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Mycobacterium tuberculosis Up-Regulates Matrix Metalloproteinase-1 Secretion from Human Airway Epithelial Cells via a p38 MAPK Switch

Paul T. G. Elkington,* Jenny E. Emerson,* Laura D. C. Lopez-Pascua,* Cecilia M. O’Kane,* Donna E. Horncastle,† Joseph J. Boyle,† and Jon S. Friedland2*

Pulmonary cavitation is vital to the persistence and spread of Mycobacterium tuberculosis (MTb), but mechanisms underlying this lung destruction are poorly understood. Fibrillar type I collagen provides the lung’s tensile strength, and only matrix metalloproteinases (MMPs) can degrade it at neutral pH. We investigated MTb-infected lung tissue and found that airway epithelial cells adjacent to tuberculosis (Tb) granulomas expressed a high level of MMP-1 (interstitial collagenase). Conditioned media from MTb-infected monocytes (CoMTb) up-regulated epithelial cell MMP-1 promoter activity, gene expression, and secretion, whereas direct MTb infection did not. CoMTb concurrently suppressed tissue inhibitor of metalloprotease-1 (TIMP-1) secretion, further promoting matrix degradation, and in Tb patients very low TIMP-1 expression was detected. MMP-1 up-regulation required synergy between TNF-α and G protein-coupled receptor signaling pathways. CoMTb stimulated p38 MAPK phosphorylation, and this is the point of TNF-α synergy with G protein-coupled receptor activation. Furthermore, p38 phosphorylation was the switch up-regulating MMP-1 activity and decreasing TIMP-1 secretion. Activated p38 localized to MMP-1-secreting airway epithelial cells in Tb patients. These data reveal a monocyte-epithelial cell network whereby MTb may drive tissue destruction, and they demonstrate that p38 phosphorylation is a key regulatory point in the generation of a matrix-degrading phenotype. The Journal of Immunology, 2005, 175: 5333–5340.

M. tuberculosis (MTb)3 infects a third of the world’s population and kills 2 million people a year (1). Pulmonary cavitation is fundamental to MTb’s global success, because it creates an immunoprivileged site within which the bacteria can multiply and then spread to new hosts (2). Pulmonary extracellular matrix (ECM) turnover results in collagen accumulation with increasing age (3). MTb must subvert pulmonary matrix homeostasis and cause the degradation of structural collagen fibers to induce cavity formation. However, the cell types responsible for this tissue destruction and the signaling pathways that regulate it are poorly understood (4).

Fibrillar type I collagen provides the lung’s tensile strength (5) and is highly resistant to enzymatic degradation, whereas type III collagen predominates in alveolar walls (6). Matrix metalloproteinase (MMP)-1 (interstitial collagenase), MMP-8 (neutrophil collagenase), MMP-13 (collagenase-3), and MMP-14 (MT1-MMP) are unique in their ability to degrade type I collagen at neutral pH (7). MMPs are a family of zinc-dependent proteases that collectively can degrade all components of the ECM, but also have multiple other immunological functions, such as cytokine and chemokine processing, defense activation, and protease-activated receptor cleavage (7, 8). Increased MMP-1 expression has been demonstrated in destructive pulmonary pathologies in humans (9, 10), and mice that overexpress human MMP-1 develop spontaneous air space enlargement (11). Pulmonary epithelial cells express MMP-1 mRNA, secrete active enzyme (12, 13), and are a primary source of MMP-1 in emphysema (9).

MMP activity is tightly controlled at multiple levels: by gene transcription, by activation of proenzymes, by compartmentalization, and by secretion of specific inhibitors, the tissue inhibitors of metalloproteinase (TIMPs) (7). MMP-1 secretion is regulated in part by the PGE2-cAMP pathway (14, 15) and MAPK pathways (12, 15, 16). TIMPs are phosphorylation-dependent, signal-transducing enzymes that are involved in many facets of cellular regulation and immune responses (17, 18). The ERK MAPKs are primarily involved in cell division, whereas p38 MAPKs are activated by cytokines and ligands for G protein-coupled receptors (GPRC) and are implicated in diseases characterized by excess inflammation (17).

