors other than cell cycle proteins appear to contribute to the activation of elastin gene transcription (19).

Brief elastase treatment of confluent, matrix-laden pulmonary fibroblast culture results in the upregulation of elastin mRNA that is accompanied by increased elastin gene transcription. The data presented above show that maximal elastin gene expression in pulmonary fibroblasts is achieved after 5–6 days in culture. The work of our group and others have shown that pulmonary fibroblasts require an additional 7–14 days for significant accumulation of insoluble elastin in the extracellular matrix (14, 30, 41) as well as proteoglycans and growth factors (4, 5, 34). Within these experiments, the amount of insoluble elastin ranged from 15 to 32 μg/75-cm² flask.

The next series of experiments were designed to study the effect of elastase treatment on 14- to 17-day fibroblast cultures containing detectable amounts of alkali-resistant, insoluble elastin (14, 30, 41). Two experimental conditions were used for these studies that involved either removal or retention of the elastase-released products within each culture flask. Figure 3A presents Northern blot analyses of elastin and histone mRNA levels after elastase treatment where elastase released products were either removed (fresh medium) or retained (retained medium), and the results were compared with mock-treated cell cultures (control). The results are interesting from several aspects. First, elastase treatment results in an initial decrease in elastin mRNA levels (30 ± 10%) followed by a gradual increase that eventually exceeds the initial level. This increase in elastin mRNA occurred regardless of retention or removal of elastase-released products. Retention of elastase products consistently showed a greater decrease in elastin mRNA and may reflect the inhibitory effect of released bFGF on cells that were not affected by the brief elastase treatment since the proteolytic damage is not uniform (34). The amount of bFGF released by elastase treatment was calculated to be in the range of 35–40 pg/ml. Mock-treated cell cultures exhibited no change in elastin or histone mRNA levels, which obviates any concern due to culture conditions. Morphological examination of the cell cultures after elastase treatment revealed a definite disruption of cell and matrix interactions as shown by rounding of some cells adjacent to elastase exposure and a clearing of matrix near the proteolytic damage.

Fig. 2. Northern blot and nuclear run-on of rat pulmonary fibroblasts in culture. A: total RNA was extracted from pulmonary fibroblasts from 2 to 8 days after seeding in culture and Northern blot analysis was performed on 20 μg of total RNA and probed with 32P-labeled cDNA for elastin and histone. rRNA was stained with methylene blue to show gel loading. B: the results from 4 separate experiments were quantitated by laser densitometer and plotted against days after seeding. Values are means ± SD. C: nuclei were isolated from 2- and 8-day cell cultures of pulmonary fibroblasts and nuclear run-on assay performed in the presence of [32P]UTP for 20 min. The 32P-labeled transcripts were hybridized to histone, elastin, and pBluescript DNA.

Fig. 3. Northern blot and nuclear run-on analysis of 14-day rat pulmonary fibroblasts cultures treated with elastase. A: total RNA was extracted from 14-day pulmonary fibroblast cultures treated with elastase at designated times after elastase treatment. Three conditions are given: 1) fresh medium, where elastase products were removed and cultures were given fresh medium; retained medium, where elastase products were added along with fresh medium (see MATERIALS AND METHODS); and 3) control, where cells were treated with buffer, and fresh medium was added. The Northern blot analysis was performed on 20 μg of total RNA and probed with elastin and histone cDNAs. rRNA was stained with methylene blue. B: nuclei were isolated from 14-day rat pulmonary fibroblasts cultures after treatment with elastase at the indicated times. Elastase-released products were retained in the medium after treatment. A nuclear run-on assay was performed, and the 32P-labeled transcripts were hybridized to actin, histone, elastin, and pBluescript cDNAs.
The decrease in elastin mRNA levels at 24 h coincides with a recovery period where some cells re-attach to the matrix and a low level of cell proliferation occurs as shown by small induction of histone mRNA. These events are followed by an increase in elastin mRNA levels. The results suggest that one of the major effects of elastase treatment is to release cells from contact inhibition, therefore reinitiating elastin gene expression.

