Understanding the Tuberculosis Granuloma: the Matrix Revolutions

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Abstract

Mycobacterium tuberculosis causes the human disease tuberculosis (TB), and remains the top global infectious pandemic after COVID-19. Furthermore, TB has killed many more humans than any other pathogen, after prolonged co-evolution to optimise its pathogenic strategies. Full understanding of fundamental disease processes in humans is necessary to successfully combat this highly successful pathogen. Whilst the importance of immunodeficiency has been long recognised, biologic therapies and unbiased approaches are providing unprecedented insights into the intricacy of the host-pathogen interaction. The nature of a protective response is more complex than previously hypothesised. Here, we integrate recent evidence from human studies and unbiased approaches to consider how Mtb causes human TB, and highlight the recurring theme of extracellular matrix turnover.

Human tuberculosis: the intricate and prolonged contest between host and pathogen

Tuberculosis (TB) is a chronic and persistent human killer, causing more deaths in total over time than any other pathogen, and currently is the most important infection after COVID-19. Furthermore, the TB pandemic is likely to worsen due to resources being diverted to SARS-CoV-2 control [1]. The causative organism, *Mycobacterium tuberculosis* (Mtb), has undergone long-term co-evolution with humans, and is an obligate human pathogen [2]. Whilst there have been significant steps forward, such as new antibiotics for drug-resistant disease, the GeneXpert for rapid diagnosis [3] and a promising new vaccine [4], standard treatment, diagnosis and vaccination strategies in most high incidence TB countries are unchanged. Partly, this reflects the fact that we still do not understand human TB sufficiently to design transformative strategies to achieve global TB control.

Accumulating evidence from biological therapeutics and genomic analyses have suggested we need to refine our concepts of the spectrum of human disease [5, 6]. Importantly, this includes confirmation in patients that an excessive immune response can be just as harmful as insufficient response, as illustrated by increased TB incidence with PD-1 inhibition in cancer immunotherapy [6-8]. These new data highlight the fine balance that exists between protection and disease, with either an insufficient or excessive immune response being harmful [9]. Furthermore, the concurrent progression and regression of lesions within the same individual highlights the intricacy of the host pathogen interaction [10, 11]. A recently emerging theme from unbiased analyses is that extracellular matrix turnover is a cardinal feature of human TB, which is well described clinically. Here, we consider human TB in light of these emerging phenomena and the accumulating "omic" datasets, interpreting these findings alongside clinical characteristics of disease.

The granuloma: the critical arena determining outcome

The Mtb human life cycle involves multiple stages and ironically for such a successful pathogen, Mtb usually reaches a dead end in most humans, failing to transmit to a new host (Figure 1) [12, 13]. Infection is spread by aerosol from an individual with pulmonary TB, and those with lung **cavities (see glossary)** are the most infectious and drive the epidemic [14]. Therefore, for efficient transmission, Mtb must cause **immunopathology** and lung matrix destruction at the apices of the lung to exit the host and spread onwards [15]. In addition, recent PET-CT data suggest that propagation of TB within the lung starts with **cavitation**, followed by the seeding of new infection foci via bronchial spread [16]. Therefore, cavitation seems central for disease progression within the host as well as transmission onwards in the population.

In initial infection, Mtb aerosol droplets are typically inhaled to the well-ventilated lower lobes and phagocytosed by alveolar macrophages, though definitive proof in humans is difficult to obtain and not all early lesions are basal. Alveolar macrophages are poor at controlling Mtb [17] and an initial

proliferation generates a large focus of infected cells, often over 5mm in diameter, as demonstrated by the Ghon focus in the lung base [18]. During this period, Mtb proliferation is unrestricted by an adaptive host immune response and it uses a range of evasion capabilities to proliferate within a range of phagocytes, such as inhibiting phagolysomal fusion [19]. Subsequently, at around six weeks, a T-cell response develops, which is delayed relative to other respiratory pathogens [20], but ultimately leads to more efficacious control of Mtb. By this stage, Mtb needs to have spread to the lung apex, from where it will exit and restart the infectious cycle [13]. How Mtb travels from base to apex is unknown [21], though likely infected phagocytes act as Trojan horses carrying the mycobacteria [22, 23]. In patients who never develop an adaptive response Mtb disseminates throughout the body [24], with **miliary** nodules across the chest-X-ray and in other organs, as described as early as 1700 by Manget [11]. This suggests Mtb spreads extensively, with the goal of forming a niche in the upper lung where factors favour persistence over immune eradication. Seminal post-mortem studies by Opie confirmed Mtb survival in apical lung lesions in otherwise healthy individuals [25]. From this niche, Mtb must then cause inflammation, immunopathology and cavitation to transmit, and although this can happen at any point, the majority of cases reactivate in the first 2 years after infection [26]. With this time frame, disease evolution is typically a slow process, and changes in the peripheral transcriptome can be detected many months before presentation of active disease [27].