In tuberculosis (Tb), the primary effector cells of the immune response are monocytes/macrophages (19). After phagocytosing MTb, monocytes recruit inflammatory cells by the secretion of cytokines and chemokines (20). Key cytokines regulating a successful immune response include TNF-α (21), IL-1β (22), and IFN-γ (23). However, excessive cytokine secretion may lead to tissue damage and lung ECM breakdown, favoring bacterial persistence and facilitating mycobacterial transmission (2). Investigation of mechanisms underlying tissue damage in vivo is challenging, because standard models, which have been extremely useful in dissecting out specific aspects of immune responses, exhibit different pathologies in terms of tissue destruction. MTb-infected...
mice develop fibrotic as opposed to cavity pulmonary pathology (24, 25); guinea pigs rarely cavitate, whereas rabbits cavitate with Mycobacterium bovis Calmette-Guérin bacillus, which is a vaccine strain that seldom cavitates in man (25). Therefore, we focused on pulmonary immunopathology in a human cellular model combined with clinical studies.

We demonstrate that pulmonary epithelial cells in patients with active Tb express MMP-1. MtB up-regulates epithelial cell MMP-1 secretion via a monocyte-dependent network, and TIMP-1 secretion is concurrently suppressed. The monocyte-epithelial signal is transmitted by TNF-α synergy with a GPCR ligand, a process that is p38 MAPK phosphorylation dependent. Inhibition of p38 MAPK activity reverses the MMP-1 up-regulation and TIMP-1 suppression, and p38 phosphorylation is specifically demonstrated in epithelial cells adjacent to pulmonary Tb granulomas. Thus, MtB drives epithelial cell MMP secretion to favor matrix degradation, and p38 phosphorylation is a key switch.

Materials and Methods

MtB culture

MtB H37Rv Pasteur was cultured in Middlebrook 7H9 medium (BD Biosciences) with 10% ADC enrichment medium (BD Biosciences), 0.2% glycerol, 0.02% Tween 80, and 2.5 μg/ml amphotericin with agitation. MtB at mid-log growth phase at OD 0.60 (Biowave Cell Density Meter; WPA) was used in all experiments. The MtB endotoxin level was measured by the amebocyte lysate assay (Associates of Cape Cod) and was <0.3 ng/ml LPS.

Monocyte purification and infection

PBMCs were isolated from single donor buffy coats (National Blood Transfusion Service, U.K.) by density centrifugation (Ficoll Paque; Amersham Biosciences), and monocytes were adherence purified. Monocyte purity was >95% by FACS analysis. Monocytes were infected with MtB in RPMI 1640 with 2 mM glutamine and 10 μg/ml ampicillin. Cell culture medium was harvested at 24 h, and MtB was removed by filtration through a 0.2-μm pore size Anopore membrane (Whatman).

Epithelial cell culture

Normal human bronchial epithelial cells were cultured in bronchial epithelial growth medium according to the supplier’s instructions (Cambrex). All experiments were performed between passages 5 and 7. A549 cells were maintained in RPMI 1640 with 10% FCS, 2 mM glutamine, and 10 μg/ml ampicillin, and experiments were performed in serum-free medium. In direct infection experiments, MtB was removed by filtration through a 0.2-μm pore size Durapore membrane (Millipore).

