Tuberculosis, Pulmonary Cavitation, and Matrix Metalloproteinases

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Abstract

Tuberculosis (TB), a chronic infectious disease of global importance, is facing the emergence of drug-resistant strains with few new drugs to treat the infection. Pulmonary cavitation, the hallmark of established disease, is associated with very high bacillary burden. Cavitation may lead to delayed sputum culture conversion, emergence of drug resistance, and transmission of the infection. The host immunological reaction to *Mycobacterium tuberculosis* is implicated in driving the development of TB cavities. TB is characterized by a matrix-degrading phenotype in which the activity of proteolytic matrix metalloproteinases (MMPs) is relatively unopposed by the specific tissue inhibitors of metalloproteinases. Proteases, in particular MMPs, secreted from monocyte-derived cells, neutrophils, and stromal cells, are involved in both cell recruitment and tissue damage and may cause cavitation. MMP activity is augmented by proinflammatory chemokines and cytokines, is tightly regulated by complex signaling paths, and causes matrix destruction. MMP concentrations are elevated in human TB and are closely associated with clinical and radiological markers of lung tissue destruction. Immunomodulatory therapies targeting MMPs in preclinical and clinical trials are potential adjuncts to TB treatment. Strategies targeting patients with cavitary TB have the potential to improve cure rates and reduce disease transmission.

Keywords: tuberculosis; cavity; matrix metalloproteinases; collagenases

Tuberculosis (TB) is one of the oldest diseases known to mankind and has been discovered in human remains more than 9,000 years old (1). It was declared a global health emergency by the World Health Organization more than 20 years ago but continues to be one of the world's leading killers, with 8.6 million new cases and 1.3 million deaths in 2012 (2). Ninety-five percent of TB cases arise from middle- and low-income countries, but affluent nations are increasingly affected (2). Drug resistance in TB is also on the rise, with the recent emergence of totally drug-resistant strains in patients (3). With the absence of effective drug treatment, these patients face the bleak prospect of either major surgery or death.

Pulmonary cavitation is the classic hallmark of TB and is the site of very high mycobacterial burden. Pulmonary cavitation is associated with antimycobacterial-drug resistance (4) and treatment failure (5). Patients with TB with pulmonary cavities are the principal source of disease transmission compared with those with noncavitary disease (6). For cavities to form, the lung parenchyma has to be destroyed, and proteases must degrade the complicated meshwork of fibrillar collagen that supports normal lung structure. The exact mechanisms resulting in pulmonary cavitation, which contributes to the global persistence of TB, are poorly understood. Understanding the pathogenesis of cavitation has the potential to yield novel strategies to decrease disease progression and spread. In this perspective, we consider emerging data in this area, focusing on pulmonary infection and the potential for therapeutically targeting tissue destruction in patients with TB to improve outcomes. Some of the data from our group and others have to date only been reported in the form of abstracts (7–11).

The Pathogenesis of TB Leading to Cavitation

Mycobacterium tuberculosis (*M.tb*) is transmitted through the aerosol route. The initial infectious droplet is deposited in the well-ventilated lower parts of the lung, where Ghon foci, representing calcified healed TB granulomas, are primarily

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observed on chest radiographs. Traditionally, it is believed that mycobacteria are first phagocytosed by alveolar macrophages (12), where *M.tb* persists in immature phagosomes by interfering with the acidification (13). After several days of infection, *M.tb* may escape into the cytosol, where they can replicate freely (14). Macrophage infection drives a localized inflammatory response recruiting mononuclear cells that contribute to host defense but may also provide new uninfected cells for mycobacterial growth (15). The inflammatory cells that die then form the caseous center of the granuloma, a feature that is pathognomonic of TB. With the arrival of lymphocytes, the granuloma acquires a more organized structure. The granuloma then becomes surrounded by lymphocytes that may be enclosed by fibroblasts demarcating the peripheral structure of the granuloma, which is proposed to contain the infection (16).

TB cavity formation occurs usually in the apices of the lungs or in the apical segment of the lower lobes. Consequently, within the TB life cycle, a dissemination event to the apices must occur from the initial deposition event in the lung bases. Once TB cavities form in the lung apex and connect with an airway, there is exponential growth of the *M.tb*, which ultimately results in the release of large quantities of viable infectious bacilli (17), which can spread to a new human host, completing the cycle (Figure 1). The mechanism of TB dissemination to the apices is unknown, but recent studies in the zebrafish suggest that infected monocytes may transport mycobacteria through the blood circulation (18). The presence of a blood-borne phase in TB is consistent with the distribution of lesions throughout the lungs and other organs observed in miliary TB and by the very occasional positive *M.tb* blood cultures in patients, particularly those coinfected with HIV.

