Regulation of Matrilysin Expression in Airway Epithelial Cells by Pseudomonas aeruginosa Flagellin*

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Matrilysin (matrix metalloproteinase-7) is expressed by mucosal epithelia throughout the body and functions in host defense by activating murine intestinal α-defensins. In normal adult human lung, matrilysin is expressed at low levels in the airway epithelium, but is markedly up-regulated in cystic fibrosis (CF). Because CF lungs support a heavy bacterial load, we assessed if relevant CF pathogens regulate matrilysin expression in human lung epithelial cells. Indeed, acute infection with Pseudomonas aeruginosa (but not Staphylococcus aureus, Haemophilus influenzae, or Klebsiella pneumoniae) induced the expression of matrilysin in Calu-3 lung epithelial cells. Increased matrilysin mRNA levels were detectable at 3 h post-infection and peaked at a 25-fold induction between 6 and 8 h. Both P. aeruginosa CF isolates and laboratory strains induced matrilysin expression to similar levels. Flagellin, the monomeric precursor of bacterial flagella, was identified as the inductive factor released by P. aeruginosa that regulated matrilysin expression. In addition, flagellin-null mutants failed to stimulate matrilysin expression in cultured cells or in lungs infected in vivo. These data show that P. aeruginosa (and specifically flagellin) potently stimulates matrilysin expression in lung epithelial cells and may mediate the overexpression of this proteinase in CF lungs.

Despite their specialization to serve distinct functions in different tissues, mucosal epithelia share common features in their response to injury and infection. Following injury, epithelial cells initiate a programmed series of coordinated responses, such as proliferation, migration, and matrix assembly, to restore the integrity of the damaged tissue. In addition, by forming a barrier at mucosal surfaces and by the release of a variety of antimicrobial products, epithelia provide the first line of innate defense against invading pathogens. Although seemingly divergent events, the epithelial programs in response to infection and injury may have co-evolved, particularly with respect to the genes selectively induced and repressed. After all, injury provides an opportunity for infection, and infection can lead to injury. Hence, many of the epithelial products associated with either of these events may actually be common to both. Matrilysin (matrix metalloproteinase (MMP)1-7), a member of the MMP gene family produced primarily by mucosal epithelia, is an example of a protein that is regulated by both infection and injury and serves essential functions in both innate defense and re-epithelialization.

As we demonstrated in an aseptic ex vivo model of airway damage, matrilysin is prominently expressed by migrating epithelial cells in wounded trachea, and re-epithelialization of the injured airway is markedly delayed in matrilysin-null mice (1). Furthermore, as discussed below and the subject of this report, the presence of bacteria, either commensal or pathogenic, is also a potent effector of matrilysin expression, and we hypothesize that the activity of this enzyme may be a common and essential component of mucosal defense mechanisms. For example, intestinal pro-α-defensins, demonstrated substrates of this MMP, are not activated in matrilysin-null mice, leading to an impaired ability to battle enteric pathogens (2). Collectively, our functional data indicate that the catalytic activity of matrilysin is an essential component of both mucosal defense and repair.

Unlike many MMPs, which are typically expressed or released in response to injury, disease, or inflammation, matrilysin is expressed by non-injured, non-inflamed exocrine and mucosal epithelia in most adult human tissues. For example, matrilysin is produced by the ductal or glandular epithelia of the skin, salivary glands, pancreas, liver, breast, intestine, urogenital tract, and other tissues (3–6). We proposed that exposure to commensal bacteria (and possibly low-level infection) regulates the widespread expression of matrilysin in healthy mucosa (7). Indeed, in mice with conventional microflora, matrilysin is prominently expressed in the Paneth cells of the small intestine, but it is nearly undetectable in germ-free mice. Expression of this MMP, however, is fully restored in ex-germ-free mice colonized with just one species of commensal bacteria (7). In addition, matrilysin expression in cultured cells and tissues is, depending on its basal levels, induced or markedly increased by exposure to several strains of pathogenic Escherichia coli (7). Thus, bacterial exposure seems to be the physiologic signal that regulates matrilysin expression in intact epithelia. However, the bacterially derived signal that regulates matrilysin expression is not known.

