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Original article

German cockroach proteases regulate matrix metalloproteinase-9 in human bronchial epithelial cells

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Background: Matrix metalloproteinases (MMPs) digest extracellular matrix proteins and may play a role in the pathogenesis of bronchial asthma. MMP-9 levels are increased in the bronchoalveolar lavage fluid and sputum of asthmatics compared with that of controls. As exposure to cockroaches is an environmental risk factor for asthma, we sought to investigate the role of German cockroach fecal remnants (frass) on MMP-9 expression.
Methods: Human bronchial epithelial cells (16HBE14o-) and primary normal human bronchial epithelial cells were treated with cockroach frass in the absence or presence of tumor necrosis factor (TNF)α. MMP-9 mRNA, protein levels and pro-MMP-9 activity were determined using real-time polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA) and zymogram assays. Pretreatment of frass with aprotinin abolished protease activity. PD98059, a chemical inhibitor of extracellular signal regulated kinase (ERK), and SLIGKV, an activator of protease-activated receptor (PAR)-2 were also used. AP-1DNA binding was determined by electrophoretic mobility shift assay (EMSA) and ERK phosphorylation by Western blot analysis.
Results: Cockroach frass augmented TNFα-mediated MMP-9 mRNA and protein expression by a mechanism dependent on active serine proteases within frass and not on endogenous endotoxin. Frass increased ERK phosphorylation, and chemical inhibition of ERK attenuated cockroaches' effects on MMP-9. Serine proteases are known to activate the PAR-2 receptor. We found that selective activation of PAR-2 using the peptide SLIGKV augmented TNFα-induced MMP-9 protein levels and increased ERK phosphorylation. Frass and SLIGKV each increased AP-1 translocation and DNA binding.
Conclusions: These data suggest that German cockroach frass contains active serine proteases which augment TNFα-induced MMP-9 expression by a mechanism involving PAR-2, ERK and AP-1.

Key words: activator protein-1; *Blatella germanica*; extracellular regulated kinase; frass; serine protease.

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Matrix metalloproteinase (MMP)-9 is a zinc- and calcium-dependent protease which can degrade collagen type IV, a major component of the epithelial basement membrane. Several observations suggest a role for MMP-9 in the pathogenesis of bronchial asthma. The level and activity of MMP-9 was increased in the sputum of severe asthmatics compared with that of patients with mild asthma or normal controls, and the level of MMP-9 was sensitive to downregulation by oral corticosteroid treatment (1). In allergic patients who underwent bronchoscopy and segmental bronchoprovocation with saline or allergen (ragweed, cat or house dust mite), MMP-9 levels were significantly increased in the bronchoalveolar lavage (BAL) fluid within 48 h (2). A second study using atopic and normal patients confirmed that allergen challenge (inhalation of house dust mite) increased MMP-9 expression in sputum from atopic patients compared with non-atopic patients (3). Together, these data suggest that MMP-9 may play a role in modulating the asthma phenotype.

Cockroach exposure has been recognized as an important cause of asthma for over 30 years. Several allergens have been cloned from German cockroach (*Blattella germanica*) and termed Bla g 1, Bla g 2, Bla g 4 and Bla g 5. Interestingly, while none of the cockroach allergens cloned to date are proteolytically active (4, 5), protease activity has been noted in the whole-body extract of German cockroach (5, 6). A variety of aeroallergens have been shown to exhibit protease activity including house dust mite (*Dermatophagoides pteronyssinus*), fungus (*Aspergillus fumigatus*) and cat (*Felis domesticus*) (7–9). This raises the question of the overall role of proteases in the development of asthma and airway hyperresponsiveness.

We have been interested in the role of German cockroach extracts in modulating human airway inflam-

matory responses. We have shown that serine proteases contained in cockroach whole-body extracts upregulate the expression of pro-inflammatory cytokines interleukin (IL)-8 and IL-6 in the presence of tumor necrosis factor (TNF)α (6, 10, 11). Protease-activated receptor (PAR)-2 is a G-protein-coupled receptor that is activated upon cleavage by serine or trypsin-like proteases. It has been shown that German cockroach extracts activate PAR-2 (10–13). Once PAR-2 is cleaved, a signaling cascade involving G-proteins, MEK [mitogen-activated protein (MAP) kinase/extracellular signal-regulated kinase (ERK)] and ERK is initiated (10).

It is conceivable that asthmatics would encounter cockroach-derived proteins in the context of airway inflammation (i.e. increased TNFα). As TNFα has been shown to regulate MMP-9 expression (14, 15), and since MMP-9 is increased in the BAL of asthmatics (16), we tested the hypothesis that German cockroach frass would modulate TNFα-induced MMP-9 expression in human bronchial epithelial cells.

