



SERIES “MATRIX METALLOPROTEINASES IN LUNG HEALTH AND DISEASE”

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Matrix metalloproteinases in tuberculosis

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ABSTRACT: Tuberculosis (TB) remains a global health pandemic. Infection is spread by the aerosol route and *Mycobacterium tuberculosis* must drive lung destruction to be transmitted to new hosts. Such inflammatory tissue damage is responsible for morbidity and mortality in patients. The underlying mechanisms of matrix destruction in TB remain poorly understood but consideration of the lung extracellular matrix predicts that matrix metalloproteinases (MMPs) will play a central role, owing to their unique ability to degrade fibrillar collagens and other matrix components. Since we proposed the concept of a matrix degrading phenotype in TB a decade ago, diverse data implicating MMPs as key mediators in TB pathology have accumulated. We review the lines of investigation that have indicated a critical role for MMPs in TB pathogenesis, consider regulatory pathways driving MMPs and propose that inhibition of MMP activity is a realistic goal as adjunctive therapy to limit immunopathology in TB.

KEYWORDS: Immunopathology, lung, matrix metalloproteinases, tuberculosis

EXTRACELLULAR MATRIX DESTRUCTION IS CRITICAL TO THE SUCCESS OF MYCOBACTERIUM TUBERCULOSIS

Tuberculosis (TB) was declared a global health emergency by the World Health Organization in 1994, but despite the resulting research and public health efforts, the annual number of cases is increasing [1]. Furthermore, the incidence of both multidrug-resistant and extensively drug-resistant TB continues to rise [2]. Standard TB treatment has remained unchanged since the early 1980s. Approximately one-third of the world's population is thought to be infected with the causative organism, *Mycobacterium tuberculosis* (Mtb) [3]. To achieve this extraordinarily high prevalence, Mtb must transmit very effectively. Lung extracellular matrix destruction is essential to TB transmission and consequently of critical importance to the global success of Mtb. The key consequences of such tissue destruction are morbidity and mortality in TB patients.

The life cycle of Mtb commences with the inhalation of infectious droplets expectorated by a patient with active pulmonary disease (fig. 1). These droplets are deposited in the well-ventilated lower sections of

the lungs. The initial immune response leads to the formation of a Ghon focus. The mechanisms whereby Mtb disseminates from the initial Ghon focus are not well understood, but studies in the zebrafish model of *M. marinum* infection suggest that Mtb is transported within infected monocytes [4]. Some patients develop active primary TB, resulting in disseminated miliary infection or TB meningitis, but these disease manifestations cause death of the host without transmission of Mtb. Mtb can only transmit when there is reactivation of infection in the lung, which usually occurs at the apices or in the apical segments of the lower lobes. The patient then develops a cough which then drives aerosol transmission. The mechanism whereby Mtb targets the lung apices is unknown, but once it reaches the lung interstitium, breakdown of the extracellular matrix must occur for the pathogen to disseminate *via* the airways. The most highly infectious patients are those who develop cavitary lung disease [1], since each cavity may contain up to 10⁹ mycobacteria [5], and these patients can be regarded as the aerosol supershedders that drive the global pandemic. Our group have demonstrated that specific patients are many times more infectious than others [6]. Cavities are often several

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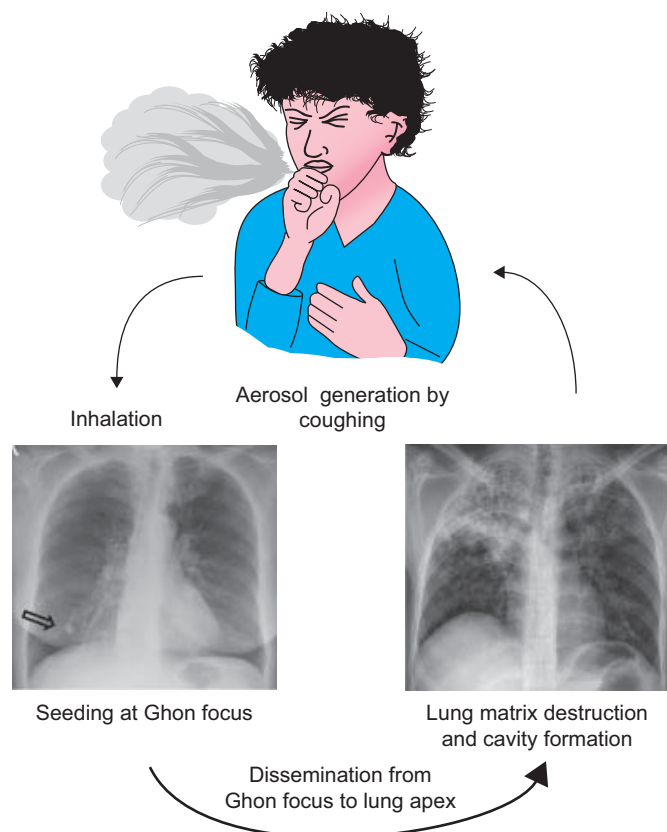