Tuberculous cavity formation is classically described as "post-primary," occurring at a later time point to the initial "primary" infection. A critical event in the formation of the TB cavity has been proposed to be liquefaction, whereby a region of caseous necrosis subsequently liquefies (19) and develops into an environment for rapid bacterial proliferation (15). The region of caseous necrosis is lipid rich (20). However, the lipid contents of the caseous center do not have enzymatic activity and are unable to break down the triple helical structure of collagen, which provides the tensile strength of the lung. Therefore, build-up of lipid-rich necrotic material must be one component of a complex pathological process. These events may include accumulation of lipids, cell death, lysis of DNA from dying host cells, and extracellular matrix breakdown.

Human Lung Structure and the Implications for Development of Tuberculous Cavities

Collagen fibrils, specifically type I, III, and IV collagen, are the major structural components of the human lung (21, 22). Fibrillar type I and III collagen are highly resistant to enzymatic activity and can only be degraded by specific proteases. Although there have been extensive studies on the role of cholesterol, lipids, and cytokines in the process of caseous necrosis, these mediators do not have enzymatic activity to cleave collagen. Therefore, the process must involve hydrolytic enzymes, such as proteinases, nucleases, and lipases. The fundamental role of hydrolytic enzymes in TB pathology has been proposed for many years by Dannenberg from his extensive work on the rabbit model (19, 23). *M.tb* infection triggers recruitment and infection of leukocytes and the activation of intercellular networks, which then result in damage, with tissue destruction and cavitation. Multiple regulatory paths are activated during this process, with the end effectors being hydrolytic enzymes and, in particular, matrix metalloproteinases (MMPs).

What Are MMPs?

MMPs comprise 25 related but distinct members of a family of zinc-containing proteases, of which 24 are found in mammals. They share structural domains but differ in substrate specificity, cellular sources, and regulation. They are subclassified on the basis of substrate specificity, such as the collagenases, gelatinases, stromelysins, matrilysin, and elastase (24). MMPs have a flexible proline-rich hinge region and a carboxy (C)-terminal hemopexin-like domain, which functions in substrate recognition. These enzymes are responsible for the turnover, degradation, catabolism, and destruction of the extracellular matrix. MMPs are either secreted or anchored to the cell surface, where catalytic activity is confined to the immediate pericellular environment. Recently, it has been proposed that MMPs may also have intracellular activity and can modulate glycoprotein (IIB/IIIA) integrins (25).

The catalytic activity of MMPs is regulated at four main points: gene expression, compartmentalization (such as in the immediate pericellular environment), proenzyme (or zymogen) activation, and enzyme inactivation. MMP activity is further controlled by substrate availability and affinity (24). MMPs are also able to modulate cytokine and chemokine activity, such as cleaving and converting them into more potent variants or inactivating them, which consequently modulates inflammatory cell recruitment (24). MMPs have specific inhibitors, the tissue inhibitors of metalloproteinases (TIMPs), that bind noncovalently in a 1:1 manner to inactivate them (26). TIMPs may also be key in the development of fibrosis (27, 28), which is characteristic of healing TB infection (29).

MMPs in TB

After *M.tb* establishes infection, the local cellular organization of the surrounding tissues is modified to facilitate leukocyte infiltration and the initiation of granuloma formation. Multiple cellular and animal studies support the concept that MMPs play a role in the cellular recruitment, tissue remodelling, and destruction (30). Our group developed the concept of a matrix degrading phenotype developing in TB in which the activities of MMPs are unopposed by TIMPs (30). MMP activity has been implicated in driving TB pathology by numerous studies, many of which are listed in Table 1, indicating that MMPs have critical immunological and pathological roles.

Insights from Animal Models into the Role of Proteases

A variety of animal models have been studied to examine the pathogenesis of TB, including zebrafish, mice, guinea pigs, rabbits, and nonhuman primates. Each of these models contributes significantly