Lungs of patients with cystic fibrosis (CF) are normal in utero, but become infected shortly after birth with a number of microorganisms, including Pseudomonas aeruginosa, Staphylococcus aureus, Haemophilus influenzae, and Klebsiella pneumoniae. Infection leads to the development of chronic inflammation, which, in turn, contributes to further tissue

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1 The abbreviations used are: MMP, matrix metalloproteinase; CF, cystic fibrosis; MT1, membrane type 1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; m.o.i., multiplicity of infection; LPS, lipopolysaccharide.
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destruction and airway obstruction. Among these bacteria, P. aeruginosa is an opportunistic microorganism recovered from the airways of most patients with CF and many with impaired host defense. Eventually, >85% of CF patients become colonized with P. aeruginosa (8, 9), an infection that is extremely resistant to eradication (10, 11). The initial step of bacterial infection, crucial for the development of permanent colonization at later stages, is the adherence of the bacteria to epithelial cells.

In lungs from patients with CF, matrilysin is prominently expressed by airway and alveolar epithelial cells (1), and here we assessed if any of the bacterial species frequently isolated from CF patients mediates the marked up-regulation of this MMP. Our data demonstrate that exposure to P. aeruginosa, but not other respiratory pathogens, is a potent stimulus of matrilysin expression in lung epithelial cells. A number of bacterial gene products stimulate pro-inflammatory responses in epithelial cells, and we identified P. aeruginosa flagellin, the monomeric component of flagella, as the factor controlling matrilysin expression. Our data demonstrate that the production of this defense-related metalloproteinase is specifically regulated in airway epithelial cells by a bacterial product.

EXPERIMENTAL PROCEDURES

Tissue Samples and Cell Culture—Specimens of recipient lungs from patients with CF were biopsied at the time of transplant surgery, and Formalin-fixed, paraffin-embedded samples (n = 16) were obtained from the Department of Pathology of Washington University (St. Louis, MO) and from Dr. James R. Yankaskas (University of North Carolina). For normal lung, we examined the tumor-free margins of lung adenocarcinoma tissue (n = 5), recipient lungs of transplant patients with primary pulmonary hypertension (n = 6), and segments of the proximal end of normal human trachea obtained from donor lungs (n = 7). The human colon adenocarcinoma cell line HT29 and the human lung carcinoma cell line Calu-3 were obtained from American Type Culture Collection (Manassas, VA). These cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum without antibiotics.

Bacteria and Other Reagents—The recombinant E. coli strain AAE185/pSH2 expresses the complete type 1 pilus operon (type 1 fimbriae) (12) and was provided by Dr. Scott Hultgren (Washington University). P. aeruginosa strains PA14 (serotype 10) lipopolysaccharide were obtained from Sigma. Gentamycin and ampicillin were present at 100 μg/ml and 25 μg/ml, respectively.

Immunohistochemistry and in Situ Hybridization—Anti-human matrilysin antiserum (13) was provided by Dr. Azzaq Belaaouaj (Washington University). H. influenzae strain K1 was provided by Dr. Michael Grunert (University of Washington). P. aeruginosa strain PA14 and PAK were obtained from American Type Culture Collection (Manassas, VA). The human colon adenocarcinoma cell line HT29 and the human lung carcinoma cell line Calu-3 were obtained from the American Type Culture Collection (Manassas, VA). These cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum without antibiotics.

Immunoblotting—Media samples from the 90-min period of infection of the epithelial cells were concentrated 28-fold by lyophilization. Aliquots of concentrated media were separated by 12% SDS-polyacrylamide gel electrophoresis and transferred by semidyry electrophoretic transfer at 15 V for 20 min to nitrocellulose membranes (Hybond ECL, Amersham Pharmacia Biotech, Buckinghamshire, United Kingdom) in 48 mm Tris, 39 mm glycine, 20% methanol, and 0.0375% SDS. Nonspecific binding sites were blocked by soaking membranes in 3% nonfat dry milk in Tris-buffered saline at 4°C overnight. Blots were incubated with a 1:10,000 dilution of anti-flagellin polyclonal antiserum (17) in blocking buffer for 1 h and washed twice with Tris-buffered saline containing 0.1% Tween 20 for 10 min. Membranes were subsequently incubated with a 1:10,000 dilution of peroxidase-linked donkey anti-rabbit IgG (Amersham Pharmacia Biotech) in blocking buffer for 1 h, washed twice, and developed with the enhanced chemiluminescence detection system (Amersham Pharmacia Biotech) according to the manufacturer’s instructions.

