Implications for matrix metalloproteinases as modulators of pediatric lung disease

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Winkler, Margaret K., Jane K. Foldes, Robert C. Bunn, and John L. Fowlkes. Implications for matrix metalloproteinases as modulators of pediatric lung disease. Am J Physiol Lung Cell Mol Physiol 284: L557–L565, 2003.—Matrix metalloproteinases (MMPs) are a large family (>20) of cation-dependent proteinases believed to be important modulators of normal human lung development and potentially harmful mediators of lung damage. Little is known about MMP production and secretion by the lung during childhood or how alterations in MMP levels may be involved in lung damage. We examined endotracheal aspirates from children (<19 years) without lung disease for the presence of MMP activity. Only gelatinase activity was detectable, and inhibitor profiles suggest they represented one or more MMPs. Comparison of gelatinase activity, MMP expression, and MMP activity in children without pulmonary disease with children who required mechanical ventilation for respiratory failure show: 1) gelatinase activity was approximately five- to sixfold higher in respiratory failure; 2) MMP-7, MMP-8, and MMP-9 concentrations and MMP-8 and MMP-9 activities were markedly elevated in respiratory failure; and 3) MMP-7, MMP-8, and MMP-9 levels were significantly correlated in children with lung disease. These studies provide compelling evidence that specific MMPs are present in the diseased lung and may participate in the pathogenesis of pediatric respiratory failure.

extracellular matrix; bronchoalveolar lavage

THE FORMATION, ASSEMBLY, AND CONSERVATION of extracellular matrix (ECM) are crucial to maintain the mechanical and functional properties of the lung and allow for normal gas exchange. Alterations in ECM physical properties or disruption of lung cell-ECM interactions may render the lung incapable of performing properly. To maintain structural integrity, the lung, like other tissues, must undergo a certain degree of tissue remodeling that involves the building of new organ architecture while old tissues are broken down and removed. Tissue turnover, in a number of organs, is regulated in part by the matrix metalloproteinases (MMPs) and their inhibitors, tissue inhibitors of MMPs (TIMPs). MMPs comprise a family of ECM-degrading enzymes that share common functional domains and activation mechanisms. More than 20 members of the MMP family have been identified, which together are capable of degrading almost all components of the ECM (2, 17, 19, 38). Recent data support a major role for MMPs in maintaining lung architecture (28). Specifically, there are several lines of evidence suggesting that MMPs are involved in normal lung development. Furthermore, overactivity of MMPs in the lung is likely to contribute to pulmonary disease processes (8, 28, 37).

By degrading ECM components, MMPs can function in both the destruction of alveolar epithelium as well as its reorganization during the repair process. Animal models as well as human studies in adults support a role for MMPs and an imbalance of MMP and TIMP homeostasis in the pathogenesis of several well-recognized pulmonary disorders, including emphysema (26), idiopathic interstitial pneumonias (29), chronic obstructive pulmonary disease (COPD) (3, 25), and chronic reactive airway disease (13, 32). These data together suggest that dysregulated cellular production, secretion, and activation of MMPs and their inhibitors are involved in pathological conditions of the lung.

Findings in the developing rabbit lung model have shown that MMPs and their inhibitors may contribute to the formation of airways and alveoli in the developing fetal lung (10). For instance, in the early stages of rabbit lung development, MMP-1, MMP-2, MMP-9, and membrane type 1-MMP (MMP-14) are distributed in epithelial cells where they appear to be involved in the formation of the airway. There is a notable correlation between morphological changes and the distribution of MMPs in the different stages of lung development. For instance, it appears that in the later stages of development and in postnatal lungs, activated MMP-2 may be localized in the alveolar epithelial cells with a dramatic increase in MMP-2 activation during later lung development (10). In addition, MMP-9 has been detected in bronchial epithelial cells and type II alveolar epithelial cells during the late stages of lung development (10). Thus it is likely that MMPs and TIMPs play an important role in the development of normal lung architecture.
Several studies in humans have shown that MMPs and TIMPs generated within the lung can be detected and measured by sampling fluids obtained from bronchoalveolar lavage (BAL), a technique requiring bronchoscopy. For instance, MMP-2, MMP-9, and TIMP-1 are upregulated in BAL fluid from adult patients with acute respiratory distress syndrome (6). However, little normative data are available on what forms of MMPs are present (if any) in fluids generated by the normal human lung, particularly in children. Herein, we use another source of lower respiratory tract fluids, endotracheal tube aspirates (ETAs), from children as source materials to identify and characterize MMPs present in pulmonary secretions from healthy children as well as from children who have suffered respiratory failure requiring mechanical ventilation.

