

## 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* (Mtb) 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 MMPs will play a central role

due 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**

Tuberculosis

Matrix metalloproteinases

Immunopathology

Lung

### **Extracellular matrix destruction is critical to the success of *Mycobacterium tuberculosis***

Tuberculosis (TB) was declared a global health emergency by the World Health Organisation in 1994, but despite the resulting research and public health efforts, the annual number of cases is increasing [1]. Furthermore, the incidence of both Multi-drug resistant (MDR) and Extensively drug resistant (XDR) TB continues to rise [2]. Standard TB treatment has remained unchanged since the early 1980's. 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  $10^9$  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 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 lipoid 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 for Mtb to proliferate [11]. Cavity formation must involve the action of proteases, specifically matrix metalloproteinases (MMPs), that 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 amongst 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 aged 20 to over 70 years old [13], demonstrating that the formation of lung cavitation may allow the host and pathogen to achieve a degree of symbiosis that permit 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 analyzing 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 ECM 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 broncho-alveolar lavage fluid (BALF) from 2 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 cerebro-spinal fluid (CSF) of patients with TB meningitis [25] and we demonstrated 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 tuberculosis**

Multiple studies have examined up-regulation of MMPs in response to Mtb infection in cell culture systems. As early as the 1970's, it was shown that stimulation of guinea pig macrophages by mycobacterial extracts increases secretion of collagenase [26]. Stimulation of human THP-1 cells up-regulates gene expression of MMP-1 and MMP-9 [18, 20, 27-28]. This up-regulation 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 consequently findings must be confirmed in primary human cells. In murine macrophages, direct mycobacterial infection also up-regulates 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 up-regulate MMP-1, -3, -7 and -10 [32]. Furthermore, Mtb was a more potent

stimulus to MMP-1 secretion than the vaccine strain *M. bovis* BCG, which does not cause lung cavitation, while up-regulation 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 (Elkington et al, *J Clin Invest* 2011 *In press*), confirming that the cellular studies reflect by 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 [34-35] (fig. 3). In lymph node TB, both epithelioid macrophages and multinucleate giant cells express MMP-9 [36-37]. In CNS TB, MMP-1, MMP-3 and MMP-9 expression has been demonstrated in cerebral TB granulomas [38-39]. In addition, astrocytes are a significant source of MMP-9 [40].

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 [41]. In combination with an MCP-1 genotype, the MMP-1 2G/2G genotype increased the likelihood of developing pulmonary TB in BCG-vaccinated individuals [42]. Similarly, the -1607G polymorphism increased the risk of severe fibrosis after treatment for TB [43]. 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 multi-lobe involvement, implicating MMP-9 activity in dissemination of infection [44].

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 up-regulates MMP-9 expression [45-46]. Broad-spectrum MMP inhibition reduces blood-borne Mtb and results in smaller granulomas with less leukocyte recruitment [47], and MMP-9 knock-out mice have reduced cellular recruitment to the granuloma [45]. Administration of BB-94, an MMP inhibitor which also inhibits TNF- $\alpha$  converting enzyme (TACE, ADAM-17) led to a deviation in the immune response to a TH<sub>2</sub> profile [48]. However, the mouse does not develop histopathological changes in the lungs that reflect disease in man. In man, alveolar septal destruction occurs, but in mice alveolar wall thickening is observed [49]. Furthermore, mice do not express an orthologue of human MMP-1 in the lung [50-51], 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 (Elkington et al, *J Clin Invest* 2011 *In press*).

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 [52]. Although MMP-9 regulates monocyte recruitment to the granuloma [53], 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 tuberculosis**

Transcriptomic approaches, which make no assumptions about the mediators involved in host defence to TB, have shown that MMPs are amongst the genes most up-regulated 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 up-regulated in patients with pulmonary TB compared to controls [54]. 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 up-regulated at late time points after Mtb infection [55]. Thus, transcriptomic approaches in cell culture systems are identifying a similar group of MMPs expressed in TB to those revealed in clinical studies.

Non-human primates develop TB pathology that reflects human disease most closely. In a recent microarray study of TB-infected macaques, multiple MMPs were up-regulated at 4 weeks after infection, with the collagenase MMP-1 being most highly up-regulated and MMP-2, -7, -9, -14 and -25 also induced by infection [56]. However, at a later time point, these MMPs were down-regulated, 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 up-regulated 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 [57], multiple MMPs were up-regulated 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 up-regulated. 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 [58].

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 up-regulated [59]. 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) [53]. 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 migration gradient for monocytes which are then recruited to the granuloma and become infected. Therefore, non-selective 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 leucocytes. 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 also from stromal cells. Our early experiments showed that monocyte-

monocyte networks increased the expression of MMP-9 [36]. Since stromal cells are more numerous in the lung and may express relatively greater levels of MMPs than monocytic cells [60], they represent a potentially important source of MMPs in TB. Monocyte-epithelial cell networks up-regulate MMP-1 and -9 [34-35], while monocyte-fibroblast networks up-regulate MMP-1 and -3 [61-62]. In our cellular model of TB meningitis, monocyte-astrocyte networks were found to up-regulate MMP-1, -2, -3, -7 and -9 gene expression [40]. 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 up-regulate MMP-1 and -3 [38].