In Vivo Infection and Reverse Transcription-Polymerase Chain Reaction—C57BL/6 mice (10–12 weeks old) were anesthetized and inoculated nasally with 50 μl of sterile saline or a suspension containing 10⁸ colony-forming units of P. aeruginosa PAK or PAK-rfC. Mice (four/group) were killed 6 h later, and total lung RNA was isolated. Matrilysin mRNA was detected by reverse transcription-polymerase chain reaction as described (15) using primers MMat405 (5’-ACTTACCTCC- GATCGTAGTG-3’) and MMa548 (5’-GTCCGATCTCATTTTGTTC-3’) and an annealing temperature of 55°C for 25 cycles. Mouse GAPDH was amplified in a separate reaction using primers MGapdh205 (5’- ATTACGCGACGACGCA-3’) and MGapdh859 (5’-GGTCCT- CAAATGTGCCCCAAG-3’). Amplification products were resolved on 8% acrylamide gels and visualized by ethidium bromide staining.

RESULTS

We previously reported that matrilysin is induced in airway epithelial cells migrating over denuded tissue in an aseptic in vivo model of tracheal injury (1) and in airway cells of intact trachea infected with pathogenic strains of E. coli (7). These findings indicate that the expression of matrilysin is regulated by both infection and injury. We also reported that matrilysin is expressed at high levels by airway epithelial cells at sites of overt mucosal damage in CF (1).

In a further examination of several CF samples (n = 16), we observed that matrilysin protein was uniformly detected in the intact epithelium of trachea (Fig. 1, A and B), bronchioles (Fig. 1, E and G), and alveoli (Fig. 1, K). No staining was seen in samples processed with preimmune IgG (Fig. 1C). In addition, a moderate to intense signal for matrilysin mRNA colocalized epithelial cells that had strong staining for the protein (Fig. 1, E and F, G and H), indicating that this MMP was actively expressed in the airway epithelia of CF lungs. In contrast, matrilysin was typically not seen in the intact airway epithelia of non-CF lung samples (Fig. 1D), except in epithelial cells at the edge of wounded mucosa, if such lesions were present. In several of the non-CF samples (8 out of 18), matrilysin protein was seen in...
produce type 1 pili (12), which potently induces matrilysin epithelial cells with various CF pathogens. Inoculi containing regulation of matrilysin seen in CF lung tissues, we infected the epithelium. Staining for matrilysin was often most intense at the apical edge of the airway epithelium. Mucous-containing cells did not stain for the protein, and signal was not seen in any interstitial or inflammatory cells. Shown are tracheal sections from two different specimens. C, no signal was seen in sections processed with preimmune serum. Shown is a section serial to that in B. D, in sections of normal trachea from donor lungs, signal for matrilysin protein was typically not seen in the intact mucosa, but was invariably seen in epithelial cells at the edge of denuded areas. E, in CF samples, strong staining (peroxidase immunostaining; brown precipitant) for matrilysin protein was also seen in the intact epithelium of distal bronchioles. F, in situ hybridization was done on a section serial to that in E. Under dark-field illumination, a strong signal for matrilysin mRNA was seen in most airway epithelial cells. Autoradiographic exposure was for 10 days. G and H, in this section of CF lung, a collapsed airway was seen surrounded by a dense inflammatory infiltrate. A prominent signal for matrilysin protein (G) and mRNA (H) colocalized to the airway epithelium. Autoradiographic exposure was for 10 days. I, in lung from a patient with primary pulmonary hypertension, moderate staining for matrilysin protein was seen in the bronchiolar epithelium. J, in situ hybridization for matrilysin mRNA was done on a serial section. No specific autoradiographic signal was seen, even after an extended autoradiographic exposure of 21 days. K, in CF samples, a prominent signal for matrilysin protein was seen in essentially all alveolar epithelial cells, whether in intact areas or at the edge of denuded tissue. L, in peripheral lung from non-CF specimens, weak staining for matrilysin was seen only in occasional alveoli cells typically at sites with overt epithelial denudation (arrowheads). Shown is a section of lung from a patient with primary pulmonary hypertension. With the exception of the dark-field micrographs, all samples were photographed using Nomarski optics.