MATERIALS AND METHODS

Materials. Purified or recombinant human MMP-2, MMP-7, MMP-8, and MMP-9 were purchased from Calbiochem (San Diego, CA). Monoclonal or polyclonal antibodies to MMP-2, MMP-7, MMP-8, or MMP-9 were purchased from Chemicon (Temecula, CA). The monoclonal antibody against human CD68 was obtained from US Biologicals (Swampscott, MA). Reagents used for SDS-PAGE were purchased from Bio-Rad (Richmond, CA). Immobilon-P polyvinylidene fluoride (PVDF) membranes were purchased from Milli-pore (Bedford, MA). Enhanced chemiluminescence blotting kits, Rainbow high-molecular-weight markers, and hyperfilm-ECL were obtained from Amersham (Buckinghamshire, UK). Porcine skin gelatin and casein hydrolysates were purchased from Sigma (St. Louis, MO). The bicinchoninic acid (BCA) protein assay kit was purchased from Pierce, Milwaukee, WI. All collections were performed with the permission of the Institutional Review Board at The University of Alabama at Birmingham.

Immunocytoc hemistry. An aliquot of ETA was placed on a glass microscope slide, and then a cytometric smear was performed. The slide was then thoroughly dried at room temperature before being used for immunocytochemistry. Parallel slides were also prepared and stained with Wright’s stain for cell counts and morphology. For immunocytochemistry, slides were first fixed with acetone. Immunocytoc hemistry was performed using the Vector ABC kit according to the manufacturer’s instructions. The CD68 monoclonal antibody was used at a dilution of 1:50.

Substrate zymography. Gelatin and casein zymography was performed according to previously reported procedures from our laboratory (9, 33). In brief, porcine skin gelatin or casein hydrolysate (1 mg/ml) was added to a 10% SDS-

<table>
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<th>Table 1. Population demographics</th>
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M, male; F, female; N, no; Y, yes; BPD, bronchopulmonary dysplasia; RAD, reactive airway disease; CP, cerebral palsy; MR, mentally retarded; RSV, respiratory syncytial virus; PHTN, pulmonary hypertension; URI, upper respiratory infection; CHD, congenital heart disease; ULD, underlying lung disease.
polyacrylamide solution before casting. ETAs were either pooled (n = 21) or run separately, then 50-µl aliquots were diluted in 4× nonreducing sample buffer, and unboiled samples were electrophoresed through 10% substrate gels at 30 mA per gel for ~1 h. After electrophoresis, gels were washed in 2.5% Triton X-100 for 1 h at 4°C and then incubated in 50 mM Tris-HCl, pH 8.0, and 5 mM calcium chloride for 16 h at 37°C. Gels were stained for 10–30 min in Coomassie blue and destained for 1–2 h. Lytic areas within the substrate gels represent MMP activity. In selected experiments, aliquots of the pooled ETA were analyzed by gelatin-substrate zymography in the presence or absence of EDTA (10 mM) or a cocktail of protease inhibitors by adding the inhibitors directly to the 37°C incubation.

**Immuno blotting.** A volume of cleared ETA equal to 25 µg of total protein (MMP-2, MMP-8, or MMP-9) or 5 µg of total protein (MMP-7) was lyophilized to dryness in a speed-vac concentrator. For pooled sampling, each pool was made from equal amounts of protein from each ETA. Dried samples were resuspended in 25 µl of 2× SDS-PAGE loading buffer minus 2-mercaptoethanol. Solubilized samples were resolved on 10% PAGE gels with or without 0.1% Triton X-100 over the bottom of the gel. Proteins were transferred onto PVDF membranes by electrical current in Tris/glycine/SDS buffer containing 20% methanol. After transfer, nonspecific antibody binding sites on the membranes were blocked by incubation in 50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.1% Tween 20 (TBST), and 5% dry milk for 1 h at room temperature.