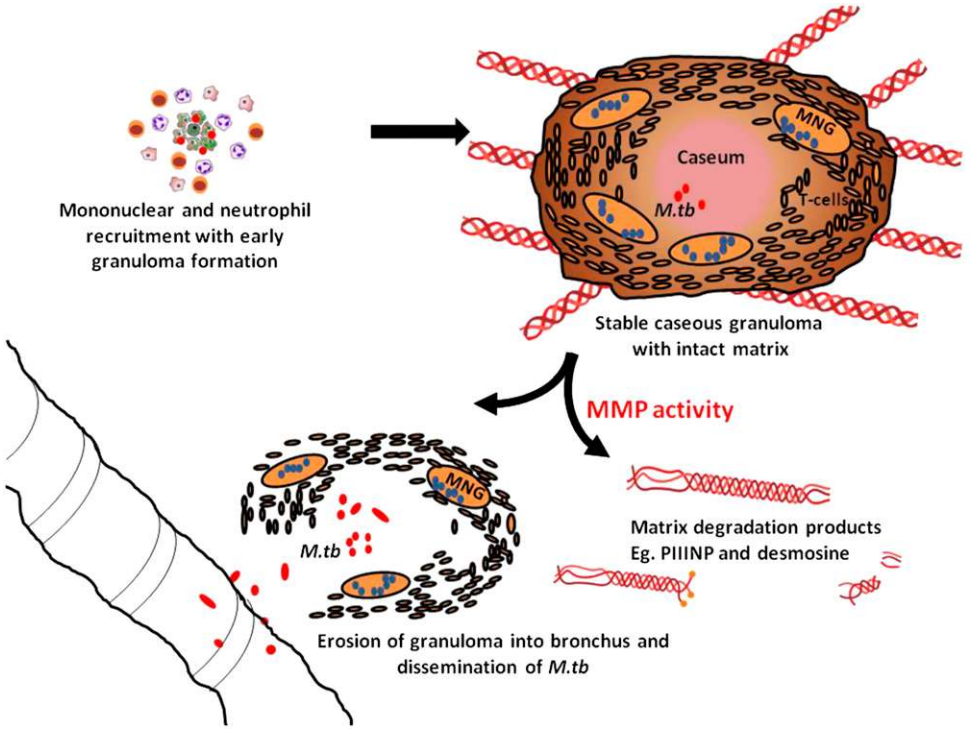

Figure 1. *Mycobacterium tuberculosis* (*M.tb*) causes granuloma formation and matrix destruction. *M.tb* infection of phagocytes causes secretion of chemokines with recruitment of macrophages, neutrophils, and T-lymphocytes, organizing themselves into the structure of a granuloma. Matrix metalloproteinase (MMP) activity results in matrix degradation with release of matrix degradation products and erosion of the granuloma into the bronchus leading to dissemination of *M.tb*. MNG = multinucleated giant cells; PIIINP = procollagen III N-terminal propeptide.

toward the understanding of pulmonary pathology. However, for a variety of reasons, none of these models completely replicate the immunopathology seen in humans (31), and therefore caution must be exercised in translating findings from one species to another.

A recent study on the zebrafish model of *Mycobacterium marinum*–induced granuloma formation revealed a crucial role of MMP-9. MMP-9 from epithelial cells, induced by the mycobacterial virulence factor ESAT-6, enhanced the recruitment of macrophages (18). This contributed to the growth of the granuloma as well as mycobacterial proliferation and underscores the potential of inhibiting MMP-9 to control the infection.

Mouse models demonstrate minimal delayed-type hypersensitivity response and do not form true caseous necrosis or cavities in response to *M.tb* (32). Consequently, the development of granulomatous necrosis has been used as a surrogate for cavity formation with *M.tb* infection (33). With this surrogate end point, a variety of host factors have been found to contribute to TB pathogenesis, including $CD3^+$, $CD4^+$ T cells, and IL-12. Humanized mice, engrafted with human hematopoietic stem cells with differentiated myeloid and lymphoid cells, contained necrotic granulomas with giant cells, human $CD68^+$ macrophages, and high bacilli numbers surrounded by a layer of $CD3^+$ T cells (34). $CD4^+$ T cells increased mycobacterial load and are associated with markers of immunosenescence (34), although the mice do not demonstrate any degree of cavitation (32). Other transgenic mice, such as those with deletions in CD4, interleukin 12 p40, IFN-γ, or αβ T-cell receptors, have less tissue necrosis compared with wild-type C57BL/6 mice, which indicates that these molecules may have a role in the process of tissue destruction that precedes cavitation (35, 36). Neutrophils are also central to TB pathology, predominating in necrotic granulomatous lesions in nitric oxide synthase–deficient mice (37), and their serine proteases contribute to control of infection by maintaining the integrity of tuberculous granulomas (38).