FIGURE 1. Lung matrix destruction is critical to the life cycle of *Mycobacterium tuberculosis* (Mtb). Mtb is spread by the aerosol route. After inhalation, infective droplets deposit at the lung bases, where the initial immune response forms the Ghon focus (arrow). Mtb then disseminates from the lung bases to the lung apices, probably haematogenously within infected monocytes. Once implanted at the apex, Mtb must engage the host immune response to drive matrix destruction, resulting in cavities within which it proliferates exponentially. The pathogen is then transmitted to new hosts by cough aerosol generation.

centimetres across, so there must be very extensive lung matrix destruction for such cavitation to occur.

Within the lung, Mtb is initially contained by granuloma formation, the characteristic host immune response to the pathogen [7]. The centre of the tuberculous granuloma may undergo caseous necrosis surrounded by activated macrophages, T-cells and fibroblasts. Caseous material is lipid-rich and derived from dead macrophages [8]. Although the classical paradigm primarily developed from the rabbit model of TB proposes that it is the rupture of caseous material into airways that leads to cavitation [9], *post mortem* studies in man suggest that cavitation starts in areas of lipid pneumonia as opposed to in association with well-formed granulomas [10]. In either case, Mtb must drive destruction of the lung extracellular matrix to spread from the interstitium to the airways and to cause cavity formation, which generates an immunodeficient site in which Mtb can proliferate [11]. Cavity formation must involve the action of proteases, specifically matrix metalloproteinases (MMPs), which are able to degrade the structural fibrils of the lung [12]. No lipid has been shown to have direct enzymatic activity.

The immunoprivileged nature of the TB cavity is revealed by clinical observations. For example, cavities can become colonised by *Aspergillus* to create a fungus ball after successful treatment for TB (fig. 2). *Aspergillus* species are environmental fungi which only cause invasive human disease when an individual has severe immunodeficiency, but even in the presence of a normal immune response, *Aspergillus* is able to colonise a pre-existing cavity. Similarly, opportunistic mycobacteria, such as *M. xenopi* and *M. avium intracellulare*, can colonise a pre-existing cavity, but Mtb is unique among mycobacteria in its ability to drive lung destruction in the face of an apparently normal immune response. Since the cavity is essentially shielded from the host immune response, it is a site where Mtb can proliferate exponentially [5]. Despite the very high bacterial load within cavities, patients can remain relatively well and continue to transmit infection for many months, or even years. In the pre-antibiotic era, there were reports of patients having fluctuating “consumption” (pulmonary TB) from age 20 to over 70 years [13], demonstrating that the formation of lung cavitation may allow the host and pathogen to achieve a degree of symbiosis that permits the pathogen to proliferate and spread while the host survives.

The lung extracellular matrix is supported by fibrillar collagens [14], which are highly resistant to enzymatic cleavage. Only MMPs can degrade these collagens at neutral pH [15], and, collectively, MMPs are able to degrade all components of the extracellular matrix. Although MMPs have been studied relatively extensively in other destructive pulmonary pathologies, such as emphysema [16], they have been comparatively overlooked in TB, despite the biochemical argument that MMP activity must be critical in driving lung matrix destruction. However, it is becoming increasingly clear that MMPs cause pathology in TB, both from studies specifically analysing MMPs in TB and also from microarray profiling studies that did not specifically aim to examine mechanisms of matrix destruction. Here, we bring together the evidence linking MMPs to TB immunopathology and build a picture of the key proteases driving extracellular matrix destruction in TB.