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 up-regulation. IL-1 $\beta$ , TNF- $\alpha$  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 up-regulation. In astrocytes, IFN- $\gamma$  synergises with IL-1 $\beta$  to increase secreted MMP-9 concentrations [63], but the function of individual cytokines may be specific to the target cell. Furthermore, pathogen-derived factors seem to amplify the host immune response [35], and evidence from the zebrafish model demonstrates that ESAT-6 drives epithelial cell MMP-9 [53]. 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 [64]. 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 to elucidate fully.

## **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. Both the p38 and ERK MAPK pathways are critical in regulating MMP secretion in multiple cell types, both as a result of direct infection and intercellular networks [34-35, 62, 65-66]. 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 PGE<sub>2</sub> and cAMP accumulation, up-regulating MMP-1 secretion [65]. It is interesting to note that p-amino salicylic acid (PAS), used to treat TB for over 60 years 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 [67]. PAS inhibits Mtb-driven PGE<sub>2</sub> accumulation and suppresses MMP-1 secretion, suggesting that in part it may act as an immunomodulator to reduce tissue destruction in TB [65]. Furthermore, it has recently been demonstrated that Mtb itself produces cAMP which accesses the host cell cytoplasm to subvert the immune response [68]. 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 NFκB, AP-1 and STAT-3 signalling pathways [38, 62, 69]. Chromatin modification has also been implicated in MMP-9 up-regulation by *M. avium* [69]. TIMP-1 lacks an NFκB promoter binding site [70] and consequently NFκB signalling may regulate the MMP / TIMP divergence which results in a matrix-degrading phenotype. In addition to the pro-inflammatory signalling, there are likely to be compensatory inhibitory pathways which 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 and a half million people per year but treatment has remained unchanged for 30 years [71]. 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 [72] and we found decreases MMP-9 concentrations early in TB treatment [73] despite having relatively little effect on other pro-inflammatory mediators [74]. Steroids are also beneficial in the HIV-TB immune reconstitution syndrome [75]. However, steroids have multiple adverse immunological effects and may be dangerous in the context of drug-resistant TB [76]. TB-related pathology will result from several processes, including direct cellular toxicity caused by infection [77], 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 United States is doxycycline, which is used in patients at sub-antimicrobial doses to reduce collagenase activity in periodontal disease [78]. Accumulating evidence demonstrates that doxycycline can suppress immune-mediated tissue damage in a variety of inflammatory conditions, such as atherosclerosis [79-80], arthritis [81-83], neurocysticercosis [84], and gram-positive infections [85-86]. 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 non-infectious diseases. For example Ro32-3555 is a potent collagenase inhibitor of proven safety in man [87]. 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 [88], 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:

- Are MMP-1 and MMP-9 the dominant MMPs driving TB pathology?
- How is the balance between MMPs and TIMPs regulated?
- What are the signalling pathways that regulate MMP activity?

- What are the best model systems to test the therapeutic effect of MMP inhibition?
- Which patients might benefit from MMP inhibition to reduce immunopathology?
- Is inhibition of specific MMPs, regulatory pathways or global MMP inhibition the best therapeutic approach?
- 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 [89]. 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 which 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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## Figure Legends

**Figure 1. Lung matrix destruction is critical to the life cycle of 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.

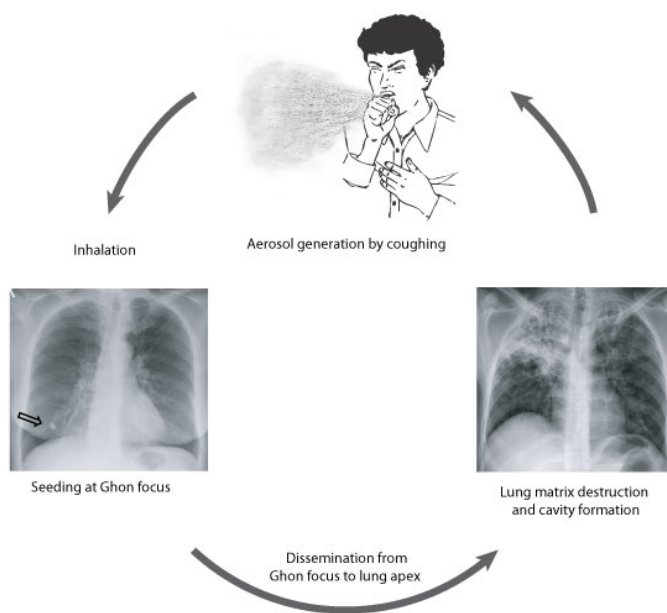


Figure 1

**Figure 2. Pulmonary cavities are immunodeficient sites.** After treatment for TB, 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.