intact epithelium (Fig. 1I); however, signal for the mRNA was not detected, even after extended autoradiographic exposure (Fig. 1J). Intense staining for matrilysin protein was also seen in intact and wound-edge alveolar type II cells in CF lungs (Fig. 1K), but only a weak immunostaining signal was detected in alveolar cells bordering sites of denudation in non-CF specimens (Fig. 1L, arrowheads). A strong signal for matrilysin protein and mRNA was also seen in the ductal cells of peritracheal and peribronchial glands in CF samples, but not in non-CF specimens (data not shown). Signal for collagenase-1 (MMP-1), stromelysin-1 (MMP-3), or gelatinase-B (MMP-9) mRNA or protein was not detected in the epithelial layer of CF or non-CF samples (data not shown). Together, these in vivo observations demonstrate that expression of matrilysin is potently and invariably up-regulated in the intact airway epithelia of CF lungs.

To determine whether infection directly mediates the up-regulation of matrilysin seen in CF lung tissues, we infected epithelial cells with various CF pathogens. Inoculi containing $1 \times 10^8$ colony-forming units of bacteria, representing a 50:1 bacterial/epithelial cell ratio, were exposed to cells for 90 min. To eliminate bacteria, monolayers were subsequently washed with phosphate-buffered saline and treated with gentamycin. Total RNA was isolated from the cells at 6 h post-infection and analyzed by Northern blotting. As a positive control for the bacterially mediated induction of matrilysin expression, we infected cells with a recombinant E. coli strain engineered to produce type 1 pili (12), which potently induces matrilysin expression in human mucosal epithelial cells (7). Infection with a P. aeruginosa strain isolated from a CF patient (ATCC 51673) markedly stimulated matrilysin expression in Calu-3 lung carcinoma (30-fold) and HT29 colon carcinoma (6-fold) epithelial cells (Fig. 2A). P. aeruginosa was a more potent stimulator of matrilysin expression than E. coli in lung-derived cells (30-fold versus 10-fold, respectively). In contrast, infection at a comparable multiplicity of infection (m.o.i.) with S. aureus, H. influenzae, and K. pneumoniae had no effect on matrilysin mRNA levels (data not shown). The up-regulation of matrilysin mRNA levels correlated with increased amounts of matrilysin protein, as detected by immunoprecipitation of the enzyme from the 24-h conditioned media of infected cells (Fig. 2B).

We infected Calu-3 lung cells for 90 min with increasing m.o.i. values of P. aeruginosa and assessed the response at different times post-infection. Even with an initial inoculum of $1 \times 10^7$ bacteria (representing a 5.1 bacteria/epithelial cell ratio), matrilysin mRNA levels were elevated 5-fold at 6 h post-infection (Fig. 2C). Up-regulation of matrilysin expression was observed as early as 3 h post-infection and peaked at 6 h; the levels of matrilysin mRNA remained elevated at 72 h post-infection (Fig. 2D). A similar time course of bacterially mediated induction of matrilysin mRNA levels was seen in HT29 colon epithelial cells (data not shown).