Materials and methods

Cockroach frass

The fecal remnants (frass) from one cage of German cockroaches were transferred to a sterile container and stored at 4°C. Frass was resuspended in endotoxin-free double-distilled water (2 h at 4°C while rocking), centrifuged to remove debris (13 000 ***g*** for 5 min at 4°C), supernatants harvested, and total protein was measured using the Bio-Rad Protein Assay Dye (Bio-Rad, Hercules, CA, USA). Protease activity was determined using the Azocoll method (17) as previously described (6). Endotoxin levels were determined by Charles River Laboratories using the Kinetic Chromogenic method (Charles River Laboratories, Charleston, SC, USA).

Cell culture

An SV40-transformed human bronchial epithelial cell line (16HBE14o-), provided by S. White (University of Chicago) was studied as described previously (18). Normal human bronchial epithelial cells (Clonetic Corp, Walkersville, MD, USA) were grown according to the manufacturers' specifications. Cells were serum-deprived prior to treatment and treated with German cockroach frass (100 ng/ml), human TNFα (3 ng/ml; R & D Systems, Minneapolis, MN, USA), aprotinin-treated cockroach extract (100ng/ml frass pretreated with 10 μg/ml aprotinin for 30 min at 37°C), the PAR-2 agonist SLIGKV (50 μM from Invitrogen, Carlsbad, CA, USA), alone or in combination as described in the figures. In some experiments, polymyxin B (50 μg/ml for 1 h; Sigma-Aldrich, St Louis, MO, USA) was added prior to treatment with frass and/or TNFα. Cells were treated for 4 h (for mRNA measurements) or 16 h (for protein measurements). A dose–concentration curve was performed for frass-treated cells and an optimal dose was determined. Cell viability was determined using the trypan blue exclusion assay.

Real-time PCR

RNA was extracted using a standard TRIzol method of phenol extraction. Total RNA is converted to cDNA by reverse transcription using the Superscript First-Strand Synthesis System kit (Invitrogen). The MMP-9 primers, designed to span an intron, are right (AAG ATG CTG CTG TTC) and left (TCG GTA CTG GAA GAC). MMP-9 was amplified by polymerase chain reaction (PCR) using SYBR Green on the iCycler (BioRad Laboratories) as follows: one cycle 95°C for 3 min, followed by 40 cycles (95°C for 5 s, 56°C for 5 s, 72°C for 10 s), 95°C for 1 min, 55°C for 1 min. The SDHA primers, designed to span an intron, are right (TGG GAA CAA GAG GGC ATC TG) and left (CCA CCA CTG CAT CAA ATT CAT G). SDHA was amplified as follows: 1 cycle 95°C for 3 min, followed by 30 cycles (95°C for 5 s, 60°C for 5 s, 72°C for 10 s), 95°C for 1 min, 55°C for 1 min. Each target gene (MMP-9) is normalized to a housekeeping or reference gene (SDHA) using the calculation $(E_{\text{ref}})^{\text{Ct ref}}/(E_{\text{tar}})^{\text{Ct tar}}$, where E is the real-time efficiency of the reference (ref) or target (tar) gene reaction and Ct is the threshold cycle of the reference (ref) or target (tar) gene (19). For MMP-9 expression in 16HBE14o-cells, the mean Ct values for MMP-9 were: untreated (29 ± 0.7; $n = 5$ for all), frass-treated (28.7 ± 0.6), TNFα-treated (25.6 ± 0.8) and TNFα plus frass-treated (23.1 ± 0.6).

ELISA

Cells were treated with cockroach frass in the presence or absence of TNFα for 16 h. In some cases, cells were pretreated with the MEK inhibitor PD98059 (30 μM for 1 h; Cell Signaling, Beverly, MA, USA) prior to the addition of TNFα and frass. Cell supernatants were collected and clarified (13 000 ***g*** for 10 min at 4°C) prior to being analyzed for total MMP-9 or TIMP-1 by enzyme-linked immunosorbent assay (ELISA) according to manufacturers' specifications (Amersham, Piscataway, NJ, USA).

Immunoblot analysis

Cells were cultured in six-well plates and serum-starved for 24 h before treatment. Selected wells were treated with frass, TNFα, or both for 10 min. Cell lysates were harvested and resolved on a 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as previously described (10). After incubation with an anti-phospho-ERK antibody (Cell Signaling), signals were amplified and visualized using enhanced chemiluminescence. The membranes were stripped (62.5 mM Tris pH 6.8, 100 mM β-mercaptoethanol, 2% SDS; 50°C for 30 min) and reprobed with an anti-ERK antibody (Cell Signaling).