As the T-cell response develops, Mtb needs to change strategy to reflect the more hostile environment of the host. The recent unpublished identification of changes in Mtb metabolism in response to IFN-γ give some insight into these events. In sensing host IFN-γ, Mtb is able to change its metabolic rate and transcriptional programme, suggesting it can respond to host immunological cues [28]. Once into this second phase of the host-pathogen interaction, Mtb must survive on a tightrope: ultimately needing to drive a host immune response that leads to cavitation whilst avoiding an effective immune response that causes its eradication. The critical structure during this “post primary” stages is the **granuloma** (Figure 2) [29]. This was historically thought to be restrictive to Mtb growth, but concepts of granuloma function and structure have more recently been questioned. For example, key studies in the *M. marinum*/zebrafish model have shown that the recruitment of monocytes to the granuloma can favour pathogen proliferation [22, 30]. Indeed, in the same model system, limiting the formation of epithelioid macrophages, which help to wall off the granuloma, actually helps to limit mycobacterial growth by allowing immune cells access to the granuloma [31]. In addition, the traditional “sphere like” structure of granulomas has been questioned by micro-CT approaches, which suggest a more complex root-like structure of interconnected areas [32], in which microenvironments may vary. Also, whether cavities emerge from the middle of caseous necrotic granulomas, or confluent areas of lipoid pneumonia, has also been disputed [33].

Despite these uncertainties, it is clear that the immune response is both necessary to control infection and also essential to drive the tissue destruction that leads to cavitation and spread [15]. Multiple types of immunodeficiency can lead to uncontrolled Mtb infection, such as advanced HIV infection, anti-TNF-α treatment and mutations within the IFN-γ/IL-12/STAT signalling pathway [19]. This has led to research that primarily focuses on identifying what is missing from the immune response to Mtb that leads to disease. However, evidence that an absence of an immunological component(s) identified in individuals who progress to active TB disease does not mean that an excess will be beneficial [34], and in fact diverse evidence shows inflammation, driven by excessive immunity, is damaging in TB. This debate is not new, and in fact dates right back to bitter disputes between Koch and Virchow [35], over whether Koch's **tuberculin** vaccine would cure infection or provoke an immune response that degraded the granuloma and enhanced disease. On one hand, human studies and animal models provide clear evidence that immunological memory from TB exposure is protective [36, 37]. However, in a seminal large scale epidemiological study, Comstock demonstrated the surprising finding that, among tuberculin reactors, those with the greatest delayed type hypersensitivity response had the highest risk of subsequent development of TB many years later [38]. One potential interpretation is that an excessive immune response to Mtb antigens is detrimental. With the onset of the HIV pandemic, the clinical features differentiating "standard" TB from immunocompromised TB proved that the immune response contributes to lung immunopathology and spread, as cavities are very rarely observed in individuals with advanced HIV-related immunocompromise, but occur on immune reconstitution with antiretroviral treatment [39]. The demonstration that T cell epitopes of Mtb are hyper-conserved compared to non-epitope regions further suggests that the pathogen derives an evolutionary benefit from the promoting the host T-cell response [40, 41].

Most recently, the accumulating evidence that anti-PD-1 treatment for cancer can activate latent TB further highlights the danger of an excessive response, with enhanced T-cell cytokine production implicated in driving immunopathology [7, 8, 42, 43]. Taken together, these observations suggest a complex interplay between innate and adaptive responses, along with mycobacterial load, determining a range of outcomes from disseminated and non-cavitary disease in the absence of an effective adaptive immune response, control/elimination with an optimal response, and matrix destruction, cavitation and spread when excessive localised inflammation occurs [9]. Along similar lines, the concept that the optimal strategy for humans might be to sequester and tolerate Mtb has been proposed, and the breakdown of this tolerance leads to active disease [44, 45]. The fraction of people defined as latently infected that actually harbour viable bacteria is debated [46], but reactivation of Mtb can occur decades after initial infection [47], suggesting this tolerant phenotype can be extremely durable.