Recent data have implicated MMPs in TB pathogenesis in murine models. We demonstrated increased matrix destruction in *M.tb*-infected human MMP-1–expressing transgenic mice compared with wild-type mice, which do not express an ortholog of human MMP-1 (39). In these transgenic mice, MMP-1 is under regulation of the scavenger receptor A promoter-enhancer, and so the collagenase is only expressed by activated macrophages (40). In an independent study, the use of a broad-spectrum MMP inhibitor BB-94 decreased blood-borne *M.tb*, resulting in smaller granulomas with reduced leukocyte recruitment (41). The Izzo group has further demonstrated that MMP-9 is involved in macrophage recruitment and granuloma development in a murine model of TB (42), consistent with conclusions from the zebrafish model (18). In MMP-9–deficient mice, reduced lung macrophage recruitment with less well-formed granuloma formation was observed, and the bacterial burden in this model was decreased (42). Together, these murine studies implicate MMPs in the development of TB immunopathology.

Table 1. Matrix Metalloproteinases Implicated in Tuberculosis

Enzyme	Common Name	Proposed Substrates (Cleavage Demonstrated *In Vitro*)	MMPs Activated	References
Collagenases				
MMP-1	Collagenase-1 (interstitial collagenase, fibroblast collagenase)	Collagen I, II, III (III > I), VII, VIII, X, gelatin, aggrecan, versican, proteoglycan link protein, L-selectin, entactin, tenascin, serpins, α_2-macroglobulin, Latent TNF	MMP-2	20, 39, 51–54, 56–58, 60–62, 64, 66, 68, 69, 70, 88, 89
MMP-8	Collagenase-2 (neutrophil collagenase)	Collagen I, II, III (I > III), VII, VIII, X, gelatin, aggrecan, fibronectin, laminin, serpins, α_2-macroglobulin	ND	9, 60–62, 77, 90
MMP-13	Collagenase-3 (rat collagenase)	Collagen I, II, III (II > I or III), IV, IX, X, XIV, gelatin, aggrecan, perlecan, fibronectin, laminin, tenascin, fibrillin, serpins	MMP-2, -9	49
Gelatinases				
MMP-2	Gelatinase A (72-kDa gelatinase)	Gelatin, collagen I, IV, V, VII, X, XI, XIV, aggrecan, versican, proteoglycan link protein, fibronectin, laminin, laminin-5, fibrillin, elastin, vitronectin, α_2-macroglobulin, latent TNF	MMP-9, -13	20, 60, 61, 71
MMP-9	Gelatinase B (92-kDa gelatinase)	Gelatin, collagen IV, V, VII, X, XIV, aggrecan, versican, proteoglycan link protein, fibronectin, elastin, vitronectin, α_1-antitrypsin, α_2-macroglobulin, latent TNF, latent TGF-β_1, latent VEGF, fibrin, NG2 proteoglycan	ND	18, 20, 55, 57, 59, 62, 63, 73, 77, 89–95
Stromelysins				
MMP-3	Stromelysin-1 (Transin-1)	Collagen III, IV, V, IX, X, gelatin, versican, aggrecan, perlecan, fibronectin, laminin, tenascin, fibrillin, latent TGF-β	MMP-1, -8, -9, -13	39, 56, 57, 60, 96
MMP-10	Stromelysin-2	Collagen III, IV, V, gelatin, nidogen, aggrecan, fibronectin, elastin	MMP-1, -7, -8, -9	20, 65
Membrane-type MMPs				
MMP-14	MT1-MMP	Collagen I, II, III, gelatin, aggrecan, fibronectin, laminin, tenascin, vitronectin, fibrillin	MMP-2, -13	20, 49, 51
Other MMPs				
MMP-7	Matrilysin	Collagen IV, gelatin, aggrecan, fibronectin, laminin, elastin, vitronectin, pro-α defensins FAS ligand, latent TNF, syndecan-1, E-cadherin, elastin	MMP-2	51, 56
MMP-12	Metalloelastase	Collagen IV, gelatin, aggrecan, fibronectin, laminin, fibrillin, elastin, vitronectin, latent TNF, α_1-antitrypsin	ND	49

Definition of abbreviations: MMP = matrix metalloproteinase; ND = not determined; TGF = transforming growth factor; TNF = tumor necrosis factor; VEGF = vascular endothelial growth factor.
This list is not exhaustive, and the authors apologize for omissions. Proposed substrates are modified from Parks and colleagues (97), Kähäri and colleagues (98), Chandler and colleagues (99), and Ashworth and colleagues (100).

