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Neutrophil Elastase Up-Regulates Cathepsin B and Matrix Metalloprotease-2 Expression

Patrick Geraghty,2* Mark P. Rogan,2* Catherine M. Greene,* Rachel M. M. Boxio,† Tiphaine Poirierr,‡ Michael O’Mahony,* Abderazzaq Belaaouaj,† Shane J. O’Neill,* Clifford C. Taggart,3* and Noel G. McElvaney*

Neutrophil elastase (NE) activity is increased in many diseases. Other families of proteases, including cathepsins and matrix metalloproteases (MMPs), are also present at elevated levels in similar disease conditions. We postulated that NE could induce expression of cathepsins and MMPs in human macrophages. NE exposure resulted in macrophages, producing significantly greater amounts of cathepsin B and latent and active MMP-2. Cathepsin B and MMP-2 activities were decreased in Pseudomonas-infected NE knockout mice compared with wild-type littermates. We also demonstrate that NE can activate NF-κB in macrophages, and inhibition of NF-κB resulted in a reduction of NE-induced cathepsin B and MMP-2. Also, inhibition of TLR-4 or transfection of macrophages with dominant-negative IL-1R-associated kinase-1 resulted in a reduction of NE-induced cathepsin B and MMP-2. This study describes for the first time a novel hierarchy among proteases whereby a serine protease up-regulates expression of MMPs and cathepsins. This has important implications for therapeutic intervention in protease-mediated diseases. The Journal of Immunology, 2007, 178: 5871–5878.

Proteases are pivotal in a wide range of disease processes, including Alzheimer’s disease, cancer, metastasis, atherosclerosis, and acute and chronic lung diseases. An understanding of the role played by proteases in these processes and their regulation may provide the opportunity for therapeutic intervention. The primary families of proteases released into the extracellular space following cell activation include members of the serine protease, matrix metalloprotease (MMP), and cysteiny cathepsin groups of proteases.

Neutrophil elastase (NE) is a 29-kDa serine protease stored in azurophil granules in its active form until it is released following neutrophil exposure to inflammatory stimuli. Once released, NE is potentially fully active because it functions optimally in a neutral environment. The main intracellular physiological function of NE is the degradation of foreign organic molecules phagocytosed by neutrophils (1). NE can degrade almost all extracellular matrix and key plasma proteins, protease inhibitors, and several proteases (2, 3). One of the most prominent families of proteases cleaved by NE is the MMP group of proteases. Serine proteases (NE, cathepsin G, and proteinase-3) have been shown to activate latent MMP-2 involving membrane type 1 MMP expression (4). MMP-2 activation by serine proteases was blocked by the elastase inhibitor α1-antitrypsin, but not by a MMP inhibitor (4).

MMPs are produced by a wide variety of cell types, including epithelium, fibroblasts, neutrophils, and macrophages. MMP-2 is secreted as an inactive, 72-kDa zymogen and is extracellularly activated by proteolytic cleavage, involving membrane type 1 MMP binding to MMP-2 on the cell membrane in a multimeric complex with tissue inhibitor of metalloproteinase-2 (5). The transcriptional regulation of MMP-2 is not well characterized, but several factors have been implicated in its regulation, e.g., TGF-β (6), intracellular calcium levels (7, 8), insulin-like growth factor-I (9), laminin, and vitronectin (10, 11).

Macrophages synthesize another group of destructive proteases called cysteiny cathepsins (12–14). Several expression patterns for cathepsins have been identified in different tissues. Cathepsin B is abundant and widely expressed in various human tissues and cells, including cancer cells (15, 16). We have shown previously that cathepsins cleave and inactivate key innate immunity proteins, including human β defensins 2 and 3 (17), secretory leucoprotease inhibitor (18), and lactoferrin (19). A number of cytokines, including IFN-γ, IL-6, and IL-13, as well as bacterial products, activate cathepsin expression (20).