Adding to the complexity of TB immunology is the fact that TB lesions can have diverse outcomes even in the same individual [11]. This was summed up neatly by Georges Canetti in 1955, based on examining thousands of tuberculous lungs before the advent of antimicrobial treatment - "Consider the bacillus in the lesion, experiencing such different fates in various foci of the same patient, and the same fate in widely different patients; destroyed in a certain histologic reaction and thriving in another nearby" [48]. Likewise, Dubos wrote in 1952 "all these processes may occur in the same person either at different times or often simultaneously….which is still almost as much a puzzle today". This concurrent progression and regression of lesions has been elegantly confirmed in modern imaging studies of infected non-human primates [10]. Consequently, it appears that the outcome of infectious foci is determined at a local granuloma level and not systemically, adding to the challenges of dissecting determinants of outcome. One proposed paradigm is that a balance within granulomas is necessary, both pro-inflammatory and anti-inflammatory mediators leading to control of infection [49, 50]. With their pivotal role in orchestrating the immune response, dendritic cells are likely to play a central role in shaping the immune response and defining outcome [51]. However, as these events occur within tissue, they are challenging to investigate, and studying the host response in the periphery is unlikely to convey sufficient granularity about individual lesions [52].

Emerging insights from unbiased analyses

Therefore, events determining outcome within individual TB granulomas remain a highly pressing question, and "omic" analyses should provide a wealth of data to give mechanistic understanding. Recently, a number of studies have reported unbiased analyses aiming to unpick the process. A strategy of comparing TB granulomas with sarcoidosis, a non-infectious granulomatous disease, was utilised to overcome the issue of cell-specific gene expression patterns [53]. Diverse analytical approaches demonstrated that the collagenase **matrix metalloproteinase-1** (MMP-1) was highly upregulated in TB, and was the most significantly differentially expressed gene between TB and sarcoidosis. Analysis of gene correlation identified a 7-gene TB-specific cluster, comprising MMP1, the monocyte chemo-attractants **CCL7** and **CCL8**, the divalent transition metal transporter **SLC11A1** (formerly known as NRAMP1), the low density lipoprotein receptor **OLR1** (formerly known as LOX1), **FAM124A**, and **LGALS17A**. Several of these genes have already been implicated in TB pathogenesis, and consideration of their known functions together informs a putative sequence of events that leads to progression of TB lesions (Figure 3, Key figure). Thus, sequencing of clinical material followed by unbiased analysis generated a hypothesised cascade of disease evolution that can be experimentally investigated. Further bioinformatic analyses in combination with a 3D biomimetic model identified that sphingosine 1 kinase inhibition suppressed Mtb growth, thereby progressing from basic disease understanding to novel therapeutic targets in an unbiased manner [53].

Using a similar transcriptomic approach, analysis of gene expression was compared in skin stimulated by tuberculin in patients with TB versus healthy controls [54]. Again, MMP1 emerged as a top divergently upregulated gene, and ingenuity pathway analysis suggested that an excessive IL-17 response was a key regulator. The IL-17/MMP1 profile resolved with treatment of infection, implying that Mtb actively primes an excessive, matrix destructive immune response that can be replicated by a distal antigenic challenge. The authors highlight the double-edged sword of IL-17 in TB, with data supporting a protective role [55, 56], and a pathological role when present in excess [54, 57]. These two recent studies have the limitation of analysing a distal compartment (mediastinal lymph node and skin), and an identical gene expression profile cannot be assumed in the lung. However, the emergence of MMP1 as a predominant mediator from these two RNAseq analyses is also consistent with several previous studies. In an early microarray analysis of re-stimulated macrophages, MMP1 was the most divergently regulated gene in patients with TB, although the authors then focused on a chemokine in validation stages [58]. Similarly, microarray analysis of lung tissue from patients failing treatment for multi-drug resistant TB found MMP1 was very highly upregulated within lesions [59]. Comparison of modular signatures in lung cancer, TB and sarcoidosis by RNAseq has shown over-representation in genes related to ECM organisation [60]. A separate RNAseq study suggested that neuroendocrine signalling was downregulated at the air-caseum interface in drug-resistant TB, whilst the complement pathway was upregulated [61]. Although MMP regulation was not directly noted, the OSM pathway was upregulated, similar to observations in the skin tuberculin study [54], and OSM can induce MMP-1 secretion [62].