Guinea pig models of TB, in contrast to the mouse, develop a more complete delayed-type hypersensitivity response toward mycobacterial antigens. However, guinea pigs demonstrate a very high mortality to *M.tb* compared with humans, in whom at least 90% of exposed individuals will contain infection (43). The advantage of the model is that granuloma formation and dissemination of infection are more similar to humans (31). Subsequent to infection with *M.tb*, guinea pigs form multiple foci of caseous necrosis, with seeding to the apical lobes of the lung. *M.tb* then multiplies rapidly before entering a plateau phase. There is progressive lung parenchymal destruction, with occasional cavity formation (44) and eventual death. Proteases are implicated in the lung parenchymal destruction, with a study demonstrating that destructive forms of TB had fourfold increased elastolytic activity and activation of cysteine protease cathepsin B in guinea pigs infected with virulent H37Rv *M.tb* (45).

The spectrum of TB disease observed in patients is better replicated in rabbits, which are naturally resistant to *M.tb* infection but can demonstrate pulmonary cavitary lesions in established disease. Cavitation is, however, more pronounced in rabbits exposed to *Mycobacterium bovis* than to *M. tb*. Tuberculous cavitation was observed more than 65 years ago when rabbits were immunized with

heat-killed *M. bovis* and subsequently reinfected with large numbers of *M.tb* (46). Significant heterogeneity in phenotype of infected animals was reported, with inbred rabbits developing larger pulmonary tubercles than outbred rabbits (47). A number of proteolytic enzymes have been implicated in rabbit models of pulmonary TB. These include proteinase (cathepsin D) and nucleases that hydrolyze solid caseous material (19). Other hydrolytic enzymes, including β-galactosidase and acid phosphatase, were also found to be present in the macrophages that surround caseating and liquefied lesions by Converse and colleagues (48). More recently, the Kaplan group has reported on MMP activity in the rabbit model. Expression of the collagenases (MMP-1, -13, and -14), gelatinases (MMP-2 and -9), stromelysin (MMP-3), and elastase (MMP-12) was up-regulated in the lungs of TB-infected rabbits with destructive pathology, which was consistent with a role for MMPs in TB immunopathogenesis (49). There are also emerging data that MMP-1 is enzymatically active and predominantly increased in the wall of TB cavities compared with granulomatous tissues (11).

Nonhuman primates develop TB pathology most closely resembling that found in man (50). The granulomas of these primates contain a central area of caseation necrosis surrounded by epithelioid macrophages and multinucleated Langerhans giant cells. Large cavitary lesions with necrosis have been observed to surround the airways in regions of tuberculous pneumonia. A recent microarray study conducted in *M. tb*-infected macaques demonstrated that collagenase MMP-1 and -14, gelatinases MMP-2 and -9, and matrilysin MMP-7 were up-regulated 4 weeks after infection (51). However, 13 weeks after infection, these genes were down-regulated. The initial tissue destructive inflammation appears to be reversed with the reprogramming of the granulomatous response toward an antiinflammatory phenotype that limits tissue destruction, which may be associated with subsequent fibrosis and healing.

Taken together, these different animal models suggest an important role for proteases in pulmonary parenchymal destruction, which may then lead to cavity formation. Key players emerging from the study of cavitation in animal models are the MMPs and the cathepsin family of proteases. However, because TB is a disease of humans, it is necessary to undertake clinical investigation of patients to more fully understand the immunopathology that underlies cavity formation.

MMPs in Human TB

A variety of MMP promoter polymorphisms identified in diverse patient populations is consistent with a key role for MMPs in TB pathogenesis. For example, the 1G MMP-1 genotype, which up-regulates MMP-1 activity, was associated with endobronchial TB (52). The consequence of a combined monocyte chemotactic protein-1 (MCP-1) genotype GG, and the MMP-1 2G/2G genotype was higher expression of both MCP-1 and MMP-1, which increased the likelihood of developing pulmonary TB in bacillus Calmette-Guérin–vaccinated individuals up to 3.9-fold (53). Similarly, the MMP-1 (-1607G) gene polymorphism is a risk factor for fibrosis after pulmonary TB, with subjects having the 1G allele secreting higher levels of MMP-1 after stimulation with IL-1β (54). In addition, MMP-9 (-1562C/T) polymorphisms, which confer a lower promoter activity, have been implicated in the dissemination of *M.tb* (55).