The ability of proteases to activate gene expression is well documented in the literature. NE, cathepsin G, and proteinase-3 can activate human gingival fibroblasts to produce IL-8 and MCP-1 through protease-activated receptor (PAR)-2 in vitro (21). NE and cathepsin G cleave the peptide corresponding to the N terminus of PAR-2 with exposure of its tethered ligand (21). In human lung epithelial cells, NE and cathepsin G deactivate PAR-2 by proteolysis of the extracellular domain downstream from the trypsin cleavage/activation site (22). However, NE does not activate PAR-1 in human mononuclear cells (23). We have demonstrated previously that NE up-regulates IL-8 gene expression in...
human bronchial epithelial cells via a non-PAR-2 pathway (24). IL-1R-associated kinase (IRAK)-1, MyD88, and TNFR-associated factor-6 were shown to be involved in NE-induced NF-κB activation and subsequent IL-8 expression. This pathway transduces signals of the IL-1R/TLR superfamily, but not PARs. There are 11 TLR family members that recognize bacterial and viral Ags leading to an immune response (25), and we have further demonstrated that IL-8 up-regulation by NE occurs in part through the cell surface membrane-bound TLR-4 (26).

In this study, we describe for the first time a novel hierarchy among proteases whereby the serine protease NE up-regulates expression of MMP-2 and the cysteine protease cathepsin B. Furthermore, knockout studies of NE demonstrated that during Pseudomonas infection, the presence of NE is necessary for the activities of these other major protease groups. Inhibition of NF-κB or TLR-4 activity or transfection of macrophages with dominant-negative IRAK-1 causes a reduction of NE-induced cathepsin B and MMP-2 expression. Such regulation by a protease of
other proteases from different families implies the existence of a protease cascade that has important implications as to how proteases function in immune responses, tissue repair, development, and disease with wide-ranging implications for many health and disease states.

Materials and Methods

Culture and stimulation of monocyte cells

Myelomonocytic cells (U937; European Collection of Cell Cultures Health Protection Agency) were cultured in RPMI 1640 medium (Invitrogen Life Technologies) and were differentiated to macrophage-like cells for 48 h with PMA. The macrophage-like cells were incubated in fresh medium for a further 2 days before stimulation. An hour prior to stimulation, cells were washed and incubated in serum-free medium. Stimulation was performed with NE (low-endotoxin elastase derived from human sputum (~50% active); Elastin Products) at doses of 0, 16, 66, 166, and 333 nM for 30 min and cultured in fresh serum-free medium for either 3 or 24 h before harvesting, pending if needed for RNA or protein isolation, respectively. NE activity levels were examined before and following stimulation to cells, and serum-free medium. Abs, PBS, and all buffers added to cells used in subsequent experiments were found not to reduce NE activity. Methoxy- succinyl-Ala-Ala-Pro-Ala-chloromethyl ketone-treated NE was used as a negative control. Cells were also treated for 1 h with 5 nM and its inactive control, SN50M (Calbiochem), or with mouse anti-human CD284 Ab (AbD serotec) and mouse IgG2a (R&D Systems) before NE stimuli to block NF-κB activity or TLR-4, respectively. The SN50 peptide contains the nuclear localization sequence of NF-κB p50 and thereby inhibits translocation of the NF-κB active complex into the nucleus.

Isolation of PBMCs

Mononuclear cells were also isolated from heparinized venous peripheral blood obtained from healthy volunteers, as described (27). Briefly, density gradient centrifugation was conducted in Ficoll-Paque (Pharmacia Biotech) to separate the red cell pellet containing the neutrophil population from the mononuclear. The mononuclear cell band was aspirated and washed three times in serum-containing RPMI 1640 medium before culture. Monocytes were enriched from the mononuclear band by selectively attaching them to 24- or 12-well plates for 60 min at 37°C. Monocytes were purified to 97% purity using the EasySep human CD14 selection mixture, as recommended by manufacturers (StemCell Technologies). Monocytes were then cultured in RPMI 1640 containing 40% autologous serum, penicillin G (final concentration 100 U/ml), and streptomycin sulfate (final concentration 100 μg/ml) at 37°C in a 5% CO2 atmosphere for 9 days (28). An hour before stimulation, cells were washed and incubated in serum-free medium. Stimulation was performed with NE (150 nM) for 30 min and cultured in fresh serum-free medium for 24 h before harvesting.