Casein zymography

Twenty microliters of cell culture medium with 5% loading buffer (0.25 M Tris (pH 6.8), 50% glycerol, 5% SDS, and bromphenol blue) were run on casein gels (Invitrogen Life Technologies) at 125 V for 2 h (buffer: 25 mM Tris, 190 mM glycine, and 0.1% SDS). Gels were incubated in 2.5% Triton X for 1 h with agitation, followed by collagenase buffer (55 mM Tris base, 200 mM sodium chloride, 5 mM calcium chloride, and 0.02% Brij; pH 7.6) for 30 min. Gels were incubated for 40 h in fresh collagenase buffer at 37°C. Caseinolytic activity was revealed by 1-h staining in 0.1% Coomassie Blue (Pharmacia Biotech) and 1-h destaining in acetic acid/methanol/water (1/3/6). MMP-1 (Oncogene; 5 ng) was run on each gel to standardize between gels. Where stated, samples were concentrated 10-fold by lyophilization before zymography. Densitometric analysis was performed by digital image acquisition (UVP) and proteolytic band quantification with National Institutes of Health Image (version 1.61).

Western blotting

For MMP-1, 40 μl of cell culture medium with 1× loading buffer (10% glycerol, 5% 2-ME, 2% SDS, 0.06 M Tris (pH 6.8), and bromphenol blue) was heat denatured and run on a 10% acrylamide gel at 200 V (running buffer: 25 mM Tris base, 192 mM glycine, and 0.1% SDS) for 3 h. Gels were electrotransferred to a nitrocellulose membrane (Amersham Biosciences) and blocked for 1 h with 5% milk protein/0.1% Tween 20. The membrane was probed with anti-human MMP-1 Ab (The Binding Site; 1/1000 dilution) overnight, washed, and incubated with peroxidase-conju-

gate secondary Ab (The Binding Site) for 1 h. Luminescence was detected with the ECL system (Amersham Biosciences) according to the manufacturer’s protocol. For MAPK analysis, cells were lysed with 200 μl of SDS sample buffer (62.5 mM Tris (pH 6.8), 2% SDS, 10% glycerol, 50 mM DTT, and 0.01% bromphenol blue) and frozen at -80°C. Forty-microliter aliquots were processed as described above and probed with primary Ab (p38, phospho-p38, ERK, and phospho-ERK; Cell Signaling Technology), then with HRP-linked anti-rabbit secondary Ab.

Immunohistochemistry

Immunohistochemistry for MMP-1, TIMP-1, and p38 was performed on paraffin-embedded lung biopsies from six nonimmunosuppressed patients with culture-proven MtB infection and six noninfected controls. Sections of 4 μm thickness were dewaxed, and endogenous peroxidase activity was blocked with 0.6% hydrogen peroxide for 15 min. Sections were micro waved for 20 min in citrate buffer (0.01 M citrate; pH 6.0) and blocked with 5% normal goat serum for 10 min. The primary Abs (MMP-1 Ab-1 (Oncogene) at 1/100 dilution, TIMP-1 Ab-485 (Novacstra) at 1/400 dilution, and p38 (Sigma-Aldrich; clone M8177) at 1/200 dilution) were applied in 0.01 M PBS/azide/BSA for 1 h at room temperature. Ab was detected with the Menarini nonbiotinylated kit according to the manufacturer’s instructions. Peroxidase activity was developed with the 3,3′-diaminobenzidine system (Menarini). Slides were counterstained with Cole’s hematoxylin, dehydrated, and mounted. Ethical consent was obtained from the Hammersmith Hospitals research ethics committee for the use of archived lung biopsies.

RNase protection assay

Epithelial cells were lysed 24 h after stimulation using Tri-Reagent (Sigma-Aldrich), and total RNA was extracted. RNA levels were analyzed by RNase protection assay according to the manufacturer’s instructions (BD Biosciences; MMP-1 template kit) and were detected by the NorthSouth chemiluminescence kit (Pierce).

Promoter-reporter assay

A549 cells were seeded in 12-well plates at 160,000 cells/well in RPMI 1640 containing 2 mM glutamine, 10% FCS (Biowest), and 10 μg/ml ampicillin. Cells were cotransfected 24 h later in serum-free medium with 0.8 μg of MMP-1 promoter DNA inserted into the firefly luciferase expression plasmid PGL2-basic (gift from Dr. I. Clarke, University of East Anglia, East Anglia, U.K.) and 0.08 μg of control reporter plasmid, PRL-TK, constitutively expressing low level Renilla luciferase activity, suspended in 2.4 μl of FuGene 6 (Roche). Cells were stimulated after 12 h, and the luciferase activity of extracts was measured using the Dual-Luciferase Reporter Assay System (Promega) analyzed with a luminometer with Bio-Orbit 1253; Labtech International). Renilla luciferase activity was used to normalize firefly activity to control for transfection efficiency. A549 cells were used in these experiments, because the methodology does not transfect primary normal human bronchial epithelial cells (NHBE).