MMPs IN TB PATIENTS: THE FIRST STUDIES

Mtb is exclusively a pathogen of man, and no animal model replicates all features of the pathology seen in human disease [17]. Consequently, clinical investigation of patients with TB produces the most robust evidence implicating MMPs in lung matrix destruction, which can then be investigated further in model systems. The first description of increased MMP concentrations in human TB was a small study of bronchoalveolar lavage fluid (BALF) from two patients showing increased MMP-9 expression compared with controls [18]. MMP-9 is the most abundant and easily measured MMP by zymography [19]. Our group developed the concept of a matrix degrading phenotype in TB in which MMP activity was not balanced by the specific tissue inhibitors of metalloproteinases (TIMPs) [20]. Subsequently, a number of studies focusing on specific MMPs have been reported. For example, MMP-1, -2, -8 and -9 were found to be increased in pleural fluid of patients with TB compared to patients with heart failure [21–23]. Circulating MMP-9 concentrations correlated with disease severity [24]. MMP-9 concentrations are also increased in the cerebrospinal fluid of patients with TB meningitis [25] and we demonstrated

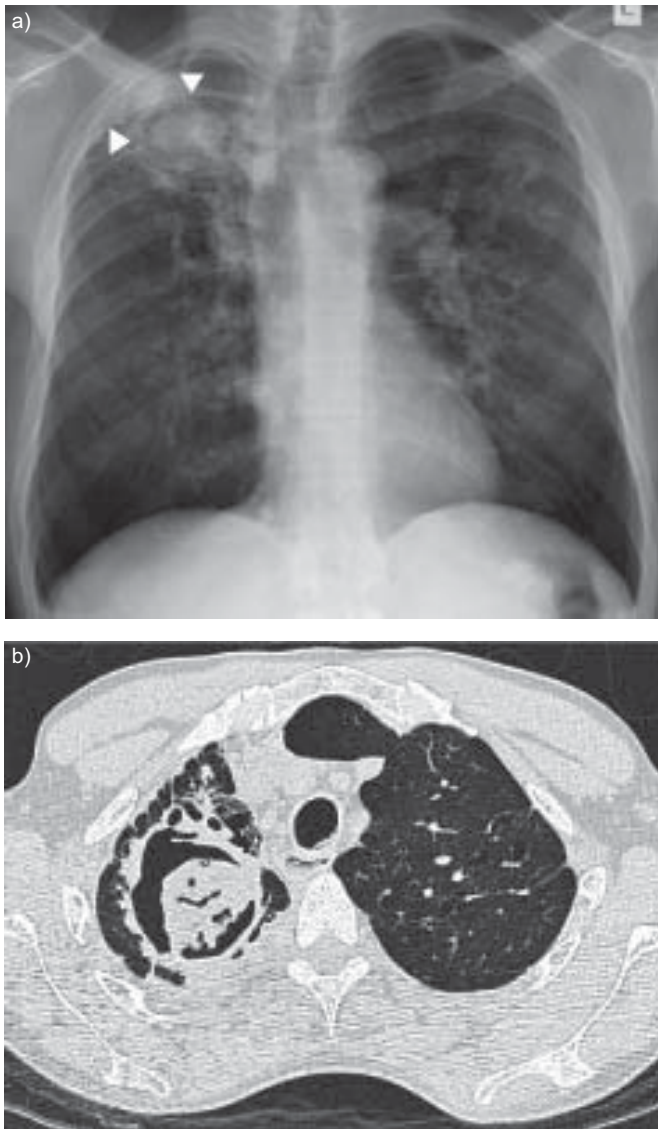


FIGURE 2. Pulmonary cavities are immunodeficient sites. After treatment for tuberculosis, residual cavities can be colonised by opportunist pathogens such as *Aspergillus*, which can form a fungus ball within the cavity (arrowheads on chest radiograph). The failure of the immune system to eradicate this low virulence saprophyte from within the cavity demonstrates the immunodeficient nature of the cavity.

that, in central nervous system (CNS) TB, MMP-9 concentrations correlated with extent of neurological compromise [20].

DEFINING THE MMPs INVOLVED IN THE PATHOGENESIS OF TB

Multiple studies have examined upregulation of MMPs in response to Mtb infection in cell culture systems. As early as the 1970s, it was shown that stimulation of guinea pig macrophages by mycobacterial extracts increases secretion of collagenase [26]. Stimulation of human THP-1 cells upregulates gene expression of MMP-1 and MMP-9 [18, 20, 27, 28]. This upregulation could be driven by lipomannans alone and was dependent on Toll-like receptor and CD14 signalling [29]. THP-1 cells are a widely used model of primary human monocytes and macrophages but they are derived from leukaemia cells [30] and