Zymography

Cells were grown to near confluence and depleted of serum for 8 h prior to treatment with frass, TNFα, or both for 16 h. Aliquots of the cell supernatant underwent electrophoresis in 8% SDS-PAGE containing 1 mg/ml gelatin (Sigma Chemical Corp, St Louis, MO, USA) under non-reducing conditions. The gel was washed for 30 min (2.5% Triton X-100, 3 mM NaN_3), rinsed for 30 min (2.5% Triton X100, 50 mM Tris-HCl, 5 mM $CaCl_2$, 1 μM $ZnCl_2$, 3 mM NaN_3, pH 7.4) and then incubated overnight at 37°C (50 mM Tris–HCl, 5 mM $CaCl_2$, 1 μM $ZnCl_2$, 3 mM NaN_3, pH 7.4). After staining with Coomassie Blue (R-250, 0.125%), gelatin-degrading enzymes were identified as clear zones against a blue background.

Electrophoretic mobility shift assay (EMSA)

All nuclear extraction procedures were performed on ice with ice-cold reagents. Cells were treated with frass in the absence or presence of TNFα for 1 h. They were harvested and nuclear proteins

isolated as previously described (11). Protein concentrations were determined by Bradford assay (Bio-Rad) and stored at −70°C until use.

The AP-1 probe [sense 5′-GAT CCG AGT GTG ATG ACT CAG GGT-3′ and antisense 5′-AAC CTG AGT CAT CAC ACT CGG ATC-3′] was synthesized from Invitrogen. The probe was labeled with [γ-^{32}P]ATP using T4 polynucleotide kinase (Gibco BRL, Gaithersburg, MD, USA) and purified in Bio-Spin chromatography columns. The gel was run using 4 μg of nuclear protein as previously described (11). Cold-specific and nonspecific probes were added at 5× the concentration of the radiolabeled probe. Gels were transferred to Whatman 3M paper, dried under a vacuum at 80°C for 1 h, and exposed to photographic film at 70°C with an intensifying screen.

Statistical analysis

When applicable, statistical significance was assessed by one-way analysis of variance (ANOVA). Differences identified by ANOVA were pinpointed by Student–Newman–Keuls' multiple range test.

Results

German cockroach frass synergistically increased TNFα-induced MMP-9 mRNA levels

We hypothesized that exposure to German cockroach fecal remnants (frass) may regulate MMP-9 expression in human bronchial epithelial cells. To test this, we treated SV40-transformed human bronchial epithelial cells (16HBE14o-) with frass in the absence or presence of TNFα and performed quantitative real-time PCR. Treatment with TNFα induced MMP-9 mRNA expression (Fig. 1A). Alone, frass had minimal effects on MMP-9 mRNA expression. When cells were treated with frass in the presence of TNFα, a synergistic increase in MMP-9 mRNA levels was detected compared with treatment with TNFα alone.

German cockroach frass synergistically increased TNFα-induced MMP-9 protein levels

To confirm that changes in MMP-9 mRNA resulted in changes in protein abundance, we performed ELISA on supernatants from cells treated with frass in the absence or presence of TNFα, and found that frass synergistically increased TNFα-induced MMP-9 protein abundance in 16HBE14o-cells (Fig. 1B) and in primary human bronchial epithelial cells (Fig. 1C). As tissue inhibitor of metalloproteinase (TIMP)-1 acts to negatively regulate MMP-9, we measured TIMP-1 protein levels. The concentration of TIMP-1 was not significantly altered by any treatment. It is important to note that in human bronchial epithelial cells, the amount of TIMP-1 secretion (control cells 202 ± 11 ng/ml; frass-treated cells 197.3 ± 13 ng/ml; TNFα-treated cells 222.8 ± 42 ng/ml; and TNFα plus frass-treated cells 223 ± 42 ng/ml) greatly exceeded the amount of MMP-9 secretion (control cells 0.42 ± 0.08 ng/ml; frass-treated cells 0.53 ± 0.07 ng/ml;