TNF-α measurement

Conditioned medium from MtB-infected monocytes (CoMtB) was analyzed using the BD Cyometric Bead Array assay (BD Biosciences) according to the manufacturer’s instructions on a FACS Calibur (BD Biosciences).

TIMP-1 and TIMP-2 ELISA

TIMP-1 and TIMP-2 levels in cell culture medium were measured by ELISA (R&D Systems) according to the manufacturer’s instructions. The lower level of sensitivity for TIMP-1 was <30 pg/ml, and that for TIMP-2 was <30 pg/ml.

Statistical analysis

Multiple intervention experiments were compared by one-way ANOVA, followed by Tukey’s multiple comparison test. Paired groups were compared by Student’s t test. A value of p < 0.05 was taken as statistically significant.

Results

Pulmonary epithelial cells express MMP-1 in patients with Tb

We first observed in patients with active, culture-proven MtB infection that airway epithelial cells adjacent to Tb granulomas express high levels of MMP-1 (Fig. 1, lower left panel). MMP-1 was
Densitometric analysis of caseinolytic bands demonstrated that MMP-1 activity secreted by CoMTb-stimulated cells was increased above that secreted by CoMCont-stimulated cells by 5.7-fold at 48 h, 7.5-fold at 72 h, and 11.0-fold at 96 h (Fig. 2C).

We next demonstrated that the MMP-1 response was dose-dependent (Fig. 3A). In contrast to the effect of CoMTb, direct infection by MTb did not alter baseline MMP-1 secretion by epithelial cells, whereas TNF-α (20 ng/ml) increased MMP-1 secretion (Fig. 3B). CoMTb up-regulated MMP-1 mRNA accumulation in epithelial cells at 24 h (Fig. 3C). mRNA accumulation was secondary to increased MMP-1 promoter activation. Transient transfection of A549 cells with the full-length MMP-1 promoter linked to a luciferase reporter demonstrated that CoMTb increased promoter activity above CoMCont levels by 2.8-fold at 12 h, 3.9-fold at 24 h, and 5.0-fold at 48 h (Fig. 3D).

CoMTb suppresses epithelial cell TIMP-1 secretion

Net MMP activity is determined by the MMP/TIMP ratio (26); therefore, we investigated whether MMP-1 up-regulation was compensated for by increased secretion of epithelial cell-derived TIMP-1 and -2 (27, 28). In contrast to the effects on MMP-1 activity, CoMTb suppressed TIMP-1 secretion to 57% of that by CoMCont-stimulated cells (Fig. 4A). TIMP-2 secretion did not differ between experimental and control stimuli (Fig. 4B). Furthermore, in TB-infected patients, we demonstrated that there was no increased TIMP-1 expression in respiratory epithelial cells adjacent to Tb granulomas. In uninfected lung tissue, epithelial cells expressed TIMP-1 at a low level (Fig. 4, row 1), and this tended to be suppressed in MTb infection (Fig. 4, row 2). The positive control (pilonidal sinus chronic granulomatous tissue) demonstrated high reactivity, excluding low test sensitivity obscuring TIMP-1 expression in Tb (Fig. 4, row 3).