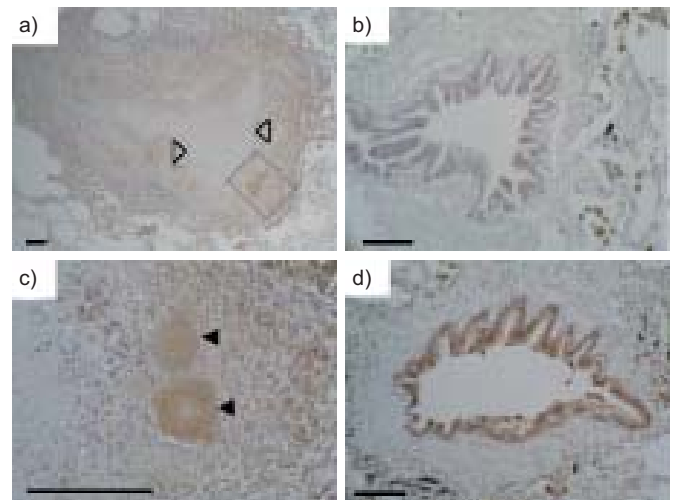


FIGURE 3. Matrix metalloproteinases (MMPs) are expressed in human tuberculosis (TB) granulomas and adjacent stromal cells. MMP-1 is expressed by epithelioid macrophages and multinucleate giant cells within human lung TB granulomas: a) a caseating TB granuloma, with central necrosis indicated by open arrowheads; c) the boxed area shown at high magnification. Arrowheads indicate Langhans' giant cells. MMP-1 is also expressed by distal epithelial cells (d), while minimal expression is demonstrated in the noninfected lung (b). Reproduced from [32, 35] with permission from the publishers.

consequently findings must be confirmed in primary human cells. In murine macrophages, direct mycobacterial infection also upregulates expression and secretion of MMP-9 [31]. Our group and collaborators undertook a systematic profiling of all known MMPs and the related A disintegrin and metalloproteinases (ADAMs) in primary human macrophages. Mtb was shown to upregulate MMP-1, -3, -7 and -10 [32]. Furthermore, Mtb was a more potent stimulus to MMP-1 secretion than the vaccine strain *M. bovis* bacille Calmette–Guérin (BCG), which does not cause lung cavitation, while upregulation of MMP-7 was equivalent. Similarly, in human peripheral blood mononuclear cells, MMP-1, -7 and -10 were induced by infection with Mtb [33]. Recently, we have demonstrated that MMP-1 and MMP-3 concentrations are increased in induced sputum and BALF of patients with TB compared to respiratory symptomatics under investigation for TB but with alternative final diagnoses [34], confirming that the cellular studies reflect MMP concentrations in the lungs of patients with TB.

Immunohistochemical studies have been used to localise MMP expression within TB granulomas. MMP-1 and -7 are expressed by epithelioid macrophages and Langhans giant cells in lung granulomas [32] and MMP-1 and MMP-9 are expressed by pulmonary epithelial cells (fig. 3) [35, 36]. In lymph node TB, both epithelioid macrophages and multinucleate giant cells express MMP-9 [37, 38]. In CNS TB, MMP-1, MMP-3 and MMP-9 expression has been demonstrated in cerebral TB granulomas [39, 40]. In addition, astrocytes are a significant source of MMP-9 [41].

MMPs are not constitutively expressed and consequently divergent gene expression may result in different disease phenotypes. MMP promoter polymorphism studies further implicate MMPs in TB pathogenesis. The 1G MMP-1 genotype was associated with developing endobronchial TB [42]. In

combination with a monocyte chemoattractant protein-1 genotype, the MMP-1 2G/2G genotype increased the likelihood of developing pulmonary TB in BCG-vaccinated individuals [43], leading the authors to propose a role for MMP-1 in TB-related tissue destruction. Similarly, the -1607G polymorphism increased the risk of severe fibrosis after treatment for TB [44]. MMP-9 polymorphisms did not differ between healthy controls and TB patients, but those TB patients with the -1562C/C genotype were more likely to develop multilobe involvement, implicating MMP-9 activity in dissemination of infection [45].

Animal models are necessary to dissect the mechanisms of tissue destruction in TB and to test the effect of new therapeutic approaches. In the mouse, *Mtb* infection upregulates MMP-9 expression [46, 47]. Broad-spectrum MMP inhibition reduces blood-borne *Mtb* and results in smaller granulomas with less leukocyte recruitment [48], and MMP-9 knockout mice have reduced cellular recruitment to the granuloma [46]. Administration of BB-94, an MMP inhibitor which also inhibits tumour necrosis factor- α converting enzyme (ADAM-17) led to a deviation in the immune response to a T-helper cell type 2 profile [49]. However, the mouse does not develop histopathological changes in the lungs that reflect disease in man. In man, alveolar wall thickening occurs but, in mice, alveolar wall thickening is observed [50]. Furthermore, mice do not express an orthologue of human MMP-1 in the lung [51, 52], and given that both human and primate studies suggest that this collagenase may be the dominant enzyme driving matrix destruction, the standard C57BL6 mouse model of TB may have limited use to dissect the role of MMPs in TB-driven immunopathology. Consistent with a central role for MMP-1 in TB-mediated tissue destruction, transgenic expression of human MMP-1 in mice leads to increased collagen destruction within TB granulomas [34].