Nuclear run-on analysis was performed to determine whether the increase in elastin mRNA seen after elastase treatment is controlled at the transcriptional level. Elastase-released products were retained in the medium after treatment. Results presented in Fig. 3B show that elastin gene transcription levels dropped to barely detectable levels within the first 2 days after elastase treatment and then returned to the initial levels. This finding is consistent with the changes observed in the elastin mRNA levels after elastase treatment.

Overall these results show that elastin gene expression increased 48–72 h after elastase treatment and that this increase is due, in part, to transcription. The fact that elastase treatment induces cell proliferation is consistent with other models of repair responses where cell migration and proliferation precede the initiation of new matrix synthesis (24, 43).

Elastin mRNA and elastin gene promoter activity levels are increased after intratracheal administration of elastase to mice. Animal experiments were designed to examine the effect of intratracheal administration of elastase on elastin mRNA in lungs of female FVB mice. Figure 4, A and B, shows photomicrographs of the light and dark field, respectively, of a representative section of lung tissue obtained 4 days after mice were given 10 μg of pancreatic elastase. Calculations of mean linear intercept values for each individual microscopic field show that 15% of the fields in the elastase-treated mice had mean linear intercept values that were 2 SD higher than that for control animals, indicating enlargement of air spaces in elastase-treated animals. In situ hybridization with elastin riboprobe demonstrates that elastin mRNA levels are increased in cells directly adjacent to the elastase injury and establishes that elastogenesis is an initial response to elastase injury in vivo.

The next set of experiments were designed to examine the response of the elastin gene promoter to elastase administration using FVB mice carrying the elastin promoter/reporter transgene. Three separate transgenic mouse lines originating from independent founders were generated with transgene copy numbers in the range of 3–5, 5–10, and 30–40 copies, as measured by Southern blot analysis. Organ-specific expression of elastin gene promoter activity was measured by CAT activity in 3- and 7-day-old mice. A representative assay of tissues isolated from skin, lung, kidney, and brain is presented in Fig. 5. These results show that the 2.2-kb elastin promoter fragment CAT activity was similar to that reported by Hsu-Wong et al. (18) for 5.2-kb elastin promoter transgene. The data further point out that elastin promoter activity in the lung is very high during the period corresponding to lung alveolarization where elastin gene expression is prominent as defined by mRNA levels (3). Three different transgenic mouse lines presented essentially the same pattern of CAT activity, which was linear with the copy number of the transgene (data not shown). To show that the CAT activity measured in tissue was reflective of endogenous elastin gene expression, we isolated pulmonary lung fibroblasts from neonatal transgenic mice and compared the level of elastin mRNA levels and CAT activity. Figure 5B provides both a Northern blot and CAT assay performed on 6-day-old mouse pulmonary fibroblasts maintained in either 10 or 0.5% serum. The results show the CAT activity mirrors the levels of elastin mRNA.

To examine the response of elastin gene promoter activity to lung injury, we instilled 10 μg of pancreatic elastase intratracheally to adult transgenic mice and analyzed lungs 3 and 14 days after treatment. Mean linear intercept values showed 21 and 60% increase at days 3 and 14, respectively. Figure 6 provides a summary of two separate experiments where elastin mRNA levels and CAT activity were determined from...
whole lung samples. It should be pointed out that the CAT activity measured in the control (saline-treated) lung tissue was 2.5% of that found in the tissue isolated from 3-day-old mice. A major increase in the level of elastin mRNA was seen 3 days after elastase treatment, and significantly, this increase was accompanied by increased elastin gene promoter activity. After 14 days, elastin mRNA remained elevated but at lower levels, whereas elastin promoter activity continued at the same elevated level. This latter finding is very interesting, since it shows that, although both elastin promoter activity and elastin mRNA are increased and remain elevated after elastase exposure, the two measurements of elastin gene expression do not parallel one another. These data suggest that posttranscriptional, as well as transcriptional, mechanisms may play a role in the elastogenic response to elastase injury. This observation is consistent with proposed developmental mechanisms regulating lung elastin gene expression (29).