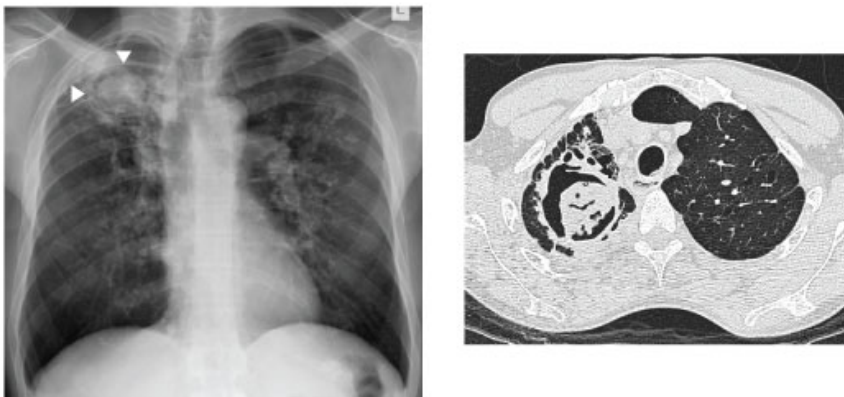


Figure 2

**Figure 3. MMPs are expressed in human TB granulomas and adjacent stromal cells.**

MMP-1 is expressed by epithelioid macrophages and multinucleate giant cells within human lung TB granulomas (A, B) (From [32] with permission). Panel A shows a caseating TB granuloma, with central necrosis indicated by open arrowheads, and the boxed area is shown at high magnification in panel B. Arrowheads indicate Langhans' giant cells. MMP-1 is also expressed by distal epithelial cells (D), while minimal expression is demonstrated in non-infected lung (C) (From [34] with permission).

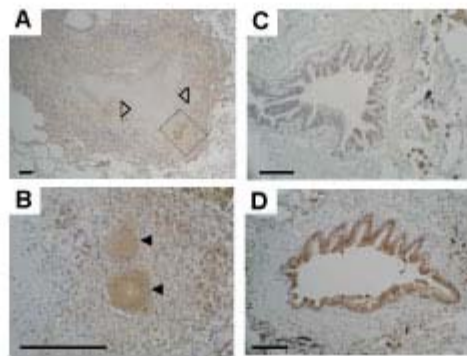


Figure 3

**Figure 4. Mycobacterial infection is attenuated in MMP-9 deficient zebrafish.** *M. 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 (From [53] with permission)

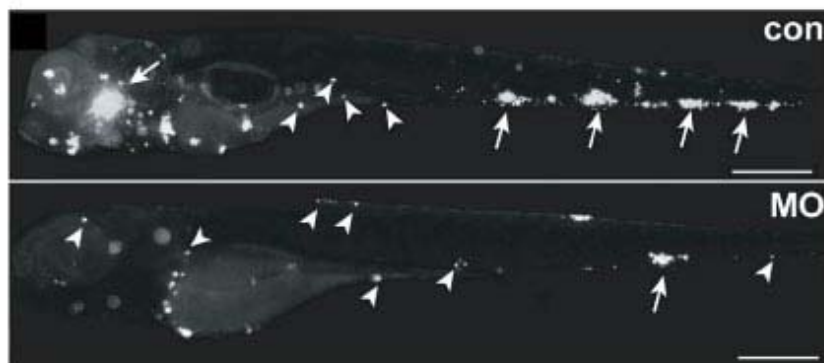


Figure 4

**Figure 5. Intercellular networks drive MMP gene expression and secretion in 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 IL-1 $\beta$ , TNF- $\alpha$  and Oncostatin M increase MMP secretion from stromal cells such as epithelial cells, fibroblasts and astrocytes. No compensatory increase in secretion of the inhibitor TIMPs occurs. This matrix-degrading phenotype results in destruction of the extracellular matrix, such as fibrillar collagens and elastin.

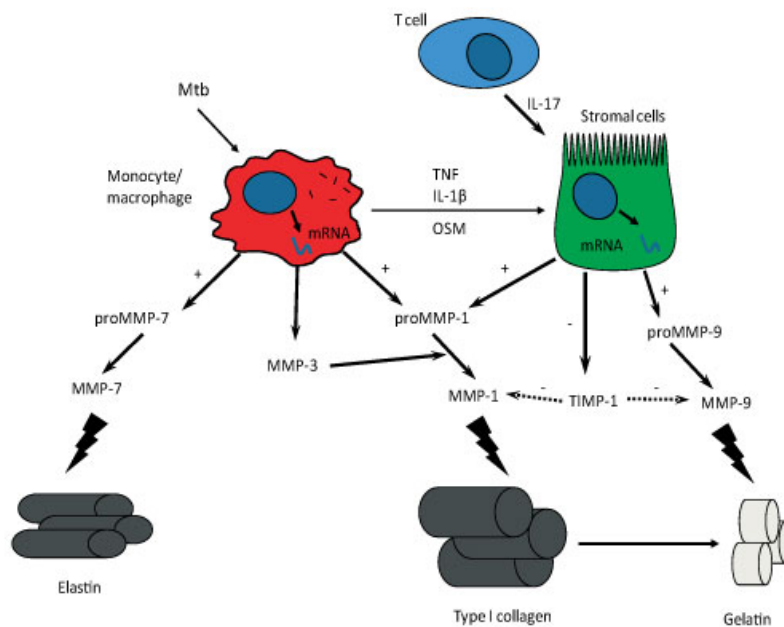


Figure 5

**Figure 6. Intracellular signalling pathways regulating MMP activity in TB.** Infection of primary human macrophages activates multiple signalling pathways, including the p38 and ERK mitogen activated protein kinase pathways, which up-regulate MMP secretion. A dominant regulatory pathway is the p38 – cyclooxygenase – PGE<sub>2</sub>-cAMP pathway, which up-regulates MMP-1 gene expression and secretion in primary human macrophages. In addition, intracellular 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.