Multiple MMPs are up-regulated in human TB, and this is supported by evidence from our group and others in both cellular models and patients. In cellular models, MMP-1 and -7 are expressed by macrophages in human granulomas, with *M.tb* being a more potent stimulus to human macrophage MMP-1 secretion than the vaccine bacillus Calmette-Guérin *in vitro* (56). Bronchial epithelial cells, which are numerous in the lung, express MMP-1, -3, and -9 in human TB (57–59). In humans, MMP-1 and -3 concentrations were increased in patients with TB compared with those with other respiratory symptoms (39), whereas MMP-1, -2, -3, -8, and -9 are raised in the induced sputum samples in patients with TB at diagnosis compared with healthy volunteers (60). The raised MMP-8 and -9 concentrations were closely associated with neutrophil markers, with MMP-8–expressing neutrophils found in the wall of TB cavities, suggesting a role of neutrophils in driving tissue destruction and cavitation in TB (7–9).

Patients with TB with more extensive tissue destructive disease on the chest radiograph had increased sputum MMP-1 concentrations compared with those with less tissue damage (61). In this study, we observed that MMP-1, -2, -3, and -8 were elevated in the induced sputum of patients with TB. However, in a subgroup analysis, concentrations were significantly lower in the induced sputum from patients with advanced HIV-TB coinfection than HIV-negative patients with TB (61). These data are consistent with the well-recognized fact that patients with TB with advanced HIV (CD4 T lymphocyte counts < 200 cells/μL) develop less tissue destruction, although they may have higher mycobacterial burden TB. This may suggest that a certain threshold level of protease activity is required to cause sufficient tissue destruction to lead to cavitation.

After commencement of TB treatment, the matrix-degrading phenotype resolves rapidly in patients with fully drug-sensitive pulmonary TB. MMP-1, -3, and -8 concentrations in sputum decrease markedly in the first 2 weeks of antimycobacterial treatment (60). Patients who remained culture positive at the second week had higher concentrations of MMP-2, -8, and -9 at diagnosis (60). The increased concentrations of multiple MMPs indicate that several proteases may contribute to tissue destruction and cavitation in TB, but their relative importance is currently undefined.

Other groups have similarly found MMPs to be up-regulated in human TB. Patients with TB pleuritis had higher concentrations of MMP-1, -8, and -9 in the pleural fluid compared with nontuberculous pleural effusions (62). TB meningitis, the most severe form of TB infection, is associated with increased MMP-9 concentrations and decreased with antituberculous treatment (63). MMP-1 was found to be an immunological correlate of delayed *M.tb* sputum conversion in African and Eurasian ancestry (64), whereas vitamin D, a recognized adjunctive therapy in human TB, suppressed MMP-7 and -9 secretion (65).

A downstream consequence of the increased MMP activity is increased tissue destruction and consequent increased release of matrix degradation products.

In patients with TB, procollagen III N-terminal peptide and desmosine, which are released during type III collagen and elastin degradation, respectively, were increased in sputum. Furthermore, plasma procollagen III N-terminal peptide concentrations correlated with both sputum MMP-1 concentrations and radiological tissue damage score (66), indicating that circulating matrix degradation products reflect lung destruction driven by dysregulated MMP activity.

Therefore, several MMPs are implicated in the pathogenesis of TB. The question arises as to why multiple enzymes are involved. Destruction of the matrix will involve several enzymes, to degrade not only the large structural fibrils, such as collagen- and elastin-cleaved, but also cross-linking fibrils and the cleavage products of the primary fibrils. Specific proteases may predominate at different points in disease pathogenesis. For example, MMP-1 may be more important in driving early macrophage-dependent collagen destruction, whereas MMP-8 (neutrophil collagenase) may predominate in advanced cavitary disease, where marked neutrophilic infiltration occurs (67). Defining such divergent roles will require the careful characterization of well-defined clinical cohorts.

The Regulation of MMP Expression in TB

Understanding the mechanisms regulating tissue destruction and cavitation in TB is critical to identify novel, more selective therapeutic targets. In *M.tb* infection, multiple intracellular signaling pathways are activated, which together drive MMP secretion (Figure 2). The p38 and extracellular signal–related kinase/mitogen-activated protein kinase (MAPK) pathways have been shown to be key in the regulation of MMP gene expression and secretion in multiple cell types, including leukocytes and stromal cells, as a consequence of both direct *M.tb* infection and activation of intercellular networks (58, 68, 69). The p38 MAPK pathway signals downstream to the cyclooxygenase pathway, with p38 activity increasing cyclooxygenase-2 accumulation, with subsequent prostaglandin (PG)E_2 and cAMP accumulation. cAMP accumulation results in the up-regulation of MMP-1 secretion (68).