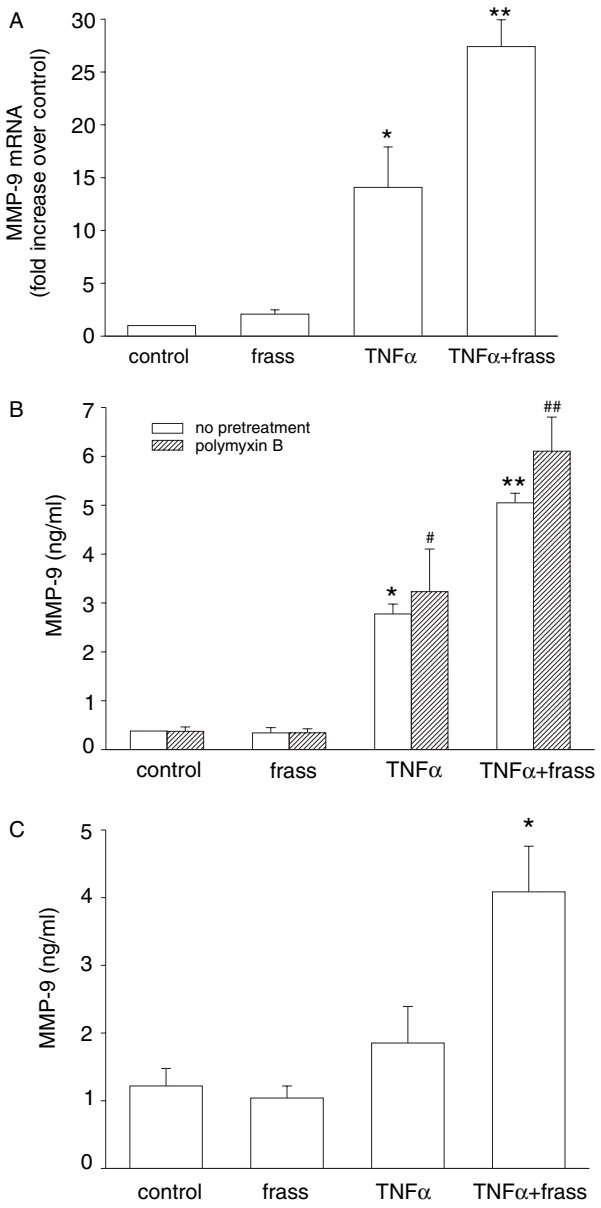

Figure 1. Cockroach frass regulates TNFα-induced MMP-9 levels. (A) 16HBE14o-cells were treated with cockroach frass, TNFα or both for 4 h. Quantitative real-time PCR was performed. MMP-9 normalized to SDHA and expressed as fold-increase over control is shown. Data represent mean ± SEM for four experiments (compared with control $*P = 0.005$; compared with TNFα alone $**P = 0.004$; ANOVA). (B) 16HBE14o-cells were given either no pretreatment or pretreated with polymyxin B (50 μg/ml for 1 h) prior to the addition of frass, TNFα or both for 16 h. Supernatant was harvested and analyzed by ELISA. Data represent mean ± SEM for four to seven experiments (compared with control $*P = 0.006$, $\#P = 0.001$; compared with TNFα alone, $**P = 0.002$, $\#\#P = 0.004$; ANOVA). (C) Normal primary human bronchial epithelial cells were treated as in (B). Data represent mean ± SEM for four experiments (compared with TNFα alone, $*P < 0.001$, ANOVA).

TNFα-treated cells 2.5 ± 0.7 ng/ml; TNFα plus frass-treated cells 4.8 ± 1.2 ng/ml).

Endotoxin does not play a role in the synergistic effect

The endotoxin level in frass was measured by Charles River Laboratories and found to be 10.9 EU/mg protein (or 1.09 μg/mg protein). Incubation of cells with 100 ng/ml frass resulted in the addition of 109 pg/ml endotoxin. To rule out the possibility that endotoxin plays a role in the upregulation of MMP-9 expression by frass, we incubated 16HBE14o-cells with 100 pg/ml endotoxin in the absence or presence of TNFα and measured MMP-9 release by ELISA. Endotoxin had no effect on MMP-9 release, alone or in the presence of TNFα, suggesting that the effect of frass was not because of endotoxin contamination (data not shown). As a result of the complex composition of frass, we also wanted to determine if removal of endotoxin from frass would alter the synergistic increase in MMP-9 protein expression. To do this, we pretreated cells with polymyxin B for 1 h prior to addition of TNFα and/or frass. Polymyxin B treatment did not affect frass-induced synergistic increase in TNFα-mediated MMP-9 release (Fig. 1B).

German cockroach proteases modulate MMP-9 expression

Frass contained active serine proteases (19 μg/mg frass). Pretreatment of frass with the serine protease inhibitor aprotinin (10 μg/ml for 30 min at 37°C) inhibited 80% of protease activity as determined by the Azocoll assay. Treatment of cells with aprotinin-treated frass failed to augment TNFα-induced MMP-9 expression (Fig. 2). These data suggest the importance of active serine proteases in frass for modulating MMP-9 protein abundance.