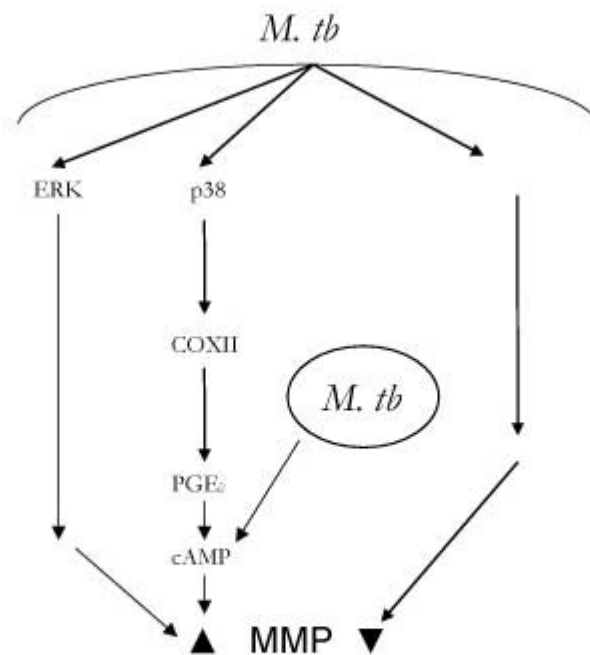


Figure 6

## References

1. Dye C, Williams BG. The population dynamics and control of tuberculosis. *Science* 2010; 328: 856-861.
2. Raviglione MC, Smith IM. XDR tuberculosis--implications for global public health. *N Engl J Med* 2007; 356: 656-659.
3. Dye C, Scheele S, Dolin P, Pathania V, Raviglione MC. Consensus statement. Global burden of tuberculosis: estimated incidence, prevalence, and mortality by country. WHO Global Surveillance and Monitoring Project. *JAMA* 1999; 282: 677-686.
4. Davis JM, Ramakrishnan L. The role of the granuloma in expansion and dissemination of early tuberculous infection. *Cell* 2009; 136: 37-49.
5. Helke KL, Mankowski JL, Manabe YC. Animal models of cavitation in pulmonary tuberculosis. *Tuberculosis (Edinb)* 2006; 86: 337-348.
6. Escombe AR, Moore DA, Gilman RH, Pan W, Navincopa M, Ticona E, Martinez C, Caviedes L, Sheen P, Gonzalez A, Noakes CJ, Friedland JS, Evans CA. The Infectiousness of Tuberculosis Patients Coinfected with HIV. *PLoS Med* 2008; 5: e188.
7. Cooper AM. Cell-mediated immune responses in tuberculosis. *Annu Rev Immunol* 2009; 27: 393-422.
8. Kim MJ, Wainwright HC, Locketz M, Bekker LG, Walther GB, Dittrich C, Visser A, Wang W, Hsu FF, Wiehart U, Tsenova L, Kaplan G, Russell DG. Cessation of human

tuberculosis granulomas correlates with elevated host lipid metabolism. *EMBO Mol Med* 2010; 2: 258-274.

9. Russell DG, Barry CE, 3rd, Flynn JL. Tuberculosis: what we don't know can, and does, hurt us. *Science* 2010; 328: 852-856.

10. Hunter RL, Jagannath C, Actor JK. Pathology of postprimary tuberculosis in humans and mice: contradiction of long-held beliefs. *Tuberculosis (Edinb)* 2007; 87: 267-278.

11. Kaplan G, Post FA, Moreira AL, Wainwright H, Kreiswirth BN, Tanverdi M, Mathema B, Ramaswamy SV, Walther G, Steyn LM, Barry CE, 3rd, Bekker LG.

*Mycobacterium tuberculosis* Growth at the Cavity Surface: a Microenvironment with Failed Immunity. *Infect Immun* 2003; 71: 7099-7108.

12. Elkington PT, D'Armiento JM, Friedland JS. Tuberculosis immunopathology: the neglected role of extracellular matrix destruction. *Sci Transl Med* 2011; 3: 71ps76.

13. Dubos R, Dubos J. The white plague : tuberculosis, man, and society. Rutgers University Press, New Brunswick ; London, 1987.

14. Davidson JM. Biochemistry and turnover of lung interstitium. *Eur Respir J* 1990; 3: 1048-1063.

15. Brinckerhoff CE, Matrisian LM. Matrix metalloproteinases: a tail of a frog that became a prince. *Nat Rev Mol Cell Biol* 2002; 3: 207-214.

16. Elkington PT, Friedland JS. Matrix metalloproteinases in destructive pulmonary pathology. *Thorax* 2006; 61: 259-266.

17. Young D. Animal models of tuberculosis. *Eur J Immunol* 2009; 39: 2011-2014.

18. Chang JC, Wysocki A, Tchou-Wong KM, Moskowitz N, Zhang Y, Rom WN. Effect of *Mycobacterium tuberculosis* and its components on macrophages and the release of matrix metalloproteinases. *Thorax* 1996; 51: 306-311.