Semiquantitative RT-PCR

After treatment, cells were harvested in TRI reagent (Sigma-Ireland), and RNA was extracted, as detailed in the manufacturer’s protocol. RNA (2 μg) was reverse transcribed at 37°C with 1 mM deoxynucleotide mix (Promega), 1.6 μg of oligo-p(dT)15 primer (Roche), and 1 μl Moloney murine leukemia virus reverse transcriptase (Promega) in a 20 μl vol, as described in the manufacturer’s protocol. A total of 2 μl of each cDNA was amplified with 1.25 U of TaqDNA polymerase, 1× PCR buffer, and 10 mM dNTPs (Promega) in a 50 μl vol containing 100 pmol each of the following primers: 5′-ATG TGG CAG CTC TGG GCC T-3′ and 5′-TAC TCA and 5′-TCC CAA GGT CCA TAG CTC ATC G-3′ for cathepsin B; 5′-ATG TGG CAG CTC TGG GCC T-3′ and 5′-TAC TCA GTG CGT GGT GAA ATT-3′ for cathepsin B; 5′-GCC CCC AAA ACG GAC AAA GA-3′ and 5′-TCC CAA GGT CCA TAG CTC ATC G-3′ for MMP-2; and 5′-AAC TCT GGT AAA GAT GAT-3′ and 5′-TAC TCA GCG CCA CCA GCA TGG-3′ for GAPDH. PCR products were quantified densitometrically at cycle numbers between 10 and 40 to determine the appropriate cycle number at which exponential amplification of products was occurring, and to identify the cycle number at which sufficient discrimination was possible to accurately quantify increases or decreases in gene expression. After a hot start, the amplification profile was 32 cycles of 1-min denaturation at 94°C, 1-min annealing at 58°C, and 1-min extension at 72°C. RT-PCR amplification of cathepsin B, MMP-2, and GAPDH generated products of 1004, 525, and 211 bp, respectively. PCR products were commercially sequenced (MWG Biotech) to verify gene identity. PCR products were resolved on a 1% agarose gel containing 0.5 μg/ml ethidium bromide (Sigma-Aldrich). The ratio of PCR fragment intensities of cathepsin B and MMP-2 relative to GAPDH was determined by densitometry.

FIGURE 2. Protease profile from peripheral blood monocytes. A, RT-PCR was conducted on mRNA from PBM treated with varying concentrations of NE (250 nM) to amplify regions of the cathepsin B, MMP-2, and GAPDH genes. The quantification of the expression of cathepsin B and MMP-2 was assessed compared with GAPDH. *, p < 0.01 when cells were exposed to NE, vs 0 nM NE. #, p < 0.01 when cells were exposed to NE vs 0 nM NE. B, Cathepsin activity in supernatant of macrophages from healthy volunteers following stimulation with NE (250 nM) compared with cells incubated in medium only. *, p = 0.03 vs control. MMP-2 activity was determined in supernatants from control (Con) and NE-treated (NE) PBM using gelatin zymography (C). Bands at 72 and 66 kDa are representative of latent MMP-2 and active MMP-2, respectively. Experiments or analyses of results were performed at least three times, and representative data and SE are shown.

NE knockout mouse analysis

NE gene-targeted mice were generated, as previously described (29). NE knockout mice and their wild-type littermates (n = 3 genotype) were intranasally challenged with PBS (50 μl) or PBS containing Pseudomonas aeruginosa H103 (4.8 × 105 CFUs). Twenty-four hours after, mouse lungs were lavaged with PBS and the protein concentration of the lavages was determined, as previously described (30). The lungs were processed for histology and immunohistochemistry. Briefly, lungs were inflated with 10% formalin in PBS. The excised lungs were then immersion fixed with 10% buffered formalin overnight, dehydrated, embedded in paraffin, and cut into 5-μm sections. Serial lung tissue sections were deparaffinized, rehydrated, and H&E stained. Lung sections were stained for cathepsin B and MMP-2 with anti-mouse cathepsin B (R&D Systems) and anti-mouse/rat MMP-2 Ab (R&D Systems), using the Cell and Tissue HRP-DAB system (R&D Systems).