Single cell RNA sequencing (ssRNAseq) analysis is now beginning to shed light on cellular subsets. One approach recently employed in the non-human primate (NHP) model of TB involved parallel ssRNAseq and quantification of viable Mtb bacilli from multiple individual granuloma [63]. This revealed a high degree of heterogeneity between different granuloma in the same individuals; and associations between T1/17 T-cells and Mtb control, and mast cells and plasma B-cells and Mtb progression. Alternatively, by comparing ssRNAseq data from lung tissue isolated from NHPs with either progressive or latent Mtb infection, active TB was found to be associated with an influx of plasmacytoid dendritic cells (pDCs), activated macrophages and T-cells, and latency with enriched CD27+ natural killer (NK)-cells [64]. In support of these data, NK cells emerged as a signature correlating with latency in a multi-omic study of human peripheral immune responses, suggesting they may play a predominantly protective role [65]. In separate studies, ssRNAseq was combined with Mtb strains containing a bacterial stress reporter to identify macrophage subsets able to induce bacterial stress *in vivo*. This revealed distinct and epigenetically constrained macrophages subsets with differential degrees of permissiveness [66]. Finally, ssRNAseq of granuloma in zebrafish revealed an unexpected association between Th2 signalling and the generation of epithelioid macrophages, which help to “wall-off” Mtb within the granuloma [67]. Interestingly, as discussed

above, the same group previously showed that partial disruption of this epithelioid barrier improved Mtb control by enhancing immune cells access [31], whereas, in this most recent study, complete abrogation of the barrier leads to increased Mtb growth. This neatly illustrates the fine balance between control and progression at the level of each individual granuloma.

In proteomic studies, a seminal laser capture study demonstrated the importance of spatial organisation within the TB granuloma [68]. A central pro-inflammatory environment was identified within the central caseous core, surrounded by a peripheral anti-inflammatory zone, with the arachidonic acid pathway playing a key regulatory role. These findings parallel earlier reports of the importance of spatial organisation within the granuloma [69]. Similarly, Multiplexed Ion Beam Imaging by Time-of-Flight (MIBI-TOF) has identified microenvironments within the TB granuloma, consistent with areas of immunosuppression [70]. A key question is whether this immunosuppression is part of the pathogen's evasion strategy or alternatively the host's tolerance to a persistent antigenic stimulus [44, 45]. In plasma proteomic studies using SOMAscan methodology, again MMP-1 emerged as one of the most divergently regulated proteins in teenagers who then progressed to TB [71], consistent with previous work identifying a critical role for MMP-1 from a hypothesis-driven approach [72, 73].

Taken together, three themes are emerging from these recent "omic" studies: i) the necessary balance between pro- and anti-inflammatory pathways in controlling TB without causing immunopathology; ii) the importance of cellular composition and cross-talk, 3D organisation and microenvironments within TB granulomas; iii) the consistently observed role for MMP-1 in TB immunopathology. A limitation to consider is most cases studied represent failed control, as clinical disease has occurred, and so dissecting out determinants of protection versus pathology is challenging.

The recurring theme of the extracellular matrix

As outlined, unbiased studies from different groups and methodological approaches have recurrently identified MMP-1 as one of the top few genes upregulated in TB. This raises the question of why MMP-1 is so predominant. Within the granuloma, the goal of Mtb cannot purely be survival, as ultimately the host will die, and the pathogen will not transmit (Figure 1). Therefore, Mtb needs to cause cavities to transmit maximally [14]. The mechanism by which this happens, however, remains poorly understood (Box 1). Strikingly, Mtb bacilli are frequently impossible to find by standard AFB staining techniques within human granulomas [74], and yet Mtb-driven inflammatory gene signatures are present through the granuloma. How Mtb causes widespread inflammation and reprogramming of granulomas in the apparent absence of high bacterial numbers is unknown. A number of potential mechanisms could explain this; self-propagating intercellular pro-inflammatory cytokine networks, swarm behaviour by immune cells [75], microvesicles leading to transfer of Mtb antigens or mRNAs

to uninfected cells [76], a progressive build-up of Mtb antigens [33], or Mtb that is not stained by standard approaches [77].

An additional factor that may contribute to excessive inflammation is that of **trained innate immunity**. Mtb evidently causes epigenetic modification [78, 79] and innate immune training [80], and one of the mechanism of protection through the vaccine BCG is thought to be via non-specific protective training [81]. The recent demonstration that MMP-1 is rapidly and highly upregulated upon PPD stimulation in the skin of TB infected individuals [54], remote to the site of lung infection, is consistent with circulating innate immune cells programmed to drive an excessive pro-inflammatory response. Interestingly, the seven gene signature within TB granulomas includes a potential innate immune training component (Figure 3) [53], as OLR1 can regulate epigenetic modification [82]. Of specific relevance to TB, OLR1 upregulation in atherosclerosis is associated with the formation of foamy macrophages [83], a cell type also induced in TB granuloma. However, the trained immune phenotype induced by TB infection that can lead to dysregulated inflammation has not been fully characterised. In addition, non-haematopoietic cells, such as fibroblasts, may play significant roles in TB progression, as they are central players in matrix turnover. For example, OSM has emerged as one central hub from unbiased analysis [54, 61] and can upregulate fibroblast MMP-1 secretion [62].