19. Elkington PT, Green JA, Friedland JS. Analysis of matrix metalloproteinase secretion by macrophages. *Methods Mol Biol* 2009; 531: 253-265.
20. Price NM, Farrar J, Tran TT, Nguyen TH, Tran TH, Friedland JS. Identification of a matrix-degrading phenotype in human tuberculosis in vitro and in vivo. *J Immunol* 2001; 166: 4223-4230.
21. Hoheisel G, Sack U, Hui DS, Huse K, Chan KS, Chan KK, Hartwig K, Schuster E, Scholz GH, Schauer J. Occurrence of matrix metalloproteinases and tissue inhibitors of metalloproteinases in tuberculous pleuritis. *Tuberculosis (Edinb)* 2001; 81: 203-209.
22. Sheen P, O'Kane CM, Chaudhary K, Tovar M, Santillan C, Sosa J, Caviedes L, Gilman RH, Stamp G, Friedland JS. High MMP-9 activity characterises pleural tuberculosis correlating with granuloma formation. *Eur Respir J* 2009; 33: 134-141.
23. Park KJ, Hwang SC, Sheen SS, Oh YJ, Han JH, Lee KB. Expression of matrix metalloproteinase-9 in pleural effusions of tuberculosis and lung cancer. *Respiration* 2005; 72: 166-175.
24. Hrabec E, Strek M, Zieba M, Kwiatkowska S, Hrabec Z. Circulation level of matrix metalloproteinase-9 is correlated with disease severity in tuberculosis patients. *Int J Tuberc Lung Dis* 2002; 6: 713-719.
25. Matsuura E, Umehara F, Hashiguchi T, Fujimoto N, Okada Y, Osame M. Marked increase of matrix metalloproteinase 9 in cerebrospinal fluid of patients with fungal or tuberculous meningoencephalitis. *J Neurol Sci* 2000; 173: 45-52.
26. Wahl SM, Wahl LM, McCarthy JB, Chedid L, Mergenhagen SE. Macrophage activation by mycobacterial water soluble compounds and synthetic muramyl dipeptide. *J Immunol* 1979; 122: 2226-2231.

27. Friedland JS, Shaw TC, Price NM, Dayer JM. Differential regulation of MMP-1/9 and TIMP-1 secretion in human monocytic cells in response to Mycobacterium tuberculosis. *Matrix Biol* 2002; 21: 103-110.
28. Rivera-Marrero CA, Schuyler W, Roser S, Ritzenthaler JD, Newburn SA, Roman J. M. tuberculosis induction of matrix metalloproteinase-9: the role of mannose and receptor-mediated mechanisms. *Am J Physiol Lung Cell Mol Physiol* 2002; 282: L546-555.
29. Ellass E, Aubry L, Masson M, Denys A, Guerardel Y, Maes E, Legrand D, Mazurier J, Kremer L. Mycobacterial Lipomannan Induces Matrix Metalloproteinase-9 Expression in Human Macrophagic Cells through a Toll-Like Receptor 1 (TLR1)/TLR2- and CD14-Dependent Mechanism. *Infect Immun* 2005; 73: 7064-7068.
30. Tsuchiya S, Yamabe M, Yamaguchi Y, Kobayashi Y, Konno T, Tada K. Establishment and characterization of a human acute monocytic leukemia cell line (THP-1). *Int J Cancer*, 1980; pp. 171-176.
31. Quiding-Jarbrink M, Smith DA, Bancroft GJ. Production of matrix metalloproteinases in response to mycobacterial infection. *Infect Immun* 2001; 69: 5661-5670.
32. Elkington PT, Nuttall RK, Boyle JJ, O'Kane C M, Horncastle DE, Edwards DR, Friedland JS. Mycobacterium tuberculosis, but Not Vaccine BCG, Specifically Upregulates Matrix Metalloproteinase-1. *Am J Respir Crit Care Med* 2005; 172: 1596-1604.
33. Coussens A, Timms PM, Boucher BJ, Venton TR, Ashcroft AT, Skolimowska KH, Newton SM, Wilkinson KA, Davidson RN, Griffiths CJ, Wilkinson RJ, Martineau AR. 1alpha,25-dihydroxyvitamin D3 inhibits matrix metalloproteinases induced by Mycobacterium tuberculosis infection. *Immunology* 2009; 127: 539-548.
34. Elkington PT, Emerson JE, Lopez-Pascua LD, O'Kane C M, Horncastle DE, Boyle JJ, Friedland JS. Mycobacterium tuberculosis Up-Regulates Matrix Metalloproteinase-1

Secretion from Human Airway Epithelial Cells via a p38 MAPK Switch. *J Immunol* 2005; 175: 5333-5340.

35. Elkington PT, Green JA, Emerson JE, Lopez-Pascua LD, Boyle JJ, O'Kane C M, Friedland JS. Synergistic up-regulation of epithelial cell matrix metalloproteinase-9 secretion in tuberculosis. *Am J Respir Cell Mol Biol* 2007; 37: 431-437.