CoMTb-dependent epithelial cell MMP-1 secretion is mediated via TNF-α and GPCR synergy

To dissect the monocyte-epithelial cell network, we first demonstrated that the mediators were heat labile (data not shown). We next investigated the potential roles of IL-1β, IFN-γ, and TNF-α, key cytokines in immunity to MTb (19). Anti-TNF-α Ab (2 μg/ml) suppressed epithelial cell MMP-1 secretion by 51%, but could not completely block up-regulation (Fig. 5A). IL-1β and IFN-γ were not involved in the network (data not shown). The TNF-α concentration in CoMTb measured by cytokine bead array was 156 ± 76 pg/ml, but TNF-α alone was a relatively weak stimulus of MMP-1 secretion, causing no up-regulation at 1 ng/ml (Fig. 5B). These data indicate that TNF-α synergizes with an additional factor(s) in CoMTb to stimulate MMP-1 secretion.
We next studied signaling through GPCRs, because in monocytes, G protein signaling pathways regulate MMP-9 secretion (29). Pertussis toxin, which inhibits GPCR signaling (30), suppressed CoMTb up-regulation of MMP-1 secretion by 85.3% at 1 ng/ml and by 100% at 10 ng/ml, without causing cell death, measured by trypan blue exclusion (Fig. 5C). This experiment was performed in growth factor-free medium, and samples were concentrated 10-fold by lyophilization before zymography.

Because anti-TNF-α and pertussis both individually inhibit MMP-1 secretion, we then studied epithelial cell stimulation using TNF-α/H9251 and cholera toxin (CT), which constitutively activates GPCRs by ADP-ribosylation (31), alone and in combination. CT had a minimal effect on MMP-1 secretion when used as a single stimulus (Fig. 5D). However, CT (1 μg/ml) in combination with TNF-α (1 ng/ml) caused 8.8-fold greater MMP-1 secretion than TNF-α alone (Fig. 5D). CT (10 μg/ml) did not drive MMP-1 secretion, indicating a bell-shaped dose-response curve. This reduced secretion was not due to cell death. These data demonstrate that CoMTb up-regulates MMP-1 secretion through TNF-α synergy with a GPCR ligand. Investigation of potential GPCR ligands secreted by MTb-infected monocytes, including CCL-2, CCL-3, CCL-5, CXCL-8, and CXCL-12, by stimulating NHBE with each cytokine alone and in combination with TNF-α did not identify the specific mediator in CoMTb (data not shown).
p38 MAPK phosphorylation is the site of TNF-α/H9251 synergy with CT. A, CoMTb stimulation causes p38 MAPK phosphorylation after 30 min. Representative data from an experiment performed on three separate occasions are shown. B, ERK is constitutively phosphorylated in NHBE. Representative data from an experiment performed on three separate occasions are shown. C, PG pathway inhibition does not reduce MMP-1 secretion. Epithelial cells were preincubated with indomethacin for 2 h, then stimulated with CoMTb. The mean ± SEM from a single experiment performed in triplicate are shown. D, CT and TNF-α synergize to cause p38 phosphorylation. A549 cells were stimulated with CoMTb, TNF-α (1 ng/ml), CT (1 μg/ml), or MTb supernatant, either alone or in combination. p38 phosphorylation was analyzed at 30 min. CT and TNF-α together caused equivalent p38 phosphorylation as CoMTb. Results from an experiment performed on two separate occasions are shown.
MAPK and PG pathways are key regulators of MMP-1 secretion (12, 15, 16) and CoMTb stimulation caused p38 phosphorylation at 30 min, returning to baseline at 120 min (Fig. 6A). CoMTb similarly drives p38 phosphorylation in the A549 respiratory epithelial cell line (data not shown). ERK1/2 was constitutively phosphorylated (Fig. 6B). PG-dependent pathways were not involved in the network, because inhibition of signal transduction by indomethacin did not block CoMTb-induced MMP-1 secretion by epithelial cells (Fig. 6C) or MMP-1 promoter activity (data not shown). Indomethacin suppressed MMP-1 secretion by stimulated macrophages, confirming the efficacy of PG pathway inhibition.