Antibodies were used at the following concentrations: monoclonal anti-human MMP-2 antibody, 1:1,000; polyclonal anti-MMP-7, 1:1,000; monoclonal anti-MMP-8, 1:1,000; monoclonal anti-human MMP-9, 1:200. All antibodies were diluted in TBST plus 5% dry milk (TBST milk) and incubated with the membrane overnight at 4°C on a rocking platform. After being incubated with primary antibodies, the membranes were washed with TBST for 15 min at room temperature with three changes of buffer. Horseradish peroxidase-conjugated anti-rabbit and anti-mouse secondary antibodies were from Amersham. Antibodies were diluted 1:2,500 in TBST and incubated with membranes for 1 h at room temperature. After being washed with TBST for 15 min at room temperature with three changes of buffer, membranes were incubated for 1 min with enhanced chemiluminescence reagent (ECL, Amersham). Membranes were removed from ECL reagent and exposed to Hyperfilm-ECL autoradiography film (Amersham).

**Quantitative analysis of MMPs by quantitative dot blot.** To quantify MMP-2, MMP-7, MMP-8, and MMP-9 levels in individual ETAs, we used a dot blot methodology described previously by our group that detects both pro- and active forms of MMPs (33). One microgram of each ETA was applied in duplicate under vacuum onto a nitrocellulose filter using a 96-well dot-blotting apparatus. To quantitate the MMPs present in each ETA, a standard curve was also applied on each blot. For standard curves, purified or recombinant MMP-2, MMP-7, MMP-8, or MMP-9 (0–50 ng) were dot blotted in a similar fashion to clinical specimens. Once all samples were applied, the filter was air-dried and then blocked in TBST. The filter was then incubated with either MMP-2 antiserum (1:100), MMP-7 antiserum (1:200), MMP-8 antiserum (1:500), or MMP-9 antiserum (1:200) overnight at 4°C. Blots were washed in TBST and then incubated for 1 h at 22°C with anti-rabbit or anti-mouse horseradish peroxidase conjugate (1:2,500). After being washed, the filters were developed using the ECL kit as described above. To quantitate MMP immunoreactivity, the films developed from the various filters were read using a standard 96-well plate reader at 630 nm. Standard curves generated from the known MMP concentrations were calculated using Bio-Tek software, and unknown ETA MMP concentrations were calculated using these standard curves. Results were expressed as nanograms per microgram of total protein. Between groups, differences in MMP concentrations were compared using Student’s t-test, and data are expressed as means ± SE for each group.

**MMP activity assays.** To measure directly the MMP activity in ETAs, we used a novel fluorescent substrate assay (R&D Systems, Minneapolis, MN) to quantitate specific, active MMPs present in ETAs from a subpopulation of healthy children (n = 8) and from children experiencing respiratory failure (n = 8). The enzymatic activities of MMP-8 and MMP-9 activities, whose protein levels are elevated in ETAs from children experiencing respiratory failure, were measured. For the activity assays, ETAs were first diluted into 1:10 for controls and 1:50 for sick children. MMPs (e.g., MMP-8 or -9) were then captured using an MMP-specific antibody precoated microtiter plate. After being washed, captured MMPs were incubated with the fluorogenic substrate for 17–20 h at 37°C in the dark. The amount of cleaved substrate was measured using a fluorometric plate reader at an excitation wavelength of 320 nm and an emission wavelength of 405 nm. The concentration of active MMP was interpolated from a standard curve using pure and activated MMP-8 or MMP-9. Samples were run in duplicate, and results are expressed as nanograms per milliliter per microgram of total protein.