MMP-9 has dominated the literature in terms of number of MMP-TB publications. However, this does not necessarily mean that it is the most important biologically or the best therapeutic target. The frequency of studies of MMP-9 in TB may reflect both its relative abundance and ease of detection by gelatin zymography [53]. Although MMP-9 regulates monocyte recruitment to the granuloma [54], it cannot degrade fibrillar collagens and so may not be the final effector of matrix destruction in pulmonary TB. MMP-1 is emerging as the primary collagenolytic MMP in TB, and therefore is most likely to be responsible for the destruction of extracellular matrix collagens.

MMPs AND GENE PROFILING IN TB

Transcriptomic approaches, which make no assumptions about the mediators involved in host defence to TB, have shown that MMPs are among the genes most upregulated in *Mtb* infection. For example, in a microarray analysis comparing macrophages from patients with pulmonary TB, TB meningitis and latently infected controls, MMP-1 emerges as the most highly divergently regulated gene, being 256-fold more strongly upregulated in patients with pulmonary TB compared to controls [55]. This implies that a tissue destructive innate immune response results in a greater risk of developing active TB, although this was not commented on by the authors. In an earlier gene expression profiling study, MMP-7, -9 and -14 were found to be upregulated at late time points after *Mtb* infection [56]. Thus,

transcriptomic approaches in cell culture systems are identifying a similar group of MMPs expressed in TB to those revealed in clinical studies.

Nonhuman primates develop TB pathology that reflects human disease most closely. In a recent microarray study of TB-infected macaques, multiple MMPs were upregulated 4 weeks after infection, with the collagenase MMP-1 being most highly upregulated and MMP-2, -7, -9, -14 and -25 also induced by infection [57]. However, at a later time point, these MMPs were downregulated, demonstrating that the initial pro-tissue destructive profile may be reversed as part of a global reprogramming of the granuloma response.

An interesting approach in man has been to combine the use of laser capture microdissection with microarray technology to identify genes upregulated in human TB-infected lung tissue [8]. Although the authors focused on lipid metabolism, since current paradigm proposes that the accumulation of lipids is the primary driver of pathology leading to cavity formation [58], multiple MMPs were upregulated by infection. MMP-1 (interstitial collagenase) was increased 606-fold, MMP-9 199-fold and expression of MMP-2, -10, and -14 were also significantly upregulated. In a microarray analysis of whole blood from patients with TB, MMP-9 was identified in an 86-transcript signature that distinguished active TB from other inflammatory and infectious diseases [59].

Studies of mycobacterial pathogenesis in the zebrafish permit manipulation of both the host and pathogen and also real-time imaging of infection. In a transcriptomic approach, virulent *M. marinum* was found to upregulate MMP-9, -13 and -14 more potently than an attenuated strain, even though the majority of genes were equally upregulated [60]. Again, this implicates MMP activity as being key in the pathogenesis of mycobacterial disease, though the dominant collagenase was MMP-13 as opposed to MMP-1 observed in mammalian studies. The zebrafish model has also demonstrated the functional role of MMP-9 in modulating cellular recruitment to the granuloma. If MMP-9 expression is suppressed, granulomas are smaller and dissemination is reduced (fig. 4) [54]. The pathogen-derived factor 6-kD early secreted antigenic target (ESAT-6) was found to drive MMP-9 secretion from epithelial cells, resulting in a

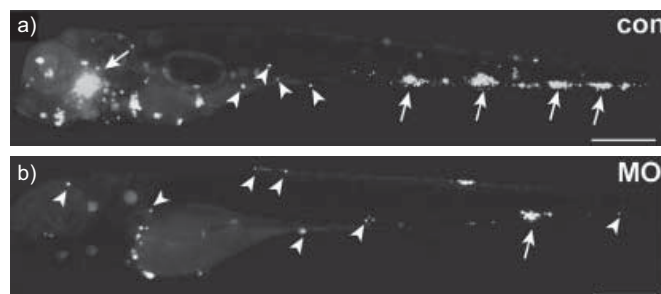


FIGURE 4. Mycobacterial infection is attenuated in matrix metalloproteinase (MMP)-9-deficient zebrafish. *Mycobacterium marinum* infection of zebrafish embryos permits visualisation of infection over time by fluorescent microscopy. In wild-type zebrafish (con), extensive dissemination of infection occurs (arrows: granulomas; arrowheads: single infected macrophages). However, in the MMP-9 morpholino (MO), where MMP-9 expression is suppressed, dissemination is significantly reduced. Reproduced from [54] with permission from the publisher.