We proceeded to investigate whether p38 might be the point of convergence for the observed TNF-α/GPCR synergy. In A549 cells, TNF-α (1 ng/ml) caused p38 phosphorylation, but to a lesser extent than CoMTb (Fig. 6D, lanes 2 and 3). Tb supernatant, used to control for Tb Ags present in CoMTb, had no effect on p38 phosphorylation (Fig. 6D, lane 4). CT (1 μg/ml) had minimal effect on p38 phosphorylation (Fig. 6D, lane 5), but in combination with TNF-α caused p38 phosphorylation of a similar magnitude to that found with CoMTb (Fig. 6D, lane 7). Similar responses were demonstrated in NHBE, identifying that TNF-α/GPCR synergy drives p38 phosphorylation.

p38 MAPK phosphorylation is the switch between increased MMP-1 and decreased TIMP-1 secretion

Next, we used specific pharmacological inhibitors of the p38 and ERK MAPK pathways (32) to demonstrate that both are necessary for up-regulation of MMP-1 secretion by CoMTb. SB203580 (10 μM), a p38 pathway inhibitor, suppressed MMP-1 secretion after CoMTb stimulation by 67%, and 10 μM PD98059, an ERK pathway inhibitor, reduced MMP-1 secretion by 83% (Fig. 7A). Neither inhibitor reduced cell viability at these concentrations (data not shown). U0126, an alternative ERK pathway inhibitor, also inhibited MMP-1 secretion (data not shown). Reduced secretion was secondary to suppressed MMP-1 mRNA accumulation in CoMTb-stimulated cells (Fig. 7B).

In addition to reducing MMP-1 gene expression and secretion, p38 inhibition prevented the suppression of TIMP-1 secretion by CoMTb (Fig. 7C). In contrast, inhibition of the ERK pathway further reduced TIMP-1 secretion (Fig. 7C). Therefore, p38 phosphorylation switches the epithelial MMP/TIMP profile to a matrix-degrading phenotype by concurrently increasing MMP-1 secretion and suppressing TIMP-1 secretion. To confirm the in vivo relevance of our cellular model, we performed immunohistochemical analysis of lung biopsies from MTb-infected patients and demonstrated that airway epithelial cells express phosphorylated p38 at the site of tissue destruction (Fig. 7D).

Discussion

In this study we demonstrate that MTb drives increased MMP-1 and reduced TIMP-1 secretion from pulmonary epithelial cells via a monocyte-dependent network, and that p38 MAPK phosphorylation is a key signaling switch. Our initial observation was that epithelial cells adjacent to pulmonary Tb granulomas highly express MMP-1, whereas in uninfected lung tissue no MMP-1 expression was demonstrated. MMP-1 secretion is driven by an intercellular network that involves TNF-α synergy with a GPCR

![Figure 7](http://www.jimmunol.org/)
Excess MMP-1 activity is implicated in other destructive pulmonary pathologies, such as emphysema (10, 11), and epithelial cells have been identified as a source of this collagenase (9). Because epithelial cells make up 25% of the total lung cells (35), their protease secretion may significantly influence the breakdown of lung matrix. Furthermore, because communication between the airways and the granuloma allows exponential growth of MTb (36), epithelial cells may be specifically involved in the tissue destruction that links the airway to the site of infection and allows MTb to generate a relatively immunoprivileged site (2). It is interesting to note that the proposed mouse orthologue of MMP-1, McolA (37), has greatly reduced activity against type I collagen, whereas human MMP-1 performs the rate-limiting step in the degradation of type I collagen (33), the primary architectural collagen of the lung (5). Pulmonary cavitation is a complex process likely to involve multiple proteases in addition to MMP-1. Cross-linking fibrils must be cleaved to unmask major fibrils before their degradation (34). In addition, other collagenases, such as MMP-14, and elastases may contribute to cavitation. These proteases may be derived from cell types in addition to epithelial cells; for example, direct infection of macrophages with MTb up-regulates MMP-1 and -7 secretion (our unpublished observations).