The PI3-kinase pathway also regulates these MMPs (Figure 2). MMP-1 and -9 are up-regulated but MMP-3 suppressed after inhibition of the PI3-kinase p110α subunit (57), whereas downstream inhibition of Akt results in suppression of MMP-1, -3, and -9. Still further downstream the PI3-kinase pathway is the mammalian target of rapamycin (mTOR)/p70S6 kinase complex; inhibiting this suppressed MMP-1 and -3 but not MMP-9 secretion (57), indicating the intricacies that may be associated with a single pathway, which may make specific therapeutic interventions complex. The relative importance of MMPs derived from inflammatory cells and stromal cells has not been defined, and it may be that the principal sources of MMPs may change during the pathological process.

Transcriptional factors such as nuclear factor (NF)-κB, signal transducer and activator of transcription 3 (STAT 3), and activator protein-1 (AP-1) are principal regulators of MMP genes in TB (69, 70). Inhibition of NF-κB using chemical inhibitors resulted in increased MMP-2 secretion in microglial cell lines, indicating that NF-κB negatively regulates MMP-2 secretion in these cells, with the p65 subunit

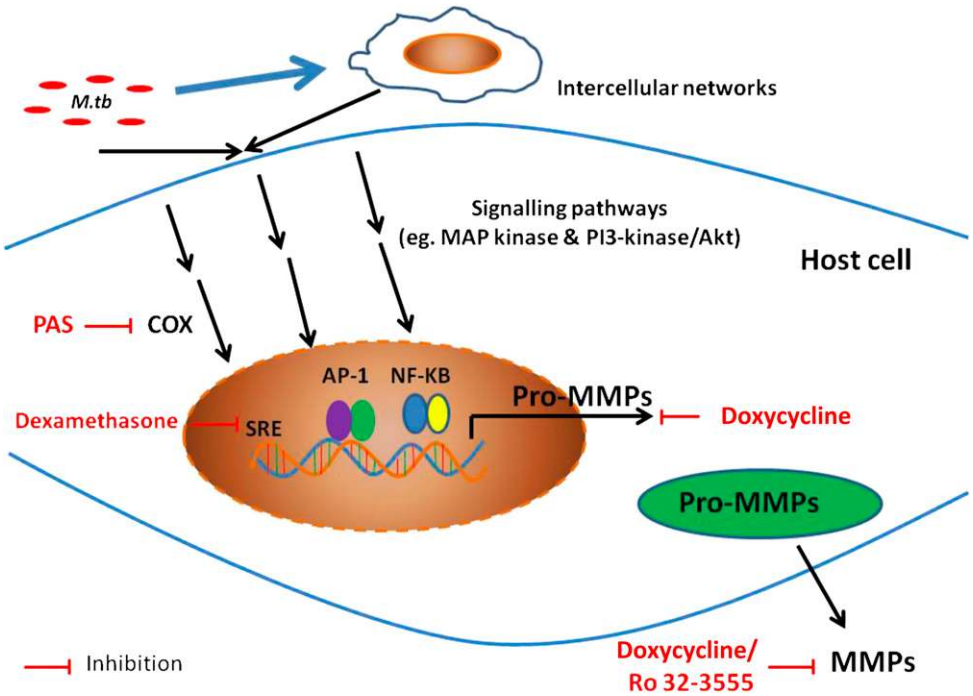

Figure 2. *Mycobacterium tuberculosis* (*M.tb*) drives matrix metalloproteinase (MMP) secretion by direct effects and intercellular networks. Secretion of MMP is regulated at multiple signaling pathways including the mitogen-activated protein (MAP) kinase and PI3-kinase/Akt pathway, and transcription factors nuclear factor (NF)-κB and AP-1. Doxycycline, Ro32-3555, dexamethasone, and P-aminosalicylic acid (PAS) act at indicated levels to inhibit MMP secretion. SRE = steroid response element.

expressed in biopsies of patients with central nervous system TB (71). NF-κB similarly regulates MMP-9 secretion in astrocytes (72). STAT-3 activation by phosphorylation is present in astrocytes in TB-associated cellular networks with IFN-γ potentiating STAT-3 activation (73). The inhibition of Jak, which is upstream of STAT, inhibited MMP-9 secretion, indicating that the JAK-STAT pathway regulates MMP secretion in astrocytes (73). Binding of AP-1 subunit nuclear translocation is up-regulated in microglial cells as well as in patient biopsy specimens (70), implicating a role in TB infection. Other novel intracellular signaling pathways have recently been identified to be regulators of MMP genes in other inflammatory conditions. For example, the AMP-activated protein kinase (AMPK) pathway was recently found to regulate MMP-2 gene expression (74). Preliminary data indicate AMPK regulates MMP secretion in neutrophils in TB (10) and may be another key regulator of MMPs because it regulates several downstream transcription factors such as Cry1, p300, and histone deacetylases (75).