To localize the cells exhibiting increased elastin promoter activity after elastase treatment, we treated an additional group of transgenic mice with an intratracheal dose of elastase (10 μg/mouse) or saline solution (as control) as described above. Five days later the mice were killed, the lungs were fixed, and sections were prepared for immunohistochemistry. Figure 7 provides a panel of sections where the localization of antibody to CAT protein in elastase-treated lung (Fig. 7A) and saline-treated lung (Fig. 7B) is compared with the light fields of the same sections (Fig. 7, C and D, respectively). Results show that CAT protein localization in the tissue sections from elastase-treated lungs (Fig. 7D) appeared in areas associated with obvious tissue damage similar to the spatial location of elastase-induced levels of elastin transcript (see Fig. 4).

DISCUSSION

Investigations into the response of lung elastin gene expression to elastolytic activity are important to our understanding of the development and progression of pulmonary emphysema. Available animal models for elastase injury rely on a single, exogenous dose of active elastolytic enzymes (39) or acute exposure to cigarette smoke (17) to produce enlargement of respiratory spaces with destruction of alveolar walls. The results of these models have provided valuable information concerning elastogenic responses to injury (26, 39) and potential cellular sources of elastase activity (17). However, human emphysema is characterized as a COPD and as such develops over a long period of time. The human disease is diagnosed in its terminal stages by clinical manifestations of compromised respiratory function. Tissue samples obtained at autopsy provide little information regarding early events precipitating the pathogenesis. There are no means available to study tissues from individuals exposed to conditions known to cause COPD, yet there are those who do not develop the disease. Experimentally, it is difficult to design whole animal experiments that involve a repetitive exposure to chemical or biological agents leading to elastolytic activity that mimic events accom-

![Fig. 5.](image-url)
panying development or protection from the human disease.

We have previously proposed that a “normal” repair of proteolytic damage to interstitial lung elastin exists and is accompanied by an upregulation of elastin gene expression in the localized area of elastin damage (34). A breakdown in this homeostatic condition might occur in chronic exposure to elastolytic enzymes, where a wide spectrum of active proteolytic enzymes released from damaged matrix and apoptotic cells exacerbate the situation. These events can destroy the structural integrity of the airway network, causing depletion and/or dissociation of elastogenic cells from important matrix signals that initiate the repair process. As a model to understand the mechanisms underlying “normal” responses of elastogenic cells to elastase exposure, we have utilized pulmonary fibroblasts cultures that produce a significant matrix containing insoluble,