Finally, it has previously been proposed that an autoimmune component may contribute to TB progression, with immune cells responding to host stress antigens or matrix neoepitopes generated by matrix breakdown [84]. This phenomenon would explain many of the unusual clinical characteristics of human TB, such as uveitis and erythema nodosum, that overlap with autoimmune diseases. Genomic analyses support this concept, such as the similarities between TB and autoimmune disease signatures in peripheral blood [85]. Similarly, immunological network analysis in HIV-infected individuals also supports an autoimmune process exacerbating pathology in TB via TH17 polarisation [57]. However, the possibility that host antigens may contribute to pathogenesis in TB remains conjectural.

Tissue-dependent considerations in studying the immune response

The majority of research on host immunity to Mtb have studied circulating immune cells [52]. However, it is becoming increasingly apparent that events within tissue may greatly differ from those in the periphery, just as they do between different TB lesions within the same lung [10, 68, 69, 86]. For example, comparison of lung versus circulating T cells showed very different immunological profiles at the site of disease relative to the periphery [55, 87]. The fact that TB reactivates at the lung apices, not the base where it initially implants, suggest that even within the same organ there are broad immunological differences, which may related to differential immune surveillance [13].

Alternatively, localisation could relate to differences in mechanotransduction across the lung, as there is an increased likelihood of collagen cleavage under tension [88]. As different lesions can progress and regress, one cannot assume that studying a single lesion is sufficient, presenting a significant experimental challenge. Once one layers the spatial immune organisation of the granuloma into this equation, comprehensive understanding of the host-pathogen interaction becomes highly challenging. We propose that cross-correlation between human disease and model systems which incorporate the extracellular matrix, by studying the immune response in 3D within relevant tissue and accompanying biomimetic models where outcomes differ stochastically, will be critical if this complexity is to be understood.

Concluding remarks

The lack of understanding of what determines protection versus pathology in tuberculosis is hindering progress (see Outstanding Questions). The debate goes back to the previously mentioned hard-fought disputes between Koch and Virchow [35], and the greater granularity provided the molecular era has further highlighted the complexity of the host-pathogen interaction. Both disputants could select recent data regarding IL-17 in human TB to support their argument that the host response is either protective or pathogenic [54, 55], and similarly, evidence about tissue microenvironments could support each position [63, 68, 70]. Ultimately, the historic concept of "good" and "bad" immune responses in TB are unlikely to be sufficient. New paradigms predicting determinants of outcome are needed, taking into consideration the multiplicity of inputs, spatial organisation, diverse outcomes, and even the potential for multiple routes to the same outcome. The wealth of data from "omic" technologies can only be successfully interpreted if analysis is framed within the clinical characteristics of human disease (Box 2). Emerging themes from the recent unbiased analyses highlight the need for a balanced immune response for Mtb control and point to aberrant extracellular matrix turnover and excessive MMP-1 activity as being a critical effector leading to disease progression (*see* Clinician's corner). Ultimately, embracing the complexity of human TB is essential to understand the central unresolved question: what determines outcome in an individual TB lesion?

Box 1: Tuberculosis and the matrix

The human lung is highly intricate, relying on the extracellular matrix to support a meshwork of alveoli to generate a total surface area the size of a tennis court [89]. Matrix destruction is fatal, as gas exchange then fails. Therefore, the basal environment of the lung is highly tolerogenic and skewed towards matrix protection. To effectively transmit, Mtb must overcome this matrix homeostasis to cause lung cavitation.

In individuals who progress to active disease, the immune equilibrium is lost and excessive inflammation develops. Diverse unbiased approaches suggest that MMP-1 is a final effector of collagen cleavage in this process. MMP-1 is secreted as a pro-enzyme requiring proteolytic activation [90]. Numerous *ex vivo* and *in vitro* studies demonstrate that Mtb induces secretion of pro-MMP-1 by host cells. In addition, Mtb secretes serine proteases [91, 92], suggesting a potential proteolytic cascade whereby Mtb may both directly induce and activate MMP-1 within its microenvironment, propagating matrix breakdown. Intriguingly, one of the antigens in the novel M72/AS01E TB vaccine, the first candidate to improve on BCG in human trials, is an Mtb serine protease [4]. It is possible, therefore, that anti-protease antibodies generated by M72/AS01E vaccination helped prevent TB reactivation by limiting MMP-1 activation and initial matrix breakdown. This is pure speculation, but if proven then matrix-protective vaccination strategies may be a novel way to prevent TB reactivation.