Presence of cathepsin B

Cathepsin B activity was determined from medium taken from macrophage-like cells 24 h after stimulation with or without NE or BAL. Cathepsin B activity was determined in 100 μl of each sample using the substrate Z-Arg-Arg-AMC (0.1 mM). A cathepsin B inhibitor CA-074 (10 μg/ml) was used as a control for the specificity of the cathepsin B substrate. The reaction buffer used for cathepsin B activity estimation was 0.2 M sodium acetate, 2 mM EDTA, 1 mM DTT, 1 μM pepstatin, and 2 mM Pefabloc (pH 5.5). The samples were incubated with substrate for 60 min at 37°C, and fluorescence (substrate turnover) was determined by excitation at 355 nm and emission at 460 nm. Results were expressed as a change in fluorescence units over a 60-min period.
Gelatin zymography was performed on medium collected from unstimulated or NE-stimulated cells and BAL samples. Samples were subjected to 7% SDS-PAGE with a gel-containing gelatin (1 mg/ml). After electrophoresis was performed, gels were incubated in 50 mM Tris (pH 7.5), 5 mM CaCl₂, 1 mM ZnCl₂, and 2.5% (v/v) Triton X-100 for 30 min. The gels were washed in the same buffer without the Triton X-100 for 5 min and then incubated at 37°C overnight in the same buffer supplemented with 1% (v/v) Triton X-100. The gels were stained with 0.125% Coomassie blue and washed with 10% acetic acid and 40% methanol in water. The presence of MMPs appears as transparent bands. Latent MMP-2 and active MMP-2 were observed at 72 and 66 kDa, respectively. Densitometry was conducted to compare the intensity of the MMP transparent bands.

Preparation of subcellular fractions

U937 cells were activated with NE, and nuclear and cytoplasmic extracts were isolated. Briefly, cells were washed and resuspended in 1 ml of ice-cold PBS and kept on ice for 5 min. Cells were lifted from plates with a cell scraper and pelleted by centrifugation at 10,000 rpm for 5 min at 4°C. The supernatant was removed, and the cell pellet was resuspended in 1 ml of hypotonic buffer (10 mM HEPES (pH 7.9), 1.5 mM MgCl₂, 10 mM KCl, 0.5 mM PMSF, and 0.5 mM DTT) (Sigma-Aldrich). Cells were pelleted by centrifugation at 14,000 rpm for 10 min at 4°C and then lysed for 10 min on ice in 20 μl of hypotonic buffer containing 0.1% Igepal CA-630. Lysates were centrifuged as before, and the cytoplasmic extract was removed. The remaining nuclear pellet was lysed in 15 μl of lysis buffer (20 mM HEPES (pH 7.9), 420 mM NaCl, 1.5 mM MgCl₂, 0.2 mM EDTA, 25% (v/v) glycerol, 0.5 mM PMSF) (Sigma-Aldrich) for 15 min on ice. After centrifugation at 14,000 rpm for 10 min at 4°C, nuclear extracts were removed into 35 μl of storage buffer (10 mM HEPES (pH 7.9), 50 mM KCl, 0.2 mM EDTA, 20% (v/v) glycerol, 0.5 mM PMSF, and 0.5 mM DTT). Protein concentrations of cytoplasmic and nuclear extracts were determined, and extracts were stored at −80°C until required for use.

Western blot

Cytoplasmic fractions from macrophages were separated by electrophoresis on 12% SDS-polyacrylamide gels and transferred to a nitrocellulose membrane (Sigma-Aldrich), and this was probed using rabbit anti-GAPDH (Santa Cruz Biotechnology) and mouse anti-IRAK-1 Ab (BD Transduction Laboratories). Analyses of results were performed at least three times, and representative data and SE are shown.

Zymography

Gelatin zymography was performed on medium collected from unstimulated or NE-stimulated cells and BAL samples. Samples were subjected to 7% SDS-PAGE with a gel-containing gelatin (1 mg/ml). After electrophoresis was performed, gels were incubated in 50 mM Tris (pH 7.5), 5 mM CaCl₂, 1 μM ZnCl₂, and 2.5% (v/v) Triton X-100 for 30 min. The gels were washed in the same buffer without the Triton X-100 for 5 min and then incubated at 37°C overnight in the same buffer supplemented with 1% (v/v) Triton X-100. The gels were stained with 0.125% Coomassie blue and washed with 10% acetic acid and 40% methanol in water. The presence of MMPs appears as transparent bands. Latent MMP-2 and active MMP-2 were observed at 72 and 66 kDa, respectively. Densitometry was conducted to compare the intensity of the MMP transparent bands.