**RESULTS**

To confirm that ETAs obtained from children represented lower respiratory tract secretions, we first examined whether the cell populations present in ETAs represent cell types typical of the lower respiratory tract. Previous studies in neonates have demonstrated that ETAs contain representative cell types present in the lung and bronchi, including epithelial cells, neutrophils, and macrophages (18). In our studies, cell counts from ETAs obtained from healthy children generally ranged from 3 × 10^5 to 3 × 10^6 cells/ml. Examination of samples by Wright’s stain demonstrated several different cell types, including the notable presence of large, uninciliated cells consistent with monocytes and/or alveolar macrophages, a cell type primarily associated with the lower respiratory tract. To confirm the identity of these cells as macrophages, immunocytochemistry utilizing a monoclonal antibody against CD68, an alveolar macrophage-specific cell surface antigen, was performed. As shown in Fig. 1, cells staining positive for CD68 were identified in ETAs from healthy children. This confirms their identification as alveolar macrophages and demonstrates that ETA samples contain lower airway cell types and are representative of lower airway secretions.

The production and secretion of MMPs from cell types located in the human lung have been described to various degrees from both in vivo sampling as well as in vitro cell culture systems (see Refs 26 and 28 for review). However, the secretion of MMPs in lower respiratory tract fluids has not been well characterized. Therefore, we next determined whether MMP...
proteins and enzymatic activities could be detected in ETAs. We examined ETAs from healthy children using gelatin substrate zymography. Figure 2A, lane 1, shows that faint bands (~72 and ~95 kDa) were identified in pooled samples of ETAs from healthy children, which are similar to the molecular masses of pro-MMP-2 and pro-MMP-9 (38). In contrast, casein-substrate zymography revealed no detectable caseinolytic activity, which, if present, could reflect the activities of stromelysins and/or collagenases (data not shown). To further characterize the gelatinases present in ETAs, control samples were incubated in the presence of several protease inhibitors. As shown in Fig. 2A, lane 3, no inhibition was observed in the presence of the serine protease inhibitor PMSF (1 mM). Similarly, no inhibition was seen when incubated in the presence of cysteine and aspartic protease inhibitors (data not shown). In contrast, the metal-dependent protease inhibitor EDTA (Fig. 2A, lane 5) completely inhibited all detected gelatinase activity. Together, these studies demonstrate that all proteases identified by gelatin-substrate zymography in control ETAs are inhibited by a metal-dependent protease inhibitor, but not by the other three classes of protease inhibitors, and based on their activities, molecular weights, and inhibitor profiles, they most likely represent members of the MMP family (19).

On the basis of our observation that gelatinase activity was detectable in ETAs from healthy children, we next explored whether gelatinase activity might be altered in lower respiratory tract secretions obtained from children who were intubated and required mechanical ventilation for the treatment of respiratory failure. As shown in Fig. 2A, lane 2, a number of distinct gelatinase bands were detected in the ETAs obtained from these children, with molecular masses ranging from ~50 to 200 kDa, most likely representing various forms of MMP-2 and MMP-9. Furthermore, casein-substrate zymography revealed that these same ETAs displayed significant caseinolytic activity over a wide range of molecular masses, even though control ETAs revealed no detectable casein-degrading activity (data not shown). Similar to control samples, EDTA completely inhibited all gelatinase activity in ETAs from sick children (Fig. 2A, lane 6), whereas serine (Fig. 2A, lane 4), aspartic, and cysteine protease inhibitors (data not shown) were without effect. Densitometric analysis of gelatinolytic bands of between ~50 and 200 kDa was used as a means to quantify the differences in gelatinase activity observed between age-, race-, and sex-matched subpopulations from each of the two groups. Total gelatinase activity in ETAs from ventilated, sick children was significantly elevated compared with specimens obtained from healthy chil-

Fig. 1. CD68-positive cells in endotracheal tube aspirates (ETAs). Aspirates were prepared as described in MATERIALS AND METHODS and stained with the alveolar macrophage-specific monoclonal antibody, CD68. CD68-positive cells appear as large black cells (denoted by arrows) when compared with other cells in the field.
dren (Fig. 2B). On average, gelatinase activity was approximately five- to sixfold higher in children experiencing respiratory failure \( (P < 0.005) \).