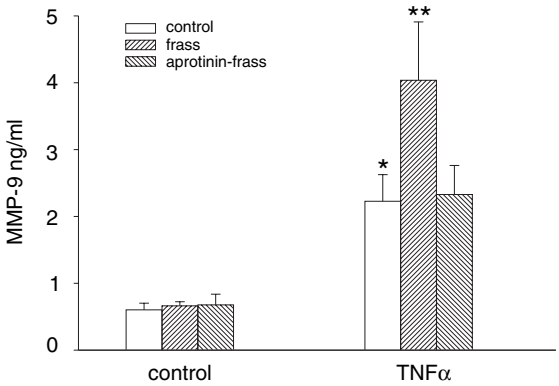

Figure 2. Protease activity in frass regulates MMP-9 protein abundance. 16HBE14o-cells were treated with frass, aprotinin-treated frass, or TNFα, alone or in combination for 16 h prior to harvest. Supernatant was collected and analyzed for MMP-9 protein by ELISA. Data represent mean ± SEM for five experiments (compared with control $*P = 0.045$; compared to TNFα alone $**P < 0.001$, ANOVA).

The effect of TNFα and cockroach frass on pro-MMP-9 activity

Gelatin zymogram assays were used to detect the 92-kDa pro-MMP-9. Treatment with frass alone had no effect on pro-MMP-9 activity. Treatment with TNFα slightly increased pro-MMP9 activity; however, treatment with frass and TNFα resulted in a synergistic increase in active pro-MMP-9 (Fig. 3). Incubation of the control sample with an MMP-9 antibody shifted the band on the zymogram validating that the band detected on the zymogram is indeed pro-MMP-9 (data not shown).

Cockroach frass does not activate NF-κB

Nuclear factor (NF)-κB is a well-known activator of MMP-9 transcription. TNFα induced NF-κB translocation and DNA binding. However, frass had no effect alone or on TNFα-induced NF-κB translocation and DNA binding as determined by electrophoretic gel shift assay (data not shown).

Cockroach frass-induced activation of MMP-9 is due to activation of ERK

Treatment of primary normal human bronchial epithelial cells (Fig. 4A) or 16HBE14o-cells (Fig. 4B,C) with frass increased ERK phosphorylation. Both ERK1 (44 kDa) and ERK2 (42 kDa) are detected in the primary cells, while ERK2 is the primary form detected in 16HBE14o-cells. Pretreatment of cells with PD98059, a chemical inhibitor of MEK, abolished frass-induced synergy (Fig. 4D). Similar observations were obtained using another commercially available ERK inhibitor, U0126 (data not shown). We also investigated the possibility that frass increased phosphorylation of p38, another mitogen-activated protein kinase. TNFα increased phospho-p38 in a statistically significant manner, but the addition of frass to TNFα-treated cells did not result in a further phosphorylation of p38. Frass had no effect on phosphorylation of p38 either alone or in the presence of

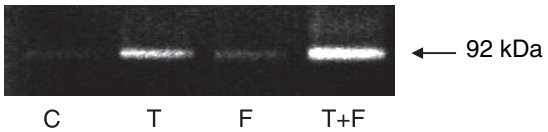

Figure 3. Frass increased TNFα-induced proMMP-9 activity. 16HBE14o-cells were treated with TNFα (T), frass (F), alone or in combination (T + F) for 16 h prior to harvest. Cell supernatants were subject to SDS-PAGE/gelatin electrophoresis under non-reducing conditions. Gelatinolytic activity is identified as clear zones on a blue background. The experiment was performed three times with similar results.