We assessed the response of lung epithelial cells to infection with a variety of P. aeruginosa strains to determine whether the regulation of matrilysin is affected by the pathogenicity of the bacteria. Infection with either P. aeruginosa CF isolates (ATCC 51673 and 39324) or with laboratory strains (ATCC 10145 and 25619) markedly stimulated matrilysin mRNA lev-
els (Fig. 2E). Similarly, matrilysin expression was also induced in HT29 colon epithelial cells infected with type 1 piliated E. coli (Ec) or P. aeruginosa (Pa; ATCC 51673) at an m.o.i. of 50. Monolayers were then washed with phosphate-buffered saline and subsequently incubated for 6 h in fresh medium containing antibiotics. Northern blot analysis was done for matrilysin (MAT) and GAPDH mRNAs. B, Calu-3 lung epithelial cells were infected with P. aeruginosa 51673 at different m.o.i. values and metabolically labeled during a 24-h post-infection period. Matrilysin was immunoprecipitated from the conditioned media with specific antibodies, and the complexes were resolved on 12% SDS-polyacrylamide gels. The pro form of the enzyme, which was selectively immunoprecipitated, migrated at ~28 kDa. Molecular mass standards are shown on the left in kilodaltons. Autoradiography was done for 72 h. C, Calu-3 lung epithelial cells were infected for 90 min with P. aeruginosa 51673 at the indicated m.o.i. values. Total RNA was isolated at 6 h post-infection, and Northern blotting was done with matrilysin and GAPDH. D, Calu-3 cells were infected for 90 min, and total RNA was prepared at the indicated times post-infection. Autoradiograms were scanned, and the densitometric signals for matrilysin mRNA were normalized to those for GAPDH mRNA. The results are expressed relative to matrilysin levels in control cells at each time point. E, monolayers of Calu-3 cells were infected for 90 min with P. aeruginosa at an m.o.i. of 50. P. aeruginosa 51673 and 39324 are strains isolated from CF patients. P. aeruginosa 10145 and 25619 are laboratory strains. Total RNA was prepared from the cells at 6 h post-infection, and Northern analysis was done for matrilysin and GAPDH mRNA.

Fig. 2. P. aeruginosa-mediated induction of matrilysin. A, human HT29 (colon) and Calu-3 (lung) carcinoma epithelial cells were infected for 90 min with type 1 piliated E. coli (Ec) or P. aeruginosa (Pa; ATCC 51673) at an m.o.i. of 50. Monolayers were then washed with phosphate-buffered saline and subsequently incubated for 6 h in fresh medium containing antibiotics. Northern blot analysis was done for matrilysin (MAT) and GAPDH mRNAs. B, Calu-3 lung epithelial cells were infected with P. aeruginosa 51673 at different m.o.i. values and metabolically labeled during a 24-h post-infection period. Matrilysin was immunoprecipitated from the conditioned media with specific antibodies, and the complexes were resolved on 12% SDS-polyacrylamide gels. The pro form of the enzyme, which was selectively immunoprecipitated, migrated at ~28 kDa. Molecular mass standards are shown on the left in kilodaltons. Autoradiography was done for 72 h. C, Calu-3 lung epithelial cells were infected for 90 min with P. aeruginosa 51673 at the indicated m.o.i. values. Total RNA was isolated at 6 h post-infection, and Northern blotting was done with matrilysin and GAPDH. D, Calu-3 cells were infected for 90 min, and total RNA was prepared at the indicated times post-infection. Autoradiograms were scanned, and the densitometric signals for matrilysin mRNA were normalized to those for GAPDH mRNA. The results are expressed relative to matrilysin levels in control cells at each time point. E, monolayers of Calu-3 cells were infected for 90 min with P. aeruginosa at an m.o.i. of 50. P. aeruginosa 51673 and 39324 are strains isolated from CF patients. P. aeruginosa 10145 and 25619 are laboratory strains. Total RNA was prepared from the cells at 6 h post-infection, and Northern analysis was done for matrilysin and GAPDH mRNA.

To assess the role of bacterial adherence, we examined the effect of type IV pili, which mediate the adherence of P. aeruginosa to eukaryotic cells (18). Calu-3 cells were infected with a wild-type strain (PAK) or with an isogenic mutant lacking the PilA subunit (PAK-NP). This mutant does not produce pili and hence adheres poorly to eukaryotic cells compared with the parental strain (17). However, matrilysin expression was stimulated to similar levels in cells infected with either strain (Fig. 3A). Because adherence of P. aeruginosa was not critical for matrilysin expression, we searched for preformed soluble bacterial factors that mediate induction of this MMP. Preparations of lipopolysaccharide (LPS) from P. aeruginosa failed to induce matrilysin in Calu-3 lung cells (Fig. 3B). In addition, the presence of gentamycin during the 90-min infection period did not prevent induction (Fig. 3B), and matrilysin expression was also induced by infection with heat-killed bacteria (data not shown). Thus, bacteria do not need to be metabolically active to influence matrilysin expression.