To date, investigating anti-protease strategies has been challenging, as standard TB mouse models do not develop the typical caseating lesions of human disease [93], and lack a functional orthologue of human MMP-1 [94]. The C3HeB/FeJ or Kramnik mouse is a notable exception, developing cavitary lesions. However, these mice are immunodeficient and develop high bacterial loads [95], and so matrix destruction may occur via distinct mechanisms. Transgenic expression of human MMP-1 in immunocompetent mice results in collagen destruction and caseation in granulomas without altering Mtb growth [96], supporting a central role for MMP-1 in initiating the cavitary process.

Multiple MMP inhibitors are available. However, MMP inhibitor therapy alone is harmful in preclinical models [97, 98], whereas when administered alongside antibiotics is beneficial [99]. We performed a phase IIB trial of doxycycline as adjunctive therapy in patients with pulmonary TB, and found that doxycycline suppressed MMP-1 and reduced cavity size without affecting mycobacterial load [100]. Notably, two weeks of doxycycline caused changes that persisted at 8 weeks, suggesting that early events in TB treatment have long-lasting impact (Figure 4), supporting the concept of host-directed therapies to improve outcome [101].

Box 2: Opportunities and challenges of "omics" data analysis for identification of novel therapies

Advances in "omics" technologies over the past three decades have opened unprecedented opportunities for the investigation of complex biological events in human tissues. For the first time, analytical techniques can dissect not only expression levels of selected genes/proteins, but also, and often simultaneously, allow for the delineation of single nucleotide polymorphisms, the whole transcriptome, proteome, epigenome and metabolome, of cell populations or single cells, and

determine their spatial arrangement in tissues. While integrated analysis across these layers could provide a complete delineation of biological processes involved, current studies often focus these state-of-the-art approaches on a single information layer, such as transcriptomics or proteomics, and aim to demonstrate usefulness for identification of therapeutic targets.

Application of network analysis has proven extremely successful in this task. Based on mathematical graph theory, it allows interrogation of biological data in hypothesis-free way, and independently of the existing curated databases. Weighted and unweighted gene co-expression analysis [102, 103] and mutual information and partial deconvolution of information analysis [104] allow delineation of underlying structure in experimental data, and identification of candidate regulators for gene/protein modules. Importantly, by assigning eigenvector values to co-expressed modules of biological features, such as transcripts, genes or proteins, it allows integration and co-analysis of clinical features and data from other high-throughput platforms.

By example, the recent study comparing TB and sarcoidosis, a non-infectious granulomatous disease, and modelling TB infection in a 3D biomimetic model, provides a proof-of-concept of how gene co-expression analyses can be used for identification of novel therapeutic targets in TB [53]. Similarly, applying unbiased co-expression network analysis to clinical trial data, we identified immunological processes regulated by treatment with doxycycline, revealing selective modulation of innate immunity [100]. As an alternative approach, a module analysis approach was employed to demonstrate central role of the IL-17 response in exacerbating TB pathology [54]. In these analyses, extrapolation to events in the lung interstitium is now needed.

While significant progress is being made in development of approaches to high dimensional data analysis, including advanced mathematical modelling for single cell and spatial data, and application of Bayes theory and machine learning/artificial intelligence methodologies, the key challenge yet to be overcome is bridging mechanistic understanding of the biological process with data analysis. As advances are being made in deriving causal network architecture from “omics” data, and developing methods for analysis of dynamic signal flow through the network, this approach is likely to become the new frontier for predictive data analysis, allowing multi-layer predictive modelling of therapeutic perturbations.

Glossary

- **Cavities:** Air-filled holes within the lung that result from complete destruction of the extracellular matrix, from the end result of the the process of **cavitation**. Mtb proliferates in

the cavity walls exponentially, leading to highly infections patients, chronic transmission and an increased risk of treatment failure