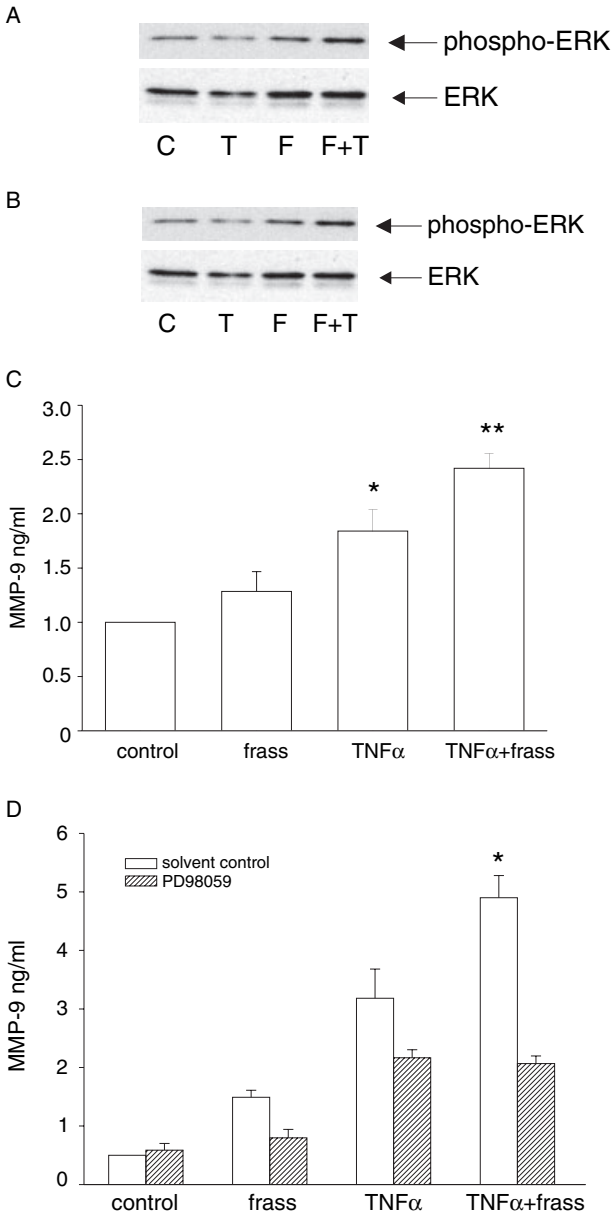

Figure 4. ERK activation is required for frass-induced MMP-9 expression. (A) Primary normal human bronchial epithelial cells were untreated (C) or treated with TNFα (T), frass (F) or both (T + F) for 10 min prior to harvest. Immunoblot analysis using an antibody against the dually phosphorylated form of ERK 1 and ERK 2 is shown in the top panel. The gel was stripped and reprobed for total ERK 1 and ERK 2 (bottom panel). This gel is representative of two separate experiments. (B) 16HBE14o-cells were treated as above. A representative gel is shown. (C) Analysis of phospho-ERK/total ERK. Data are means ± SEM, $n = 4$ (compared with control $*P = 0.042$, $**P = 0.004$) (D). Cells were pretreated with PD98059 (30 μM for 1 h) prior to the addition of frass, TNFα or both. Data represent mean ± SEM for four experiments (compared with control $*P < 0.001$; compared with TNFα alone $*P < 0.001$, ANOVA).

TNFα (data not shown). In addition, pretreatment with SB202190, a specific p38 inhibitor attenuated TNFα-induced MMP-9 expression, but did not affect synergy when frass was also added (data not shown). Furthermore, inhibition of protein kinase C (with calphostin C) or phosphatidylinositol 3-kinase (with wortmannin) did not affect frass-induced synergy of TNFα-mediated MMP-9 production (data not shown).

PAR-2 increases MMP-9 synthesis and activation of ERK

Activation of PAR-2 using the selective PAR-2-activating peptide SLIGKV resulted in the increased expression of MMP-9 (Fig. 5A). In addition, treatment of cells with SLIGKV increased ERK activation (Fig. 5B).

Cockroach frass and SLIGKV increase AP-1 translocation

To test for the involvement of AP-1, another transcription factor important in MMP-9 regulation, we performed an electrophoretic gel shift assay using an oligonucleotide probe designed against the AP-1 binding site on the IL-8 promoter. Treatment of cells with frass

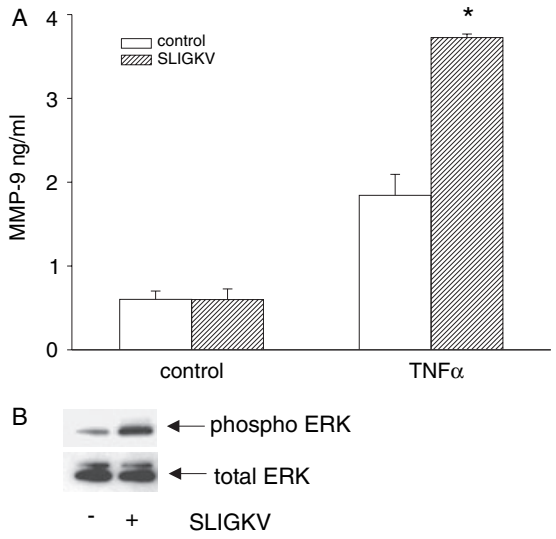

Figure 5. Selective activation of PAR-2 increased TNFα-induced MMP-9 expression and activated ERK. (A) 16HBE14o-cells were treated with the selective PAR-1 activator SLIGKV alone or in the presence of TNFα. Supernatant was harvested and analyzed by ELISA. Data represent mean ± SEM for four experiments (compared with control $*P = 0.004$; compared with TNFα alone, $**P = 0.009$, ANOVA). (B) Cells were untreated or treated with SLIGKV for 10 min prior to harvest. Immunoblot analysis using an antibody against the dually phosphorylated form of ERK is shown in the top panel. The gel was stripped and reprobed for total ERK (bottom panel). This was repeated twice and a representative gel is shown.