To identify which MMPs are prevalent in ETAs in children with lung disease compared with healthy children, we immunobotted pooled samples \( (n = 18) \) from each group with antisera specific for MMP-2, -3, -7, -8, -9, and -12. These MMPs were chosen for analyses because previous reports demonstrated their expression in cell types present in the lung. MMP-2, -7, -8, and -9 were detected in both pooled sputum samples, and all four MMPs appeared to be elevated in the pooled sample from sick children compared with the control pool (Fig. 3). Furthermore, each of the MMPs was detected as multiple bands, some of which were detected at higher-molecular-weights relative to the pro-MMP, and some of which were observed at lower-molecular-weights than their proforms. Higher-molecular-weight species, especially of MMP-9, likely represent a combination of homodimeric MMP-9 and complexes of MMP-9 with TIMPs and/or neutrophil gelatinase-associated lipocalin. Lower-molecular-weight species likely represent activated forms of each of the MMPs. In contrast, MMP-3 and MMP-12 were not readily detected in either of the pooled sputum samples (data not shown).

To further evaluate alterations in MMP expression in ETAs from healthy children and sick children, we performed quantitative dot blots with antisera specific to individual MMPs (MMP-2, -7, -8, and -9). Figure 4A shows that the amount of MMP-2 did not significantly differ in lower respiratory tract fluids obtained from either group. In contrast, MMP-7 (Fig. 4B), MMP-8 (Fig. 4C), and MMP-9 (Fig. 4D) were all significantly elevated in samples obtained from children with respiratory failure compared with healthy children. Interestingly, regression analyses comparing MMP concentrations in ETAs from the sick children revealed that MMP-7, MMP-8, and MMP-9 displayed a high degree of correlation, demonstrating that there is coordinate secretion of these MMPs during the early phase of respiratory failure in these children (Table 2). Also of interest was that patients who had a chronic, underly-
ing pulmonary condition before experiencing respiratory failure had statistically higher MMP-8 levels than those individuals who had no known underlying lung disease preexistent to their requirement for mechanical ventilation (Table 3). In contrast, MMP-7 and MMP-9 levels were not significantly different in patients with respiratory failure who had preexisting lung disease compared with those who did not (Table 3). Together, these data support the conclusion that MMPs may be differentially and acutely regulated in the lungs of children with severe lung disease requiring mechanical ventilatory support.

Although MMP-7, MMP-8, and MMP-9 protein concentrations were all markedly elevated in the secretions of children experiencing respiratory failure, these levels may not yield an increase in MMP activity. MMP activity is highly regulated by MMP inhibitors, such as TIMPs, and broad-spectrum protease inhibitors, such as α2-macroglobulin, as well as intramolecular mechanisms by which propeptides inhibit the catalytic site (38). Therefore, to assess the relationship between elevated MMP-8 and MMP-9 concentrations and the net enzymatic activity of each, we used a fluorogenic substrate assay to specifically assess MMP-8 and MMP-9 activity. As seen in Fig. 5A, MMP-8 activity was on average 40-fold higher in ETA samples collected from individuals suffering from respiratory failure compared with control children. Likewise, MMP-9 activity was consistently and significantly elevated in ETAs from children who were ventilated with respiratory disease (Fig. 5B). Together, these studies clearly demonstrate that MMP activity of two proinflammatory MMPs, MMP-8 and -9, is markedly increased in pulmonary secretions from children with acute respiratory failure.

DISCUSSION

Little is known about the roles of MMPs in normal human lung development, growth, and maintenance, especially in children. Recognizing that the lung parenchyma, under normal homeostatic conditions, will experience a modest rate of remodeling and regeneration, we have explored the possibility that MMPs may

Table 2. Linear regression

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Linear regression analyses of matrix metalloproteinase (MMP)-8 compared with MMP-2, or MMP-7, or MMP-9 in endotracheal tube aspirates (ETAs) obtained from children requiring mechanical ventilation for respiratory failure. Regression analyses were performed using PRISM software (GraphPad Software, San Diego, CA). P < 0.05 was considered a highly significant association. r², regression analysis.