- **CCL7** and **CCL8**: C-C Motif Chemokine Ligand 7 and 8. Secreted chemokines that recruit monocytes to areas of inflammation
- **FAM124A:** Family With Sequence Similarity 124 Member A. Although poorly characterised, may interact with NFκB activating protein, consistent with a role propagating inflammation
- **Gene co-expression analysis:** a bioinformatic approach based on mathematical graph theory, where clusters/modules of co-expressed genes are identified in an unbiased way based on the correlations in level of expression between each pair of genes/transcripts across study samples
- **Granuloma:** an organised collection of inflammatory cells, including activated macrophages, T cells, B cells and fibroblasts, that forms in response to Mtb infection
- **Immunopathology:** the adverse outcome of the host immune response to persistent Mtb infection, involving cell death, matrix destruction and impaired tissue function due to excessive cellular infiltration
- **LGALS17A:** Galectin 14 Pseudogene. The function has not yet been defined, and so the role in TB pathogenesis is uncertain
- **Matrix metalloproteinases**: A family of enzymes with the collective ability to degrade all fibrillar components of the extracellular matrix at neutral pH, in particular the triple helix of type I collagen, which provides the tensile strength of the lung
- **Miliary tuberculosis**: Disseminated infection, with appearance of Millet seeds across the chest X-ray, and frequently accompanied by central nervous system involvement
- **OLR1:** Oxidized Low Density Lipoprotein Receptor 1, formerly known as LOX1. Initially identified through its role in atherosclerosis, this receptor has a wide range of functions including propagating inflammation and regulating foamy macrophage formation
- **SLC11A1:** Solute Carrier Family 11 Member 1, formerly known as NRAMP1. The first gene linked to TB susceptibility in population studies, when known as Natural Resistance-Associated Macrophage Protein 1. The functions include divalent cation transport and also regulation of macrophage activation
- **Trained innate immunity**: the modulation of innate immune responses over time by epigenetic reprogramming
- **Tuberculin:** a sterile protein extract from cultures of Mycobacterium tuberculosis, typically used to test for immunological memory by intradermal injection and measurement of resulting swelling 3 days later.

Clinician's Corner

- The increase in TB incidence with both immunosuppressive anti-TNF-α and immuno-activating anti-PD-1 treatment highlights that both an insufficient or excessive immune response to Mtb can be harmful to patients, and further emphasises the complexity of the host-pathogen interaction. Clinicians need to be alert to reactivation of latent infections during cancer immunotherapy, which may mimic disease progression.
- Several recent investigations using emerging unbiased methodologies have identified exaggerated inflammation and extracellular matrix destruction as a critical pathological processes in human TB, including disproportionate upregulation of the collagenase matrix metalloproteinase-1.
- New approaches to reduce immune-mediated tissue destruction in TB are currently being advanced using host-directed therapies such as statins, metformin, imatinib and doxycycline. Non-specifically inhibiting lung matrix breakdown in TB patients with doxycycline can suppress tissue-damaging collagenases and accelerate cavity resolution without affecting bacterial load.
- Matrix-preserving strategies may not only reduce long term lung damage, but also enhance immunological control of infection, just as cavity collapse through plombage, artificial pneumothorax and thoracoplasty were successful in the pre-antibiotic era.

- These recent studies demonstrate the power of combining network-based "omic" analysis of human diseased tissue and relevant control tissue with advanced 3-dimensional cell culture modelling to understand disease mechanisms and identify new therapeutic approaches. This *tissue sequencing → bioinformatics → cellular modelling → clinical intervention* pipeline can be used to investigate diverse human diseases and accelerate translation to new therapeutic interventions.

Additional files: uploaded separately

- Highlights
- Outstanding questions

Figure legends

Figure 1: The human TB life cycle. A patient with pulmonary TB generates an aerosol by coughing, which is inhaled into the lower part of the lungs. Initial proliferation occurs, often leading to a Ghon focus visible on the chest X-ray (circle). In the absence of an efficacious immune response, disseminated miliary TB develops, with mycobacterial proliferation in many organs, but this is a dead end for the pathogen. Once the adaptive immune response activates, Mtb proliferation is controlled, and a period of latency typically occurs. Infection can reactivate in other organs, such as lymph nodes, but again this does not typically transmit. In approximately 6% of individuals, typically those with a robust immune response in the age 20-25, Mtb drives extensive lung inflammation, leading to lung matrix destruction, cavitation and transmission to new hosts. However, even extensive lesions can regress, with approximately 1/3 of "consumptives" spontaneously healing in the pre-antibiotic era. Part of this figure was created with BioRender.

Figure 2: Human TB granulomatous inflammation. Haematoxylin and Eosin staining of a lung nodule that cultured Mtb. The typical granuloma in the centre is an organised structure of activated macrophages, T cells and fibroblasts. Star: central caseous necrosis; Arrowheads: ring of epithelium macrophages. However, much of the surrounding inflammation is much more poorly organised than typically represented in schematics, and the concept of individual spherical granulomas being the source of TB cavities is being challenged.

Key figure

Figure 3: Potential sequence of events in TB granuloma progression identified by gene co-expression analysis. Unbiased analysis of RNAseq data identified a seven gene cluster unique to TB lymph nodes when compared to a non-infectious granulomatous disease, sarcoidosis. Several of these genes have previously been implicated in TB pathogenesis. Considering their function together leads to a proposed sequence of events starting with excessive monocyte recruitment, which are then epigenetically reprogrammed to propagate

inflammation, ultimately leading to excessive MMP-1 expression which causes matrix destruction. Part of this figure was created with BioRender.