migration gradient for monocytes which are then recruited to the granuloma and become infected. Therefore, nonselective approaches are independently implicating multiple MMPs in the immunopathology of TB. MMP-1 and -9 seem to be emerging as key MMPs in TB, consistent with data from more targeted approaches.

MMPs DRIVEN BY INTERCELLULAR NETWORKS

Directly infected cells are not the only source of MMPs in TB, and may not necessarily be the most important. Within the TB granuloma, there are often relatively few Mtb bacilli compared to the number of resident and influxing leukocytes. These leukocytes can also interact with stromal cells, whose importance in immune responses is frequently underestimated. Thus cell-cell interactions are likely to amplify immune responses from both immune cells and from stromal cells. Our early experiments showed that monocyte-monocyte networks increased the expression of MMP-9 [37]. Since stromal cells are more numerous in the lung and may express relatively greater levels of MMPs than monocytic cells [61], they represent a potentially important source of MMPs in TB. Monocyte-epithelial cell networks upregulate MMP-1 and -9 [35, 36], while monocyte-fibroblast networks upregulate MMP-1 and -3 [62, 63]. In our cellular model of TB meningitis, monocyte-astrocyte networks were found to upregulate MMP-1, -2, -3, -7 and -9 gene expression [41]. Secretion of MMP-9 was the most readily detected, though the secretion of the other MMPs may not have been detected due to the relative insensitivity of casein zymography to detect MMP-1, -3 and -7 [19]. More recently, investigating central nervous system TB, we have found that monocyte-microglial cell networks upregulate MMP-1 and -3 [39].

In such model systems, multiple pro-inflammatory mediators are responsible for driving MMP gene expression and some are necessary, but none are individually sufficient, for maximal MMP upregulation. Interleukin (IL)-1β, tumour necrosis factor-α and oncostatin M have been implicated in different studies driving increased MMP secretion, and G-protein coupled receptor signalling is also necessary for maximal MMP upregulation. In astrocytes, interferon-γ synergises with IL-1β to increase secreted MMP-9 concentrations [64], but the function of individual cytokines may be specific to the target cell. Furthermore, pathogen-derived factors seem to amplify the host immune response [36], and evidence from the zebrafish model demonstrates that ESAT-6 drives epithelial cell MMP-9 [54]. Since ESAT-6 is encoded in the RD1 region of Mtb, a key determinant of pathogenicity, this implies that Mtb deliberately induces host MMP-9 from stromal cells to subvert the host immune response to facilitate its dissemination [65]. There are multiple cellular sources of MMPs in TB driven by diverse mechanisms, both from immune cells and stromal cells (fig. 5). The relative contribution of directly infected cells and intercellular networks has not been fully dissected and requires extensive *in vivo* modelling supported by human investigations for its complete elucidation.

REGULATION OF MMP EXPRESSION

Regulation of MMP secretion is cell, tissue and stimulus dependent. In the context of Mtb, multiple cellular pathways are activated which together drive maximal MMP secretion.