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Laboratories). Binding was detected using the appropriate HRP-conjugated secondary Ab and visualized by chemiluminescence (Pierce).

**IL-8 and NF-κB activity ELISA**

IL-8 protein concentrations in the cell supernatants were determined by ELISAs (R&D Systems). The effect of NE on NF-κB activity was determined using the TransAM NF-κB ELISA (Active Motif), using nuclear protein fractions.

**Dominant-negative IRAK-1 transfection**

U937 cells were seeded at $1 \times 10^5$ on 12-well plates in the presence of PMA for 48 h and were incubated in fresh medium for a further 2 days before transfection. Transfections were performed with JetPei transfection reagent (Polyplus-transfection) using 1 μg of a *Renilla* luciferase reporter gene, pRLSV40. In combination with the luciferase reporter gene, dominant-negative expression vector IRAK-1Δ (a gift from Tularik Inc., San Francisco, CA) was cotransfected into the cells. IRAK-1Δ is a truncated death domain-containing N terminus version of the IRAK-1 protein that lacks the kinase-binding domain. The total amount of DNA introduced into the cells was kept constant by supplementation with the relevant empty vectors. Transfection efficiencies were quantified using a *Renilla* luciferase vector (Promega). Transfections were left untreated for 24 h and were stimulated with NE (as before). After 24 h, supernatants were recovered for cathepsin B and MMP-2 activity estimation. Cells were lysed with reporter lysis buffer (Promega), protein concentrations were determined, and reporter gene activity was quantified by luminometry on a Wallac Victor2 1420 multilabel counter (PerkinElmer) using the Promega luciferase assay system. Data are expressed as the relative luciferase activity ± SE.

**Densitometric analysis**

Gels were analyzed by densitometry and compared in a semiquantitative manner using the GeneGenius Gel Documentation and analysis system and GeneSnap and GeneTools software. All expression values were verified by at least three independent experiments.

**Statistical analysis**

Data were analyzed with the PRISM 3.0 software package (GraphPad). Results are expressed as the mean ± SE and were compared by t test. When more than two groups were being compared, an ANOVA test was
used, followed by a Tukey’s post hoc test. Differences were considered significant at $p \leq 0.05$.

**Results**

**NE induces cathepsin B and MMP-2 release from macrophages**

We hypothesized that NE could induce a protease cascade. To test this hypothesis, the effect of NE on macrophage protease gene expression was examined. U937-differentiated cells were exposed to NE (0, 16, 66, 166, 333, 500 nM) for 30 min in serum-free medium before removing the NE and incubating the cells for a further 3 h. Cathepsin B and MMP-2 mRNA expression levels were investigated by RT-PCR (Fig. 1A) and were observed to significantly increase when cells were stimulated with NE at concentrations of 166 nM and higher ($p = 0.02, p < 0.01$, and $p < 0.01$ for cathepsin B, and $p = 0.02, p = 0.01$, and $p < 0.01$ for MMP-2 expression between control cells (0 nM) and cells stimulated with 166, 333, and 500 nM NE, respectively). Cathepsin B and MMP-2 activities were measured in the supernatants 24 h after NE stimulation, and elevated cathepsin B and MMP-2 activity was observed in NE-treated cell supernatants compared with nonstimulated control cells (Fig. 1, B and C). Both latent and active MMP-2 were significantly different to the control in the presence of NE (166 nM or greater). NE was also observed to activate MMP-9 (data not shown), as described previously (31). NE treated with chloromethyl ketone before incubation with cells resulted in no increase in cathepsin B or MMP-2 activation (data not shown), showing that the effect by NE on cathepsin B and MMP-2 expression is dependent on its activity.

**Protease profile from NE-stimulated peripheral blood monocyte-derived macrophages (MDM)**

To investigate this increase in macrophage protease production, MDM extracted from the blood of healthy volunteers were exposed to NE. Increased cathepsin B and MMP-2 gene expression (Fig. 2A) were again observed following stimulation of MDM with NE in serum-free medium, as before. Protease activity levels were also increased as before (cathepsin B, Fig. 2B; $p = 0.03$ and MMP-2, Fig. 2C) following stimulation of MDM.