36. Price NM, Gilman RH, Uddin J, Recavarren S, Friedland JS. Unopposed Matrix Metalloproteinase-9 Expression in Human Tuberculous Granuloma and the Role of TNF- $\alpha$ -Dependent Monocyte Networks. *J Immunol* 2003; 171: 5579-5586.

37. Zhu XW, Price NM, Gilman RH, Recavarren S, Friedland JS. Multinucleate giant cells release functionally unopposed matrix metalloproteinase-9 in vitro and in vivo. *J Infect Dis* 2007; 196: 1076-1079.

38. Green JA, Elkington PT, Pennington CJ, Roncaroli F, Dholakia S, Moores RC, Bullen A, Porter JC, Agranoff D, Edwards DR, Friedland JS. Mycobacterium tuberculosis Upregulates Microglial Matrix Metalloproteinase-1 and -3 Expression and Secretion via NF- $\kappa$ B- and Activator Protein-1-Dependent Monocyte Networks. *J Immunol* 2010; 184: 6492-6503.

39. Gupta RK, Haris M, Husain N, Saksena S, Husain M, Rathore RK. DTI derived indices correlate with immunohistochemistry obtained matrix metalloproteinase (MMP-9) expression in cellular fraction of brain tuberculoma. *J Neurol Sci* 2008; 275: 78-85.

40. Harris JE, Nuttall RK, Elkington PT, Green JA, Horncastle DE, Graeber MB, Edwards DR, Friedland JS. Monocyte-astrocyte networks regulate matrix metalloproteinase gene expression and secretion in central nervous system tuberculosis in vitro and in vivo. *J Immunol* 2007; 178: 1199-1207.

41. Kuo HP, Wang YM, Wang CH, He CC, Lin SM, Lin HC, Liu CY, Huang KH, Hsieh LL, Huang CD. Matrix metalloproteinase-1 polymorphism in Taiwanese patients with endobronchial tuberculosis. *Tuberculosis (Edinb)* 2008; 88: 262-267.
42. Ganachari M, Ruiz-Morales JA, Gomez de la Torre Pretell JC, Dinh J, Granados J, Flores-Villanueva PO. Joint effect of MCP-1 genotype GG and MMP-1 genotype 2G/2G increases the likelihood of developing pulmonary tuberculosis in BCG-vaccinated individuals. *PLoS One* 2010; 5: e8881.
43. Wang CH, Lin HC, Lin SM, Huang CD, Liu CY, Huang KH, Hsieh LL, Chung KF, Kuo HP. MMP-1(-1607G) polymorphism as a risk factor for fibrosis after pulmonary tuberculosis in Taiwan. *Int J Tuberc Lung Dis* 2010; 14: 627-634.
44. Lee SH, Han SK, Shim YS, Yim JJ. Effect of matrix metalloproteinase-9 -1562C/T gene polymorphism on manifestations of pulmonary tuberculosis. *Tuberculosis (Edinb)* 2009; 89: 68-70.
45. Taylor JL, Hattle JM, Dreitz SA, Troudt JM, Izzo LS, Basaraba RJ, Orme IM, Matrisian LM, Izzo AA. Role for matrix metalloproteinase 9 in granuloma formation during pulmonary Mycobacterium tuberculosis infection. *Infect Immun* 2006; 74: 6135-6144.
46. Rivera-Marrero CA, Schuyler W, Roser S, Roman J. Induction of MMP-9 mediated gelatinolytic activity in human monocytic cells by cell wall components of Mycobacterium tuberculosis. *Microb Pathog* 2000; 29: 231-244.
47. Izzo AA, Izzo LS, Kasimos J, Majka S. A matrix metalloproteinase inhibitor promotes granuloma formation during the early phase of Mycobacterium tuberculosis pulmonary infection. *Tuberculosis (Edinb)* 2004; 84: 387-396.
48. Hernandez-Pando R, Orozco H, Arriaga K, Pavon L, Rook G. Treatment with BB-94, a broad spectrum inhibitor of zinc-dependent metalloproteinases, causes deviation of the

cytokine profile towards type-2 in experimental pulmonary tuberculosis in Balb/c mice. *Int J Exp Pathol* 2000; 81: 199-209.

49. North RJ, Jung YJ. Immunity to tuberculosis. *Annu Rev Immunol* 2004; 22: 599-623.

50. Nuttall RK, Sampieri CL, Pennington CJ, Gill SE, Schultz GA, Edwards DR. Expression analysis of the entire MMP and TIMP gene families during mouse tissue development. *FEBS Lett* 2004; 563: 129-134.

51. Balbin M, Fueyo A, Knauper V, Lopez JM, Alvarez J, Sanchez LM, Quesada V, Bordallo J, Murphy G, Lopez-Otin C. Identification and enzymatic characterization of two diverging murine counterparts of human interstitial collagenase (MMP-1) expressed at sites of embryo implantation. *J Biol Chem* 2001; 276: 10253-10262.

52. Leber TM, Balkwill FR. Zymography: a single-step staining method for quantitation of proteolytic activity on substrate gels. *Anal Biochem* 1997; 249: 24-28.

53. Volkman HE, Pozos TC, Zheng J, Davis JM, Rawls JF, Ramakrishnan L. Tuberculous granuloma induction via interaction of a bacterial secreted protein with host epithelium. *Science* 2010; 327: 466-469.