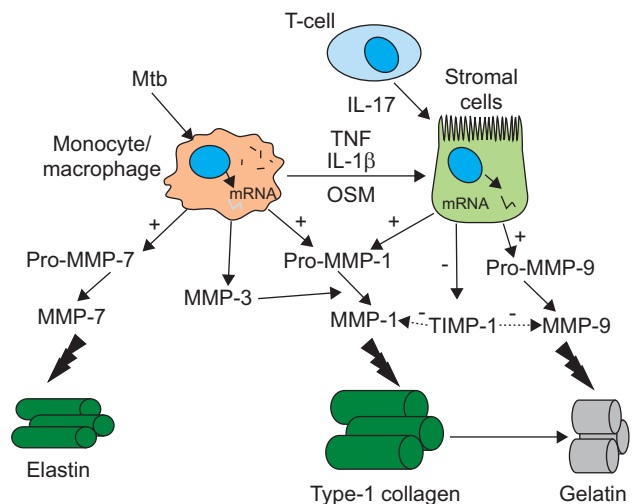


FIGURE 5. Intercellular networks drive matrix metalloproteinase (MMP) gene expression and secretion in tuberculosis (TB): a schematic representation of the intercellular signalling events that upregulate MMP secretion in TB. Direct infection of monocytes and macrophages induces gene expression and secretion of MMP-1, -3 and -7. In addition, pro-inflammatory cytokines, such as interleukin (IL)-1β, tumour necrosis factor (TNF)-α and Oncostatin M (OSM) increase MMP secretion from stromal cells such as epithelial cells, fibroblasts and astrocytes. No compensatory increase in secretion of the inhibitor tissue inhibitors of metalloproteinases (TIMPs) occurs. This matrix-degrading phenotype results in destruction of the extracellular matrix components, such as fibrillar collagens and elastin. Mtb: *Mycobacterium tuberculosis*.

Both the p38 and extracellular signal-related kinase/mitogen-activated protein kinase (MAPK) pathways are critical in regulating MMP secretion in multiple cell types, both as a result of direct infection and intercellular networks [35, 36, 63, 66, 67]. Although p38 MAPK pathway activation has multiple effects, including mRNA stabilisation, in primary human macrophages one critical downstream effector is the cyclooxygenase (COX) pathway. p38 activity increases COX-II accumulation, which leads to prostaglandin (PG)E₂ and cAMP accumulation, upregulating MMP-1 secretion [66]. It is interesting to note that p-amino salicylic acid (PAS), used to treat TB for over 60 yrs but with a poorly defined mechanism of action, is derived from the original COX inhibitor salicylic acid and only differs by the addition of an amino group [68]. PAS inhibits Mtb-driven PGE₂ accumulation and suppresses MMP-1 secretion, suggesting that, in part, it may act as an immunomodulator to reduce tissue destruction in TB [66]. Furthermore, it has recently been demonstrated that Mtb itself produces cAMP which accesses the host cell cytoplasm to subvert the immune response [69]. One action of this pathogen-derived cAMP may be to increase MMP secretion to drive the tissue destruction that is necessary for pathogen dissemination (fig. 6).

Key transcriptional regulators of MMPs in TB are the nuclear factor (NF)-κB, activator protein-1 and signal transducer and activator of transcription 3 signalling pathways [39, 63, 70]. Chromatin modification has also been implicated in MMP-9 upregulation by *M. avium* [70]. TIMP-1 lacks an NF-κB promoter binding site [71] and consequently NF-κB signalling may regulate the MMP/TIMP divergence which results in a matrix-degrading phenotype. In addition to the pro-inflammatory

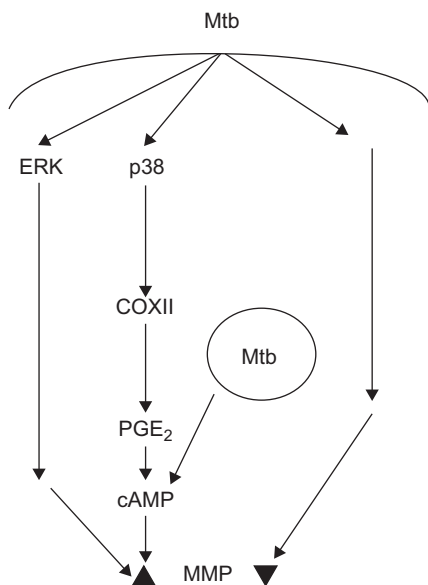


FIGURE 6. Intracellular signalling pathways regulating matrix metalloproteinase (MMP) activity in tuberculosis. Infection of primary human macrophages activates multiple signalling pathways, including the p38 and extracellular signal-related kinase (ERK) mitogen-activated protein kinase pathways, which upregulate MMP secretion. A dominant regulatory pathway is the p38/cyclooxygenase (COX)/prostaglandin (PG)_{E₂}/cAMP pathway, which upregulates MMP-1 gene expression and secretion in primary human macrophages. In addition, intracellular *Mycobacterium tuberculosis* (Mtb) secretes cAMP into the cytosol of macrophages, suggesting that it may subvert host immune responses to drive excessive MMP secretion to facilitate dissemination. Inhibitory pathways are likely to limit the pro-inflammatory phenotype but these have not yet been identified.

signalling, there are likely to be compensatory inhibitory pathways that have not yet been well characterised, which should be a priority for further cellular research. Potentially, Mtb may subvert the host immune response by inhibiting the brakes, in addition to activating the accelerators, of inflammation.