Fig. 7. Immunofluorescent localization of CAT protein on elastase-treated and control transgenic mice. Transgenic mice carrying the 2.2-kb human elastin gene promoter/CAT reporter transgene were treated with an intratracheal dose of pancreatic elastase (10 μg) or saline solution (control). Five days after treatment, mice were killed and tissue was fixed as described. Sections were incubated with CAT antibody. B (control) and D (elastase treated) show immunofluorescence associated with CAT protein. A (control) and C (elastase treated) show the light fields of the same sections.
cross-linked elastin (6) as well as matrix-bound growth factors (4, 34). This model allows controlled exposure to elastase and an opportunity to investigate the specific cellular responses to proteolytic damage and ligands released by elastase treatment. In the present study we focused on determining the level at which elastin expression is upregulated after elastase exposure (14). The results are significant from several aspects. First, the results demonstrate that upregulation of elastin gene expression after elastase injury is associated with an increase in the level of transcription both in vitro and in vivo. Others have proposed that elastin gene expression is regulated at the posttranscriptional level (42). Mauviel et al. (28) demonstrated transcriptional regulation in lung fibroblasts from transgenic mice containing the 5.2-kb elastin transgene with human recombinant IL-1. It now appears that elastin regulation is complex and involves multiple levels of regulation whose individual contribution may be independent of age but rather dependent on specific circumstances surrounding tissue damage or exposure to various ligands. Second, the fact that the cell system mimics events observed within whole lung tissue affords investigators the opportunity to use the cell model for dissection of the molecular mechanism regulating elastin upregulation. For example, we previously showed that bFGF, a major growth factor released by elastase treatment of pulmonary fibroblasts (34), affects proliferating and confluent pulmonary fibroblast cultures differently in terms of the duration of MAPK activation and the impact on elastin gene expression (7). The differential effects of bFGF on proliferating vs. confluent cells could result from differences in the level of heparan sulfate proteoglycans that can bind bFGF. Indeed, we have recently observed a dramatic increase (approximately twofold) in the level of elastase releasable bFGF that is stored within the extracellular matrix of these cultures from days 4–8 (5). Thus at confluence the cells and their corresponding matrix may be positioned to bind and sequester bFGF, leading to distinctly different kinetics of cell stimulation. Interestingly, elastase treatment causes a dramatic decrease in the heparan sulfate levels and bFGF binding to confluent pulmonary cells without dramatically altering FGF receptor levels (4). Hence, cells after elastase treatment and at subconfluence with limited and/or damaged extracellular matrix might be tuned to respond to the proliferative effects of bFGF. These latter results, in combination with data from the current study, suggest that bFGF may be an important ligand in initiating cell proliferation after elastase treatment similar to a situation described in pulmonary artery smooth muscle cell cultures (43). Future studies will address the role of bFGF in elastase-treated cultures in an attempt to understand the role that this growth factor plays in controlling repair mechanisms in the fibroblast cultures.

The results from this study show that the increase in elastin mRNA levels following elastase treatment is accompanied by an increase in elastin gene transcription. Currently, very little information is available on how elastin gene transcription is increased. The majority of published studies on specific cis-acting elements and cognizant trans-acting factors have focused on the downregulation of transcriptional levels (2, 21, 23, 28, 32, 34). On the other hand, only two cis-acting elements within the elastin gene promoter have been shown to convey increases in transcriptional initiation. These include a Sp1/Sp3 sequence in the proximal promoter region that possesses similarities to a retinoblastoma element sequence (8) and an NF1 sequence located in the distal promoter region (10). The former site controls upregulation by insulin-like growth factor I in aortic smooth muscle cells through a mechanism of derepression (8, 20). The latter site appears to control the level of elastin gene transcription via the binding of different NF1 family members, although specific factors regulating the differential binding of family members in elastogenic cells is unknown (10). It is interesting to point out that a recent report by Rafaty and Khachigian (31) demonstrates that there is cross talk between NF1 and Sp1 factors. Future studies will focus on determining the possible involvement of these two sites in regulating elastin gene transcriptional responses to elastase injury.

In conclusion, the data presented in this study demonstrate that transcription plays a major role in the response of elastin gene expression to elastase exposure both in vitro and in vivo. Furthermore, the similarity between cell culture and lung tissue responses to elastase treatment supports the use of cell cultures to dissect molecular events accompanying in vivo conditions.

The authors acknowledge the superb technical assistance of Valerie Verbitzki for isolating and maintaining pulmonary fibroblasts.

DISCLOSURES

This work was supported by National Heart, Lung, and Blood Institute Grant HL-46902.

REFERENCES

7. Carreras I, Rich CB, Jaworski JA, Dicamillo SJ, Panchenko MF, Goldstein R, and Foster JA. Functional components of basic fibroblast growth factor signaling that inhibit lung...


Downloaded from http://ajplung.physiology.org/ by 10.220.33.6 on April 12, 2017