We next investigated the effect of proteins released by P. aeruginosa on matrilysin expression in lung epithelial cells.
Proteins in the supernatants of 1- and 2-day cultures were concentrated by ammonium sulfate precipitation and analyzed by SDS-polyacrylamide gel electrophoresis. The pattern of proteins secreted by the bacterial cells was overtly different from proteins secreted by the bacteria in a time- and dose-dependent manner during the infection period. Treatment of Calu-3 cells with 0.2 μg/ml purified flagellin for 6 h resulted in a 12-fold induction of matrilysin mRNA levels (Fig. 4D). Based on the purification yield and the comparison of band staining intensities by Western blotting and protein staining, 0.2 μg/ml flagellin was roughly equivalent to direct infection at an m.o.i. of 200. Furthermore, the induction of matrilysin obtained with purified flagellin was comparable to that seen with intact bacteria (Fig. 3; see Fig. 5 below), suggesting that flagellin is the major bacterial factor regulating expression of MMP. As we found for infection with *P. aeruginosa*, flagellin did not affect expression of MT1-MMP, collagenase-1 (Fig. 4E), and gelatinases A (MMP-2) and B (MMP-9) (data not shown).

To corroborate further that flagellin is the bacterial factor responsible for *P. aeruginosa*-mediated induction of matrilysin, we infected Calu-3 cells with mutants of *fliC*, the gene that encodes flagellin (16). Exposure for 90 min to the mutant strains PAK-*fliC* and PAK-NP-*fliC*, in which an interrupted sequence (17), did not affect matrilysin mRNA levels, whereas infection with the wild-type strain PAK and the pilin mutant PAK-NP resulted in a 10-fold induction (Fig. 5A). In addition, we prepared crude supernatants (i.e. 40% ammonium sulfate-precipitated proteins from the 2-day culture supernatants) from the wild-type PAK strain and the isogenic flagellin PAK-*fliC* mutant. The protein patterns from these supernatants differed only in the presence or absence of flagellin (Fig. 5B). Treatment of Calu-3 cells with PAK (but not PAK-*fliC*) supernatants up-regulated matrilysin expression by 8-fold (Fig. 5C), further identifying flagellin as the major factor that promotes matrilysin expression.

Finally, to determine whether flagellin is relevant to the induction of matrilysin expression in vivo, we infected mice intranasally with wild-type *P. aeruginosa* (PAK) or with the
were inoculated with sterile saline. Control mice (Cntl) were inoculated with acrylamide gels and detected by ethidium bromide staining. The results shown in separate reactions. Amplified products were resolved on acrylamide transcriptions-Polymerase chain reaction. GAPDH mRNA was amplified Matrilysin stimulates the expression of this MMP. In contrast, infection with the mutant PAK-infected animals compared with the levels seen in control mice (Fig. 6). In contrast, infection with the mutant PAK-strain had no detectable effect on matrilysin mRNA levels.

**DISCUSSION**

We have demonstrated that matrilysin is prominently and invariantly expressed in the airway and respiratory epithelia of lungs from patients with CF and that exposure of lung epithelial cells to *P. aeruginosa*, a relevant CF pathogen, markedly stimulates the expression of this MMP. In contrast, *S. aureus*, *H. influenzae*, and *K. pneumoniae*, other pathogens found in CF lungs, did not influence matrilysin expression. The increase in matrilysin mRNA levels was rapid and detectable at low m.o.i., suggesting that epithelial cells readily respond to bacterial exposure by up-regulating this MMP. Furthermore, the specificity of the response to *P. aeruginosa* suggests that this bacterium selectively triggers signaling pathways that control matrilysin expression in epithelial cells. Because most adult CF patients are permanently colonized with one or more *P. aeruginosa* strains, these findings may explain the pattern of increased expression of matrilysin we observed in CF lung tissues (Fig. 1).

The pathogenesis of the airway infection in CF follows a pattern in which initial colonization by non-mucoid variants of *P. aeruginosa*, often combined with more transient infections with *H. influenzae*, *K. pneumoniae*, and *S. aureus*, almost invariably results in chronic colonization by mucoid variants of *P. aeruginosa* (10). Our results show that both CF isolates and non-CF strains of *P. aeruginosa* induce the expression of matrilysin in comparable levels, suggesting that the up-regulation of matrilysin could occur early in the establishment of the disease. Indeed, we found that a short infection led to rapid and sustained expression. In addition, the marked sensitivity of lung epithelial cells to *P. aeruginosa* exposure indicates that even low levels of initial colonization by the bacterium could turn on the expression of the enzyme. However, it is currently unclear whether matrilysin is initially engaged in tissue repair and innate defense mechanisms or whether the enzyme contributes to tissue damage from the onset of the infection, further facilitating the adherence of *P. aeruginosa* to injured tissue (20).