Table 3. Preexisting lung disease

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<td>MMP-2</td>
<td>1.23 ± 0.9 ng/µg Total protein*</td>
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<td>MMP-7</td>
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<td>MMP-9</td>
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Values are means ± SE. Comparisons of MMP-2, MMP-7, MMP-8, or MMP-9 concentrations in ETAs obtained from children requiring mechanical ventilation for respiratory failure. Comparisons were made between children who had no known preexisting lung disease with those known to have preexisting lung disease (e.g., asthma or bronchopulmonary dysplasia). Group comparisons were performed using Student’s t-test, and a significant association was defined as P ≤ 0.05.
be present in fluids produced by the lungs of healthy, growing children. Previous studies examining MMPs in the setting of lung disease have analyzed fluids taken directly from the lower respiratory tract and alveolar spaces (i.e., BAL) or fluids that may reflect lung fluid contents, such as induced sputum (13, 25, 29, 32). Because BAL is a relatively invasive procedure, requires special instrumentation, and poses some degree of risk to subjects (20), neonatal studies have avoided these concerns and risks by routinely performing “blind” suctioning with a small suction catheter inserted into the endotracheal tube to sample lower respiratory tract secretions. This ETA or lavage has been shown through several measures to reflect lower respiratory tract fluids (34). Indeed, our own studies revealed that ETAs contain cells expressing CD68, a marker of alveolar macrophages, a cell type that resides in the lower respiratory tract. ETAs, as a source material to measure MMPs, has recently been explored in neonates, where BAL and ETAs were shown to consist of similar components (4, 30, 31). Both methods yield similar findings regarding the presence of MMPs in respiratory tract secretions (4, 30, 31). On the basis of such studies, we chose to utilize ETAs as a simple and safe source of lung secretions from which we could detect and measure MMPs from healthy children and from children with respiratory failure.

Composite data from a number of studies show that several types of cells present within the lung, including alveolar macrophages and airway epithelial cells, produce MMPs (26–28). Additional sources of MMPs in the lung may be inflammatory cells that migrate into the lung during an inflammatory response (12). To date, a comprehensive examination of MMPs produced by lung-derived cells has not been performed in the normal human lung or in pathological conditions associated with lung injury. Initial studies using casein zymography revealed no detectable proteinase activity in ETAs from children who were healthy, suggesting that stromelysins and collagenases are absent or present at very low concentrations in these samples. In contrast, gelatin-substrate zymography identified gelatinase activity consistent with MMP-2 and MMP-9 (23) in ETAs from healthy children, a finding that was confirmed by Western blot analyses. Although it is possible that induction of MMP-2 and MMP-9 activity in the healthy children occurred in response to pressures generated within the lung from mechanical ventilation, the ventilation used for these children was brief (i.e., no more than 60 min), and we compared these samples with several samples obtained at the time of intubation for elective surgery and revealed no difference in MMP levels (data not shown).