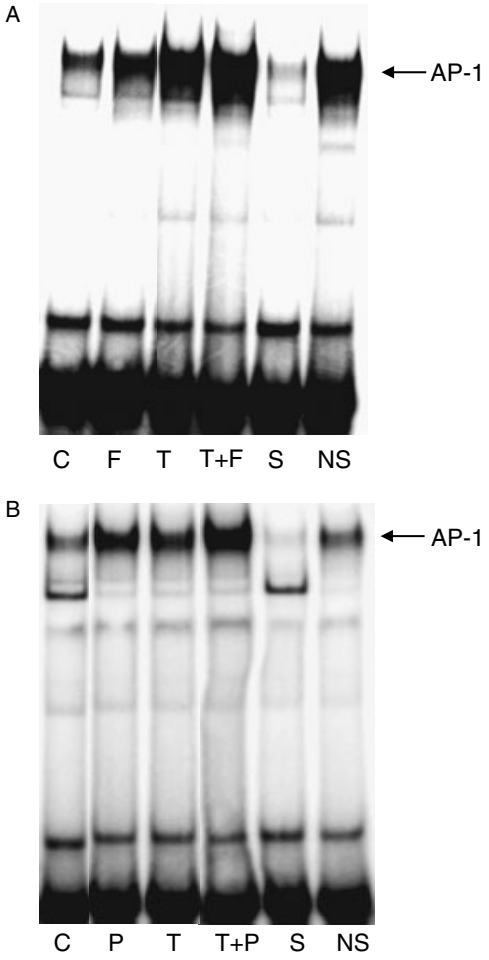

Figure 6. Frass and SLIGKV increased AP-1-DNA binding. (A) 16HBE14o-cells were untreated (C), treated with frass (F), TNFα (T), or TNFα and frass (T + F). (B) Cells were treated with the PAR-2 agonist SLIGKV (P) in the presence or absence of TNFα (T). Nuclear extracts were obtained, incubated with a ^{32}P end-labeled double stranded AP-1 oligonucleotide, and resolved on a gel. Cold AP-1 probe (S) and cold NF-κB probe (NS) were used to show specificity. Each experiment was repeated twice with a representative experiment shown.

(Fig. 6A) or SLIGKV (Fig. 6B) alone increased AP-1 translocation and upregulated TNFα-induced AP-1-DNA binding.

Discussion

The airway epithelium plays a dynamic role in the asthmatic response with its ability to synthesize important metabolically active cytokines and other proteins. Airborne proteases may come in direct contact with airway cells and may play an important role in modulating inflammation and repair processes. In this report, we find that cockroach frass contains serine proteases which modulate TNFα-induced MMP-9 expression in a mechanism dependent on PAR-2, ERK, and AP-1 translocation and DNA binding. The human MMP-9 promoter contains NF-κB, AP-1, AP-2, SP-1 and Ets binding sites (20). An absolute requirement for NF-κB in MMP-9 transcription has been previously reported (21). That frass has no effect alone but augments TNFα-induced MMP-9 expression is likely because frass does not activate NF-κB. The activation of AP-1 may not be essential for induction but may be required for maximal stimulation (21). Frass-induced activation of ERK and AP-1 further increases translocation and DNA binding of AP-1 to the MMP-9 promoter to cause an upregulation of MMP-9. Frass-induced synergy was not altered by pretreatment with inhibitors to p38, phosphatidylinositol 3-kinase or PKC. As MMP-9 plays an important role in tissue remodeling, understanding the mechanism of regulation could lend insights into therapeutic interventions.

In this report, we used cockroach frass to determine the effects on MMP-9 expression. Frass is a likely source of allergen exposure as desiccated fecal remnants may easily be incorporated in house dust. It has been shown that after disturbance, dust particles containing cockroach proteins >10 μm in diameter may be deposited in the airways (22). Once in the airways, proteins elute from these particles and may reach high local concentrations. In addition, we have preliminary data to suggest that proteases remain active in dust collected from carpeting in homes (K. Page and B. Lanphear, unpubl. obs.). This suggests that the proteases may continue to be active even after lying dormant for a period of time.