**Cathepsin B and MMP-2 activity in NE$^{+/+}$ and NE$^{-/-}$ mice**

A deficiency of NE could alter the production of cathepsin B and MMP-2 responses. To investigate this, cathepsin B and MMP-2 activities were measured in BAL fluid from NE$^{+/+}$ and NE$^{-/-}$ mice i.v. challenged with *P. aeruginosa*. Mice possessing NE (NE$^{+/+}$) produced greater quantities of cathepsin B (Fig. 3A; $p = 0.03$) and MMP-2 (latent and active MMP-2; $p = 0.02$ and $p = 0.02$, respectively) than knockout mice (NE$^{-/-}$) (Fig. 3B). Analysis of lung tissue from NE$^{+/+}$ and NE$^{-/-}$ mice for cathepsin B and MMP-2 expression, by immunohistochemistry, further examined these protease levels confirmed greater levels of positive staining for cathepsin B and MMP-2 in NE$^{+/+}$ mice compared with NE$^{-/-}$ mice (Fig. 3, C–F).

**IRAK-1 degradation, NF-κB activation, and IL-8 protein production in U937 macrophages stimulated with NE**

Time course studies demonstrated that 100 nM NE induced maximum NF-κB activation at 30 min (Fig. 4B). NE-induced NF-κB nuclear translocation was increased 5-fold compared with control. Western blotting of cytoplasmic extracts was performed using anti-IRAK-1 Ab. Stimulation with NE resulted in degradation of IRAK-1 (Fig. 4A). Our group has shown previously that NE induces IL-8 gene up-regulation in bronchial epithelial cells through an IRAK signaling pathway, resulting in nuclear translocation of NF-κB (24). NE-induced IL-8 protein levels in cell supernatants from U937s were quantified by ELISA (Fig. 4C). U937s produced a mean basal level of IL-8 of 347.6 ± 51.89 pg/mg protein. Dose-response experiments demonstrated that 100 nM NE maximal IL-8 protein production from U937 cells, increasing IL-8 levels to 1002 ± 122.9 pg/mg protein ($p = 0.01$).

**Inhibition of NF-κB TLR-4, or transfection of dominant-negative IRAK-1 leads to a reduction of NE-induced cathepsin B and MMP-2**

SN50, a cell-permeable peptide that inhibits NF-κB nuclear translocation downstream of IKK, and its mutant peptide, NF-κB SN50M, were used to investigate whether inhibition of NF-κB could reduce NE-induced protease expression. SN50 was able to prevent the effects of NE on cathepsin B (Fig. 5A; $p = 0.02$) and MMP-2 (Fig. 5B) protein activity, demonstrating that NE signals via NF-κB to induce cathepsin B and MMP-2 expression in macrophages. Inhibition of TLR-4 with the aid of mouse anti-human CD284 was also able to prevent the effects of NE on cathepsin B (Fig. 5C; $p = 0.01$) and MMP-2 (Fig. 5D) protein activity. Transfection of dominant-negative IRAK-1 also leads to a reduction in NE-induced protease expression (Fig. 6; $p < 0.01$ for cathepsin B). The empty vector had no effect on the effects of NE on cathepsin B and MMP-2.

**Discussion**

Elevated levels of proteases are typically observed at many sites of inflammation, leading to a multitude of effects, including tissue destruction, tissue remodeling, and cleavage of soluble innate factors. We have shown previously that NE can induce expression of IL-8 via the NF-κB pathway of activation in human bronchial epithelial cells (24). We postulated that increased extracellular NE
activity may induce expression of other proteases such as cathepsins and MMPs, which have been demonstrated previously to be present along with NE in conditions such as emphysema and cystic fibrosis (17, 32). This study demonstrates that NE can induce increased cathepsin B and MMP-2 expression and activity in macrophages. Previous studies have observed increased levels of protease activity in the presence of raised NE levels (4, 33–36), but have not demonstrated corresponding increased protease gene expression. This study provides molecular and animal model data that support the view that NE presides over a novel hierarchy in protease regulation. Cathepsin B and MMP-2 gene expression and activity were both increased in macrophages exposed to NE. Increased cathepsin B and MMP-2 levels were observed in wild-type mice compared with NE knockout mice i.v. challenged with P. aeruginosa. This study illustrates a potential novel method for NE to cause tissue destruction, particularly in diseases associated with high NE burden.