Is There a Role for Immunotherapy in TB?

The emerging data outlined above demonstrate that MMPs are important components of the innate inflammatory response leading to tissue destruction in TB. The roles of MMPs in TB range from the physiological migration of leukocytes through to pulmonary cavitation, which facilitates replication and spread of infection. Because lung pathology occurs primarily due to the host response, there is a potential role for immunomodulatory therapy. So is there any evidence that modifying MMP activity has the potential to improve outcomes? P-aminosalicylic acid (PAS) has been used to treat TB for more than 60 years, yet we found that it has minimal effects on TB growth in culture. However, PAS did inhibit the accumulation of PGE_2 driven by *M.tb*, resulting in the suppression of MMP-1 secretion (68). These data suggest that PAS, currently a second-line treatment reserved for those with drug-resistant disease, may represent one of the earliest examples of the use of an immunomodulator to decrease tissue destruction in TB. Another established adjunctive therapy, dexamethasone, a corticosteroid, has proven mortality benefit in specific forms of TB particularly affecting the central nervous system (76). Dexamethasone was found only to decrease cerebrospinal fluid MMP-9 concentrations (77), while not affecting other cytokines or chemokines (78), although it was discovered recently to modulate tumor necrosis factor (TNF) levels in individuals dependent on their LTA4H genotype (79).

Interestingly, anti-TNF therapy has been used with success on patients with TB-HIV coinfection (80) and those who are refractory to steroids (81), although it is also known to reactivate TB (82), indicating a potential "Goldilocks phenomenon," where these host factors should fall within a margin and not in the extreme. *In vitro* studies indicate that one action of anti-TNF is to inhibit MMPs (8). However, the cost of anti-TNF treatment is currently prohibitive in resource-poor settings, although this may change if the rise in extensively drug-resistant TB continues at its current rate (2). The ideal immunomodulatory therapy needs to be efficacious; have a good safety profile with few or no side effects; not require frequent administration; and be affordable, easily available, and transportable in resource-poor countries where the largest TB burden lies.

Doxycycline is a tetracycline antibiotic and is the only MMP inhibitor licensed by the U.S. Food and Drug Administration in the United States. Doxycycline has been used in sub-antimicrobial doses to decrease the concentration of collagenases in periodontal disease (83). Its use has also been shown to improve pulmonary function in chronic obstructive pulmonary disease (84) and patients with asthma when added to standard therapies (85). We have demonstrated that doxycycline reduced MMP-1 and -3 secretion from human macrophages (61). Interestingly, doxycycline also inhibited *M.tb* growth *in vitro* as well as in guinea pigs (61). Consequently, doxycycline may have the potential to be a novel adjunctive agent to administer with standard therapy having two beneficial actions, both inhibiting MMP expression and limiting mycobacterial proliferation. It is cheap and widely available in resource-poor settings, although it does have a side-effect profile that prohibits use in children.

More tantalizingly, there are a range of other MMP inhibitors, such as batimastat and marimastat that had been previously developed for use in cancer (86), or Ro32-3555, trialed for rheumatoid arthritis, and these compounds should be reevaluated for the potential use in TB. One major issue of batimastat and marimastat was pronounced adverse effects on the musculoskeletal system, such as inflammatory arthritis, which may lead to nonadherence to the drugs, especially when the drugs were given for a prolonged duration (87). This may explain the lack of positive outcome in previous cancer trials. Specific anti-MMP drugs may thus merit further testing, especially in the field of infection, where the duration of drug treatment is considerably shorter. Inhibitors of the p38 MAPK pathways have also been trialed in prostate cancer, and the future availability of specific small molecules directed may permit specific targeting of individual signaling paths. With the emerging data implicating MMPs in TB pathogenesis, the time is right to move the inhibition of MMPs from the research laboratory into the clinic, while the search for specific switch points that may be future therapeutic targets continues.

Conclusions

Substantial evidence is accumulating from cellular, animal, and human studies demonstrating that the host inflammatory response drives tissue destruction leading to cavity formation in TB. This process is dependent on host proteases, and MMPs are key enzymes. Clinical studies have demonstrated that MMPs are increased in TB, driving the release of matrix degradation products, and are associated with disease severity. Multiple signaling pathways and transcription factors regulate MMP secretion from a variety of innate immune cells and may serve as potential therapeutic targets. Immunomodulatory therapy, specifically targeting MMP activity and signaling pathways, deserves evaluation as an adjunct to standard antituberculous therapy in this era of increasing drug resistance to antimycobacterial agents. ■

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