MMP-9 protein levels were increased following treatment with TNFα and augmented with frass and TNFα treatment; however, these treatments did not significantly affect TIMP-1 levels. TIMP-1 is a natural inhibitor of MMP-9 and is thought to bind to MMP-9 in a 1:1 ratio. There was a significant excess of TIMP-1 compared with MMP-9 in these cells. This is likely why we were only able to detect the latent pro-MMP-9 (92 kDa) form of MMP-9 and not the active (88 kDa) form by zymogram. It is conceivable that the ratio of MMP-9 and TIMP-1 synthesized from different types of cells would be different. We have data to suggest that in neutrophils, which can degranulate and release MMP-9, the secretion of MMP-9 is in excess over TIMP-1 (K. Page and V.S. Hughes, unpublished data). The excess of TIMP-1 secretion from airway bronchial epithelial cells could be a mechanism to prevent disruption of the type IV collagen basement membrane. In a study comparing sputum samples from non-asthmatics, stable asthmatics and acute asthmatics, it was shown that pro-MMP-9 (92 kDa) activities were higher in asthmatic patients (1). They were unable to detect the active form of MMP-9 (88 kDa) in any patient, but rationalized that the concentration of active MMP-9 was small compared with the pro-MMP-9 concentration. Their data suggested that

airway inflammation following an asthma exacerbation correlates with overproduction of MMP-9.

The consequence of increased MMP-9 expression following inhalation of cockroach proteases could be to aid in the migration of inflammatory cells. MMP-9 has been shown to be important for human polymorphonuclear neutrophil (PMN) and eosinophil migration across Matrigel-coated micropore membranes (23, 24). The transmigration of dendritic cells, an important antigen-presenting cell in asthma, was impaired in a MMP-9 knockout mouse. In addition, MMP-9 deficiency inhibited allergic airway inflammation characterized by a decrease in PMN and eosinophilic infiltration (25). In another study using MMP-9 knockout mice, lymphocyte and PMN infiltration was decreased following exposure to allergen (26). However, at least one study showed that neutrophil migration is not altered in MMP-9 null mice (27). Recent evidence suggests that pores exist within the basement membranes, and matrix degradation may not be as important as previously expected (28, 29). Additional studies are needed to clarify the importance of MMP-9 in cellular migration.

The MMP-9 could also play a role in the repair response in asthmatics. Some growth factors (such as platelet-derived growth factor, basic fibroblast growth factor and transforming growth factor) are stored in the extracellular matrix by being bound to proteoglycans (30, 31). It has been shown in the endobronchial biopsies of patients with asthma that MMP-9 immunoreactivity is localized in the extracellular matrix of the bronchial submucosa (32). In support of this, it has been shown that MMP-2, -3 and -7 have been shown to degrade decorin, a proteoglycan found in the extracellular matrix (33). It is possible that MMP-9-induced degradation of the collagen type IV in the basement membranes would release growth factors which could stimulate airway smooth muscle cell proliferation, a feature found in the asthmatic airway.

Our previous work has shown that serine proteases in cockroach whole body extract synergistically increased TNFα-induced IL-8 and IL-6 expression (6). IL-8 is a potent neutrophil chemoattractant, and can cause degranulation of recruited neutrophils. Van den Steen (20) has shown that MMP-9 derived from primary human neutrophils cleaves the amino terminus of IL-8 to increase its potency by more than 10-fold. It is conceivable that airway epithelial cells which encounter both cockroach proteases and a pro-inflammatory mediator such as TNFα. TNFα binds to a receptor and causes an intracellular signaling cascade which results in the increase of NF-κB activation. Cockroach proteases bind and cleave the PAR-2 receptor, which triggers its own intracellular signaling cascade resulting in activation of G-coupled protein activation, Ras, MEK (10), ERK and AP-1 (11). The end result is the increase in IL-8, IL-6 and MMP-9 secretion from human bronchial epithelial cells. Neutrophils will be attracted to the airways by the secretion of IL-8 and may also degranulate and release more MMP-9 into the airway. The amount of MMP-9 secretion from the human bronchial epithelial cell is minimal compared to what a neutrophil could secrete, but may play an important role in mediating the early stages of airway inflammation or repair.

Overall, our data suggest that the active serine proteases in cockroach frass play an important role modulating airway responses in the presence of an inflammatory stimulus, like TNFα. Inhalation of active proteases from cockroach frass, or from other sources, may elicit local inflammatory events in the airways, thus causing asthma exacerbations. Further understanding of the role of proteases in modulating airway remodeling and inflammation could result in novel therapeutic interventions for the treatment of asthma.

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