Increased levels of NE have been demonstrated in many disease processes characterized by an inflammatory response (34, 35). It is estimated that ~250 mg of NE is turned over kilogram of body weight per day in normal individuals, demonstrating the requirement for a large anti-NE-protective screen in the body (37). NE-driven diseases also tend to exhibit reduced levels of antiprotease levels (18). Shapiro and Senior (38) postulated that NE is meant to protect important respiratory tract innate immune proteins such as secretory leucoprotease inhibitor, human β defensins 2 and 3, and lactoferrin (17, 19, 32). In this study, we show a novel pathway for cathepsin B activation. Cathepsin B released in response to stimulation by NE causes may cause degradation of the extracellular matrix, generating the emphysema seen in lung disease, as well as impact on the function of important antimicrobial proteins and peptides. It has been well documented that NE-burden conditions such as cardiopulmonary bypass demonstrate increased plasma levels of NE and MMPs, which cause pulmonary injury. Inhibition of both NE and MMPs in this condition can prevent pulmonary injury (35, 40), and, interestingly, increased MMP levels correlate with NE levels in cystic fibrosis patients (34).

MMPs are up-regulated during allergic inflammation, but participate in the formation of many lung diseases (20, 41). Previously, NE has been shown to activate MMP-9 (31). MMP-9 and MMP-12 have been implicated in the pathogenesis of chronic lung injury, particularly in emphysema. This is shown in MMP-12 knockout mice, which do not develop air space enlargement in response to smoke exposure (42). We observed an increase in active MMP-9, but MMP-9 gene expression was unchanged (data not shown), unlike NE activation of MMP-2, which was observed to occur at the level of gene expression. MMP-2 has an important anti-inflammatory role, playing a central role in the IL-13-dependent regulatory loop that has been shown to be responsible for dampening airway inflammation (43). Parenchymal inflammatory cells egress into the airway lumen in a MMP-2-dependent manner, and MMP-2−/− mice are also more susceptible to lethal asphyxiation using a model of allergic inflammation, indicating the importance of MMP-2 in leukocyte infiltration (43).

Previously, NF-κB has been shown to mediate cathepsin B and MMP-2 activation by doxorubicin treatment (44) and LPS (45), respectively. Interestingly, our study shows that inhibition of the NF-κB pathway (with SN50) will result in decreased cathepsin B and MMP-2 expression. Furthermore, a TLR-4-neutralizing Ab or transfection of macrophages with dominant-negative IRAK-1 abrogates NE-induced cathepsin B and MMP-2 expression. We have therefore demonstrated that NE induces IL-8, cathepsin B, and MMP-2 production through an IRAK-1/TLR-4-mediated pathway in macrophages. PAR-2 has been shown to cause activation of NF-κB in human keratinocytes, resulting in up-regulation of cell adhesion molecules such as ICAM-1 (46). However, PAR-2 has not been shown to interact with TLR-4/IRAK-1 pathway, thereby indicating that PAR-2 does not play a role in the NE-induced protease production observed in this study. The role of TLR-4 in the NE activation of cathepsin B and MMP-2 by macrophages is still unclear, and further research into this area may be beneficial.

The data in this study demonstrate that extracellular NE can induce a protease cascade involving cathepsin B and MMP-2 expression. Elucidation of such a hierarchy in protease control and regulation coupled with identification of key protease/proteases central to direct tissue destruction or activation of other proteases represents an important advancement in protease biology. This would greatly enhance our understanding of these proteases and could lead to potential new therapeutic strategies to treat protease-mediated diseases. Neutralization of NE activities may be sufficient to lessen the overall protease burden without the need for inhibition of all proteases. Investigating the effect of other serine proteases on expression levels of different protease families may highlight other areas of interest.

Disclosures

The authors have no financial conflict of interest.

References