Although multiple pathways regulate MMPs in different cell types, it has not yet been established which pathway is dominant nor whether signalling can be effectively targeted therapeutically in the context of TB infection. Therapeutic inhibition of intracellular signalling pathways will have wide-ranging immunological effects, such as modulating the expression of pro- and anti-inflammatory cytokines. Consequently, the outcome *in vivo* is difficult to predict and the validity of this approach must be studied in appropriate model systems before translation into man.

MMPs AS THERAPEUTIC TARGETS TO LIMIT IMMUNOPATHOLOGY IN TB

TB continues to kill over 1.5 million people per yr but treatment has remained unchanged for 30 yrs [72]. Patients die from immunopathology resulting from excess inflammatory tissue destruction. We must understand this process more thoroughly to identify new therapeutic strategies. Immunomodulatory strategies that reduce pathology are currently limited to adjunctive corticosteroid treatment. Dexamethasone improves early outcomes in TB meningitis [73] and, we found, decreases MMP-9 concentrations early in TB treatment [74], despite

having relatively little effect on other pro-inflammatory mediators [75]. Steroids are also beneficial in the HIV–TB immune reconstitution syndrome [76]. However, steroids have multiple adverse immunological effects and may be dangerous in the context of drug-resistant TB [77]. TB-related pathology will result from several processes, including direct cellular toxicity caused by infection [78], T-cell driven pathology [7] and MMP activity causing matrix destruction. Although there has been substantial growth in our understanding of the biology of macrophage infection and T-cell responses in TB, this knowledge has not identified strategies to reduce immunopathology in TB. We propose that targeting MMP activity as the final common effector of pathology may generate interventions that are clinically useful in the era of drug-resistant TB [12].

The only licensed MMP inhibitor in the USA is doxycycline, which is used in patients at sub-antimicrobial doses to reduce collagenase activity in periodontal disease [79]. Accumulating evidence demonstrates that doxycycline can suppress immune-mediated tissue damage in a variety of inflammatory conditions, such as atherosclerosis [80, 81], arthritis [82–84], neurocysticercosis [85] and Gram-positive infections [86, 87]. Therefore, doxycycline represents a potential agent to globally suppress MMP activity in TB and is cheap, safe and readily available in resource-poor settings. More specific MMP inhibitors than doxycycline have been developed by the pharmaceutical industry, usually for noninfectious diseases. For example, Ro32-3555 is a potent collagenase inhibitor of proven safety in man [88]. Although a clinical trial in rheumatoid arthritis did not reduce joint symptoms, this does not prove that it will not have a beneficial effect in TB. Multiple other MMP inhibitors were developed as potential therapies for cancer [89], and now deserve re-evaluation as adjunctive agents to reduce immunopathology in TB.

CONCLUDING REMARKS AND FUTURE QUESTIONS

Consideration of the biochemistry of the extracellular matrix predicts that MMPs must be involved in TB-driven lung matrix destruction. Experimental data is accumulating, both from transcriptomic and hypothesis-driven approaches, implicating multiple MMPs but particularly MMP-1 and MMP-9 in TB pathogenesis. The questions which we believe require addressing are as follows.

- 1) Are MMP-1 and MMP-9 the dominant MMPs driving TB pathology?
- 2) How is the balance between MMPs and TIMPs regulated?
- 3) What are the signalling pathways that regulate MMP activity?
- 4) What are the best model systems to test the therapeutic effect of MMP inhibition?
- 5) Which patients might benefit from MMP inhibition to reduce immunopathology?
- 6) Is inhibition of specific MMPs, regulatory pathways or global MMP inhibition the best therapeutic approach?
- 7) Does MMP expression vary between different clinical and mycobacterial phenotypes?

In a recent review on novel conceptual approaches to TB vaccination, the primary risk of all five proposed strategies was collateral damage caused by an exaggerated immune response [90]. The mechanism underlying this immunopathology was not considered, but the final effector of tissue damage must involve MMP activity. Combining MMP inhibition with therapeutic vaccination to realign the host immune system to effectively control mycobacterial growth may permit disease control without causing immunopathology, achieving the goal of a post-exposure vaccine that could reduce the incidence of TB reactivation.

MMP inhibition studies are now required in appropriate model systems that develop matrix destruction similar to man as a prelude to clinical trials. Given the ongoing mortality of TB that results from immunopathology, such studies are a priority as they have the potential to translate directly to new treatments to limit morbidity and mortality and possibly open the route to therapeutic vaccination of latently infected individuals.

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STATEMENT OF INTEREST

None declared.

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