We demonstrated a major role in this intracellular network driving MMP-1 activity for GPCRs and TNF-α, a key cytokine in the immune response to MTb (21, 38). Excess TNF-α activity is implicated in many of the immunopathological effects of MTb, including up-regulating MMP-9 secretion from monocytes (29). The role of GPCRs in MTb is less well defined. GPCRs transmit chemokine signaling, which is important in cell recruitment to the granuloma (20), but we were unable to identify chemokine involvement in this network despite an extensive search of likely candidates. Many predicted GPCRs have no identified ligands (39); therefore, the GPCR ligand may be an as yet unidentified mediator, a ligand of an orphan GPCR, or a microparticle, which have all been shown to up-regulate MMP expression (40).

PG signaling did not regulate MMP-1 secretion in epithelial cells, demonstrating divergent signaling compared with monocytes and fibroblasts (41, 42). However, it is more consistent with data from synoviocytes, in which PG exerts a down-regulatory effect on MMP-1 secretion (43). In the lung, PGs may perform differently from synoviocytes, in which PG exerts a down-regulatory effect on MMP-1 secretion (43). In the lung, PG signaling did not regulate MMP-1 secretion in epithelial cells, demonstrating divergent signaling compared with monocytes and fibroblasts (41, 42). However, it is more consistent with data from synoviocytes, in which PG exerts a down-regulatory effect on MMP-1 secretion (43). In the lung, PGs may perform differently from synoviocytes, in which PG exerts a down-regulatory effect on MMP-1 secretion (43).

We found a central role in this intracellular network driving MMP-1 activity for GPCRs and TNF-α, a key cytokine in the immune response to MTb (21, 38). Excess TNF-α activity is implicated in many of the immunopathological effects of MTb, including up-regulating MMP-9 secretion from monocytes (29). The role of GPCRs in MTb is less well defined. GPCRs transmit chemokine signaling, which is important in cell recruitment to the granuloma (20), but we were unable to identify chemokine involvement in this network despite an extensive search of likely candidates. Many predicted GPCRs have no identified ligands (39); therefore, the GPCR ligand may be an as yet unidentified mediator, a ligand of an orphan GPCR, or a microparticle, which have all been shown to up-regulate MMP expression (40).

PG signaling did not regulate MMP-1 secretion in epithelial cells, demonstrating divergent signaling compared with monocytes and fibroblasts (41, 42). However, it is more consistent with data from synoviocytes, in which PG exerts a down-regulatory effect on MMP-1 secretion (43). In the lung, PGs may perform differently from those at other sites, acting in an anti-inflammatory manner (44). The effects of the PG pathway on MMP-1 secretion appear to be cell and tissue specific.

We demonstrated that TNF-α- and GPCR-linked pathways combine to drive p38 phosphorylation. Although both are known to induce p38 phosphorylation as individual stimuli (17), this is the first report to demonstrate synergy between these two pathways at this level. In addition, p38 phosphorylation was the critical event in the switch between increased MMP-1 and decreased TIMP-1 secretion. p38 MAPK is a critical regulator of inflammation (47), and a regulatory role has been demonstrated in immune responses to other infectious diseases, including Leishmania (48) and Toxoplasma (49). However, a key role in regulating protease secretion in infectious disease has not been previously described. Because p38 MAPKs have been proposed as a pharmacological target in inflammatory disease (50), these data suggest another target by which p38 inhibition may reduce immunopathology.

In summary, our data demonstrate a novel mechanism by which MTb may drive pulmonary cavitation in the lung and thus create a niche within the host for its survival and transmission. MTb subverts the host immune response toward tissue destruction by driving epithelial cell MMP-1 secretion while suppressing the inhibitor, TIMP-1. The monocyte-epithelial cell network involved in regulating protease secretion in a GPCR ligand, and these signaling pathways converge at the level of p38 MAPK phosphorylation. Because p38 is the point of synergy between TNF-α and the GPCR ligand and regulates the switch to matrix degradation, it may represent a potential therapeutic target to inhibit immunopathology in Tb.

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Disclosures
The authors have no financial conflict of interest.

References