54. Thuong NT, Dunstan SJ, Chau TT, Thorsson V, Simmons CP, Quyen NT, Thwaites GE, Thi Ngoc Lan N, Hibberd M, Teo YY, Seielstad M, Aderem A, Farrar JJ, Hawn TR. Identification of tuberculosis susceptibility genes with human macrophage gene expression profiles. *PLoS Pathog* 2008; 4: e1000229.

55. Volpe E, Cappelli G, Grassi M, Martino A, Serafino A, Colizzi V, Sanarico N, Mariani F. Gene expression profiling of human macrophages at late time of infection with *Mycobacterium tuberculosis*. *Immunology* 2006; 118: 449-460.

56. Mehra S, Pahar B, Dutta NK, Conerly CN, Philippi-Falkenstein K, Alvarez X, Kaushal D. Transcriptional reprogramming in nonhuman primate (rhesus macaque) tuberculosis granulomas. *PLoS ONE* 2010; 5: e12266.

57. Russell DG, VanderVen BC, Lee W, Abramovitch RB, Kim MJ, Homolka S, Niemann S, Rohde KH. Mycobacterium tuberculosis wears what it eats. *Cell Host Microbe* 2010; 8: 68-76.
58. Berry MP, Graham CM, McNab FW, Xu Z, Bloch SA, Oni T, Wilkinson KA, Banchereau R, Skinner J, Wilkinson RJ, Quinn C, Blankenship D, Dhawan R, Cush JJ, Mejias A, Ramilo O, Kon OM, Pascual V, Banchereau J, Chaussabel D, O'Garra A. An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. *Nature* 2010; 466: 973-977.
59. van der Sar AM, Spaink HP, Zakrzewska A, Bitter W, Meijer AH. Specificity of the zebrafish host transcriptome response to acute and chronic mycobacterial infection and the role of innate and adaptive immune components. *Mol Immunol* 2009; 46: 2317-2332.
60. Cury JD, Campbell EJ, Lazarus CJ, Albin RJ, Welgus HG. Selective up-regulation of human alveolar macrophage collagenase production by lipopolysaccharide and comparison to collagenase production by fibroblasts. *J Immunol* 1988; 141: 4306-4312.
61. O'Kane CM, Elkington PT, Friedland JS. Monocyte-dependent oncostatin M and TNF-alpha synergize to stimulate unopposed matrix metalloproteinase-1/3 secretion from human lung fibroblasts in tuberculosis. *Eur J Immunol* 2008; 38: 1321-1330.
62. O'Kane CM, Elkington PT, Jones MD, Caviedes L, Tovar M, Gilman RH, Stamp G, Friedland JS. STAT3, p38 MAPK, and NF-kappaB drive unopposed monocyte-dependent fibroblast MMP-1 secretion in tuberculosis. *Am J Respir Cell Mol Biol* 2010; 43: 465-474.
63. Harris JE, Fernandez-Vilaseca M, Elkington PT, Horncastle DE, Graeber MB, Friedland JS. IFNgamma synergizes with IL-1beta to up-regulate MMP-9 secretion in a cellular model of central nervous system tuberculosis. *Faseb J* 2007; 21: 356-365.
64. Agarwal N, Bishai WR. Subversion from the sidelines. *Science* 2010; 327: 417-418.

65. Rand L, Green JA, Saraiva L, Friedland JS, Elkington PT. Matrix metalloproteinase-1 is regulated in tuberculosis by a p38 MAPK-dependent, p-aminosalicylic acid-sensitive signaling cascade. *J Immunol* 2009; 182: 5865-5872.
66. Harris JE, Green JA, Elkington PT, Friedland JS. Monocytes infected with *Mycobacterium tuberculosis* regulate MAP kinase-dependent astrocyte MMP-9 secretion. *J Leukoc Biol* 2007; 81: 548-556.
67. Lehman J. para-Aminosalicylic acid in the treatment of tuberculosis. . *Lancet* 1946; i: 15-16.
68. Agarwal N, Lamichhane G, Gupta R, Nolan S, Bishai WR. Cyclic AMP intoxication of macrophages by a *Mycobacterium tuberculosis* adenylate cyclase. *Nature* 2009; 460: 98-102.
69. Basu S, Pathak S, Pathak SK, Bhattacharyya A, Banerjee A, Kundu M, Basu J. *Mycobacterium avium*-induced matrix metalloproteinase-9 expression occurs in a cyclooxygenase-2-dependent manner and involves phosphorylation- and acetylation-dependent chromatin modification. *Cell Microbiol* 2007; 9: 2804-2816.
70. Clark IM, Swingle TE, Sampieri CL, Edwards DR. The regulation of matrix metalloproteinases and their inhibitors. *Int J Biochem Cell Biol* 2008; 40: 1362-1378.
71. Chan ED, Iseman MD. Current medical treatment for tuberculosis. *Bmj* 2002; 325: 1282-1286.
72. Thwaites GE, Nguyen DB, Nguyen HD, Hoang TQ, Do TT, Nguyen TC, Nguyen QH, Nguyen TT, Nguyen NH, Nguyen TN, Nguyen NL, Vu NT, Cao HH, Tran TH, Pham PM, Nguyen TD, Stepniewska K, White NJ, Farrar JJ. Dexamethasone for the treatment of tuberculous meningitis in adolescents and adults. *N Engl J Med* 2004; 351: 1741-1751.
73. Green JA, Tran CT, Farrar JJ, Nguyen MT, Nguyen PH, Dinh SX, Ho ND, Ly CV, Tran HT, Friedland JS, Thwaites GE. Dexamethasone, cerebrospinal fluid matrix

metalloproteinase concentrations and clinical outcomes in tuberculous meningitis. *PLoS One* 2009; 4: e7277.