The adherence of *P. aeruginosa* to lung epithelial cells depends on the expression of type IV pili (21), and mutants of the pili subunit gene PilA induce a diminished inflammatory response (22). Our data demonstrate that the lack of PilA did not affect matrilysin induction by *P. aeruginosa*. Therefore, we hypothesized that a preformed soluble bacterial factor was responsible for the induction of matrilysin expression. Indeed, we found that a factor was present in the supernatant of overnight bacterial cultures and that it was heat-stable and sensitive to proteinase degradation. We identified flagellin, the monomeric component of bacterial flagella (16, 23), as the secreted protein that induced matrilysin expression. Similarly, flagellin from *Salmonella*, enteraggregative *E. coli*, and *Pseudomonas* species induces a proinflammatory mediator in intestinal and lung epithelial cells (17, 24–27). Although adherence may not be directly critical for induction of matrilysin, binding of bacteria to tissue could potentiate the host response signal by increasing the pericellular bacterial density and hence the concentration of flagellin at the epithelial cell surface.

Previous reports suggested that bacterial soluble virulence factors can induce MMP activation or expression (28, 29); however, to our knowledge, this is the first example of flagellin up-regulating the expression of an MMP. In this regard, *P. aeruginosa* LPS had no significant effect on matrilysin expression, corroborating our previous finding that the factor responsible for the *E. coli*-mediated up-regulation of matrilysin in colon epithelial cells is not LPS-related (7). LPS can induce the expression of genes encoding innate defense molecules in airway epithelial cells, such as the mucin muc2 (30). However, human BD-2, an inducible β-defense, does not respond strongly to LPS in a variety of epithelial cells, but is up-regulated by bacteria, interleukin-1β, and other proinflammatory mediators (31–33). As we reported (7), matrilysin is induced in epithelial cells treated with interleukin-1β, tumor necrosis factor-α, and interleukin-6, albeit to a much lesser extent than in infected cells. Thus, matrilysin and human BD-2 may share common regulatory mechanisms and respond to similar stimuli. Indeed, expression of human BD-2 in human colon epithelial cells is induced by *Salmonella enteritidis* flagellin (34). Insight into the response of respiratory epithelial cells to airway pathogens has recently been provided by microarray analysis of genes differentially expressed after infection with *P. aeruginosa* and *Bordetella pertussis*, illustrating the potent proinflammatory response mounted by epithelial cells upon interaction with bacteria (35, 36). Therefore, our data include matrilysin in the panoply of genes induced early and strongly by bacterial exposure. (Incidentally, matrilysin cDNA is not included in the arrays used in these studies).

Finally, it is tempting to speculate that matrilysin and other innate defense molecules, such as mucins and defensins, are part of a general response of epithelial cells to bacterial exposure and that these molecules play pivotal roles in host defense. However, high levels of production or activity of these molecules can be detrimental and could be major contributors to the processes of tissue destruction and airway obstruction that eventually kill CF patients. For example, a high level of activity of matrilysin, an MMP capable of acting on a variety of extracellular matrix molecules, could result in the exaggerated degradation of any of these substrates and alterations in tissue architecture. Similarly, an excessive production of mucins causes clogging of the CF airways due to the accumulation of material that cannot be efficiently removed by the mucociliary clearance mechanism. Finally, the concentration of defensins in CF sputum/fluid could be so high as to be cytotoxic to airway epithelial cells (37–39). In sum, we provide evidence that flagellin, a potent virulence factor, strongly up-regulates the expression of matrilysin in lung epithelial cells and that this host enzyme is an early marker of bacterially induced inflammation. Work in progress aims to elucidate the role of matrilysin activity in the relationship between injury and repair in the pathogenesis of cystic fibrosis.
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REFERENCES

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