Several studies suggest that overproduction of MMPs may be involved in the destruction of normal lung architecture commonly observed in conjunction with acute lung injury in humans. For instance, MMP-9 has been shown to be present in BAL fluid obtained from patients with cancer, inflammatory lung disease, pneumonia, COPD, and asthma (reviewed in Ref. 39). In these same patients, MMP-1, MMP-2, and MMP-3 levels were generally 8–30 times less than MMP-9 levels; however, other forms of MMPs, such as MMP-7 and MMP-8, were not measured (39). Our studies in children experiencing respiratory failure also demonstrated a marked increase in MMP-9 concentrations compared with control values, whereas levels of the other gelatinase, MMP-2, did not change in a significant way. Indeed, MMP-9 concentrations were approximately fivefold higher than MMP-2 in ETAs from ill children. This is the opposite situation of that seen in human serum in which MMP-2 serum concentrations are ~70 times higher compared with those of MMP-9 (39). This inverse correlation suggests that in the sick lung, gelatinases detected in lung fluids do not represent simply a transudate of serum but reflect active production and secretion of MMPs by cells that reside in the lung. MMP-9 is expressed by injured epithelial cells in distal airways (1) and by alveolar macrophages (24); however, a major source of MMP-9 in the damaged or inflamed airway may arise from activated neutrophils because MMP-9 is readily released from stimulated neutrophils (35). Indeed, recent evidence shows that in airway secretions from patients with an asthma exacerbation, MMP-9 concentrations were elevated in pulmonary secretions, and this increase correlated with total macrophage, neutrophil, and eosinophil counts (15). MMP-7 (matrilysin) and MMP-8 (neutrophil collagenase) were also significantly elevated in ETAs from children with respiratory failure compared with healthy children, and all were highly correlated with MMP-9 in their expression. MMP-7 has previously been demonstrated to be expressed in adult human lung epithelial cells lining peribronchial glands and conducting airways (7, 21). Normal airway cells constitutively secrete MMP-7, which may function in the innate immunity of the lung epithelium due to its effects on defensins (21, 36). However, MMP-7 is also observed at sites of overt lung damage in emphysema, fibrotic lung disease, pneumonia, following bone marrow transplantation, and most prominently in lungs from patients with cystic fibrosis (7) and is increased in airway epithelial cells after exposure to a bacterial stimulus (16). MMP-8 is made by several cell types within and outside the lung, yet it is primarily produced and secreted by neutrophils and is released upon activation (5, 11, 22). Some clues about the cellular source of MMP-8 may come from the different molecular weight forms identified in ETAs. For instance, MMP-8 species in ETAs from children experiencing respiratory failure were primarily detected at a relative molecular weight of ~80 kDa, which represents MMP-8 of neutrophil origin. In addition, several less intense immunoreactive species were identified at ~40–60 kDa, which likely represents MMP-8 emanating from sources other than neutrophils, such as bronchial epithelial cells, glandular cells, or macrophages (22).

In addition to increased MMP-8 and MMP-9 protein concentrations, we found that the enzymatic activity of both MMPs was significantly elevated in ETAs from children suffering from respiratory failure. These data
provide a mechanism by which active forms of MMPs present in lower respiratory tract secretions can directly impact on lung surfaces, thereby causing increased ECM turnover within the lung. These studies do not, however, establish whether this acute enhancement in MMP activity functions positively or negatively in lung repair or lung damage, respectively. In acute lung injury, polymorphonuclear cells are rapidly sequestered in the vascular tree, become activated after adhesion to endothelial surfaces, and secrete a wide variety of proinflammatory molecules stored in cytoplasm granules (14). Such is the case for both MMP-9 and neutrophil-derived MMP-8; however, MMP-9 is stored in C-type granules (tertiary granules), and MMP-8 is stored in specific (secondary) granules (35). If one assumes that neutrophils may be a significant source of the acute rise in MMP-8 and MMP-9 concentrations and activity observed at the time of respiratory failure, compartmentalization may help explain why patients in our study who had preexisting lung disease before their respiratory failure had overall higher concentrations of MMP-8 in their ETAs than those observed in patients who had no known underlying lung disease before intubation and mechanical ventilation. Conversely, no significant differences were detected in MMP-9 concentrations between these two groups. Further investigations will be necessary to clarify which cell types may be involved in production and secretion of MMPs in these individuals, and longitudinal studies will be required to determine whether MMPs are indeed associated with progressive lung damage.

Together, these data demonstrate that cells emanating from the lungs of healthy children express several different MMPs, which can be identified, differentiated, and measured in ETAs. Because dramatic alterations in certain MMPs (i.e., MMP-7, -8, and -9) were observed in children experiencing respiratory failure from a variety of different underlying mechanisms, measurements of MMPs in such a readily available source as ETAs may become a useful diagnostic tool for the detection and progression of pulmonary diseases in patients receiving mechanical ventilation. In addition, as we determine more fully which MMPs are expressed by cells present in the lung under both normal and pathological conditions, a more complete understanding will be possible regarding which MMPs may be involved in normal lung healing and repair vs. those that may be involved in lung damage and long-term complications.

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