74. Simmons CP, Thwaites GE, Quyen NT, Chau TT, Mai PP, Dung NT, Stepniewska K, White NJ, Hien TT, Farrar J. The clinical benefit of adjunctive dexamethasone in tuberculous meningitis is not associated with measurable attenuation of peripheral or local immune responses. *J Immunol* 2005; 175: 579-590.

75. Meintjes G, Wilkinson RJ, Morroni C, Pepper DJ, Rebe K, Rangaka MX, Oni T, Maartens G. Randomized placebo-controlled trial of prednisone for paradoxical tuberculosis-associated immune reconstitution inflammatory syndrome. *AIDS* 2010; 24: 2381-2390.

76. Meintjes G, Rangaka MX, Maartens G, Rebe K, Morroni C, Pepper DJ, Wilkinson KA, Wilkinson RJ. Novel relationship between tuberculosis immune reconstitution inflammatory syndrome and antitubercular drug resistance. *Clin Infect Dis* 2009; 48: 667-676.

77. Behar SM, Divangahi M, Remold HG. Evasion of innate immunity by *Mycobacterium tuberculosis*: is death an exit strategy? *Nat Rev Microbiol* 2010; 8: 668-674.

78. Gapski R, Hasturk H, Van Dyke TE, Oringer RJ, Wang S, Braun TM, Giannobile WV. Systemic MMP inhibition for periodontal wound repair: results of a multi-centre randomized-controlled clinical trial. *J Clin Periodontol* 2009; 36: 149-156.

79. Lindeman JH, Abdul-Hussien H, van Bockel JH, Wolterbeek R, Kleemann R. Clinical trial of doxycycline for matrix metalloproteinase-9 inhibition in patients with an abdominal aneurysm: doxycycline selectively depletes aortic wall neutrophils and cytotoxic T cells. *Circulation* 2009; 119: 2209-2216.

80. Curci JA, Mao D, Bohner DG, Allen BT, Rubin BG, Reilly JM, Sicard GA, Thompson RW. Preoperative treatment with doxycycline reduces aortic wall expression and



activation of matrix metalloproteinases in patients with abdominal aortic aneurysms. *J Vasc Surg* 2000; 31: 325-342.

81. Hanemaaijer R, Sorsa T, Konttinen YT, Ding Y, Sutinen M, Visser H, van Hinsbergh VW, Helaakoski T, Kainulainen T, Ronka H, Tschesche H, Salo T. Matrix metalloproteinase-8 is expressed in rheumatoid synovial fibroblasts and endothelial cells. Regulation by tumor necrosis factor-alpha and doxycycline. *J Biol Chem* 1997; 272: 31504-31509.

82. Smith GN, Jr., Yu LP, Jr., Brandt KD, Capello WN. Oral administration of doxycycline reduces collagenase and gelatinase activities in extracts of human osteoarthritic cartilage. *J Rheumatol* 1998; 25: 532-535.

83. Shlopov BV, Smith GN, Jr., Cole AA, Hasty KA. Differential patterns of response to doxycycline and transforming growth factor beta1 in the down-regulation of collagenases in osteoarthritic and normal human chondrocytes. *Arthritis Rheum* 1999; 42: 719-727.

84. Alvarez JI, Krishnamurthy J, Teale JM. Doxycycline treatment decreases morbidity and mortality of murine neurocysticercosis: evidence for reduction of apoptosis and matrix metalloproteinase activity. *Am J Pathol* 2009; 175: 685-695.

85. Meli DN, Coimbra RS, Erhart DG, Loquet G, Bellac CL, Tauber MG, Neumann U, Leib SL. Doxycycline reduces mortality and injury to the brain and cochlea in experimental pneumococcal meningitis. *Infect Immun* 2006; 74: 3890-3896.

86. Krakauer T, Buckley M. Doxycycline is anti-inflammatory and inhibits staphylococcal exotoxin-induced cytokines and chemokines. *Antimicrob Agents Chemother* 2003; 47: 3630-3633.

87. Hemmings FJ, Farhan M, Rowland J, Banken L, Jain R. Tolerability and pharmacokinetics of the collagenase-selective inhibitor Trocade in patients with rheumatoid arthritis. *Rheumatology (Oxford)* 2001; 40: 537-543.

88. Coussens LM, Fingleton B, Matrisian LM. Matrix metalloproteinase inhibitors and cancer: trials and tribulations. *Science* 2002; 295: 2387-2392.
89. Kaufmann SH. Future vaccination strategies against tuberculosis: thinking outside the box. *Immunity* 2010; 33: 567-577.