Figure 4: Early therapeutic intervention in TB has persistent effects. Lung inflammation continues for the first weeks after the start of efficacious TB antibiotic therapy, which results in ongoing damage. A short-term intervention of doxycycline for two weeks still had significant effects on gene expression and protein concentrations of tissue-damaging MMPs at 8 weeks, demonstrating if the immune response is diverted towards health early in treatment, this trajectory is maintained. This reduction in inflammation reduces the overall lung immunopathology during the course of treatment. This figure was created with BioRender.

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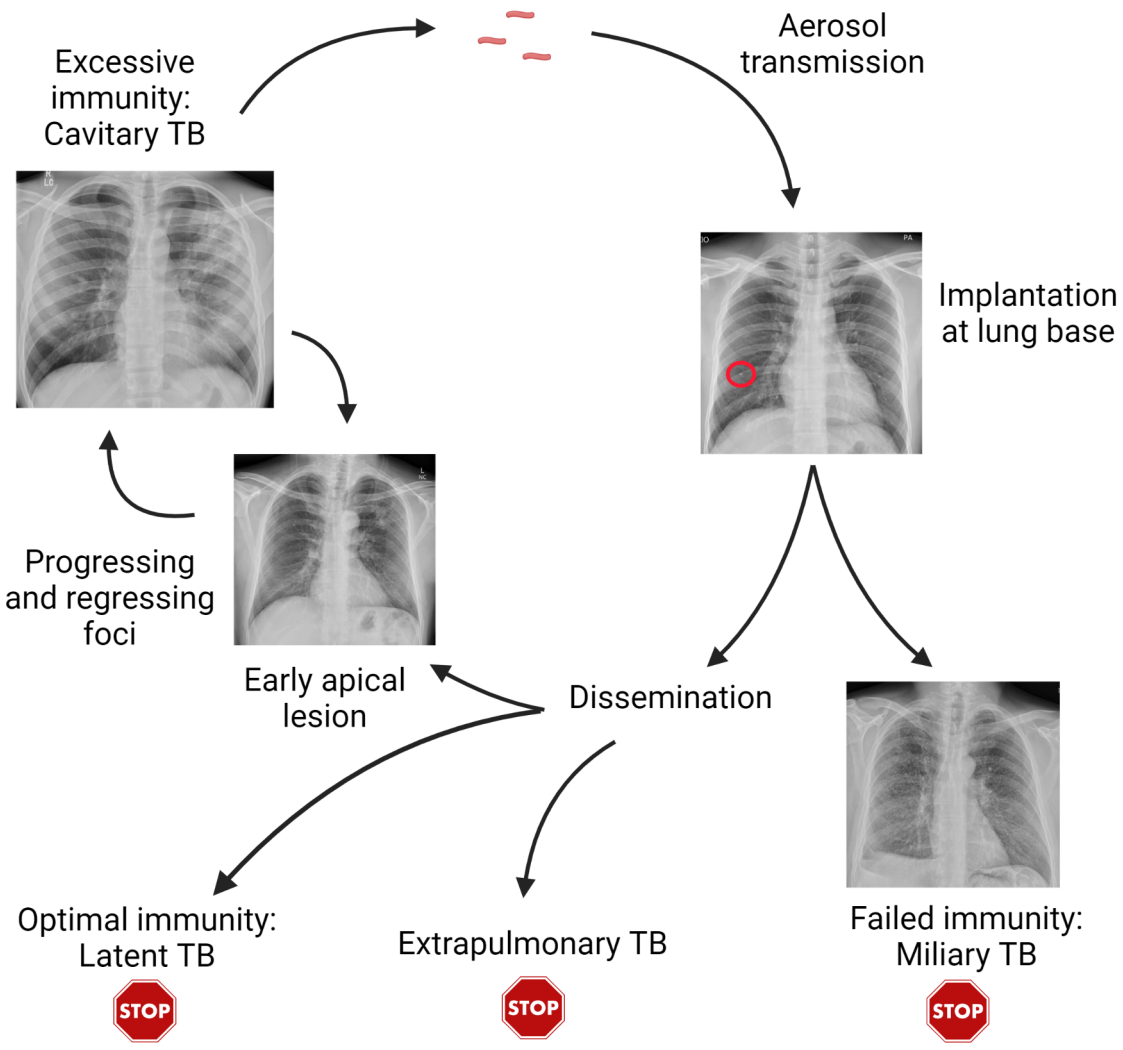
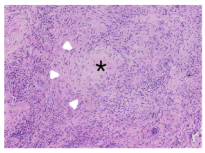
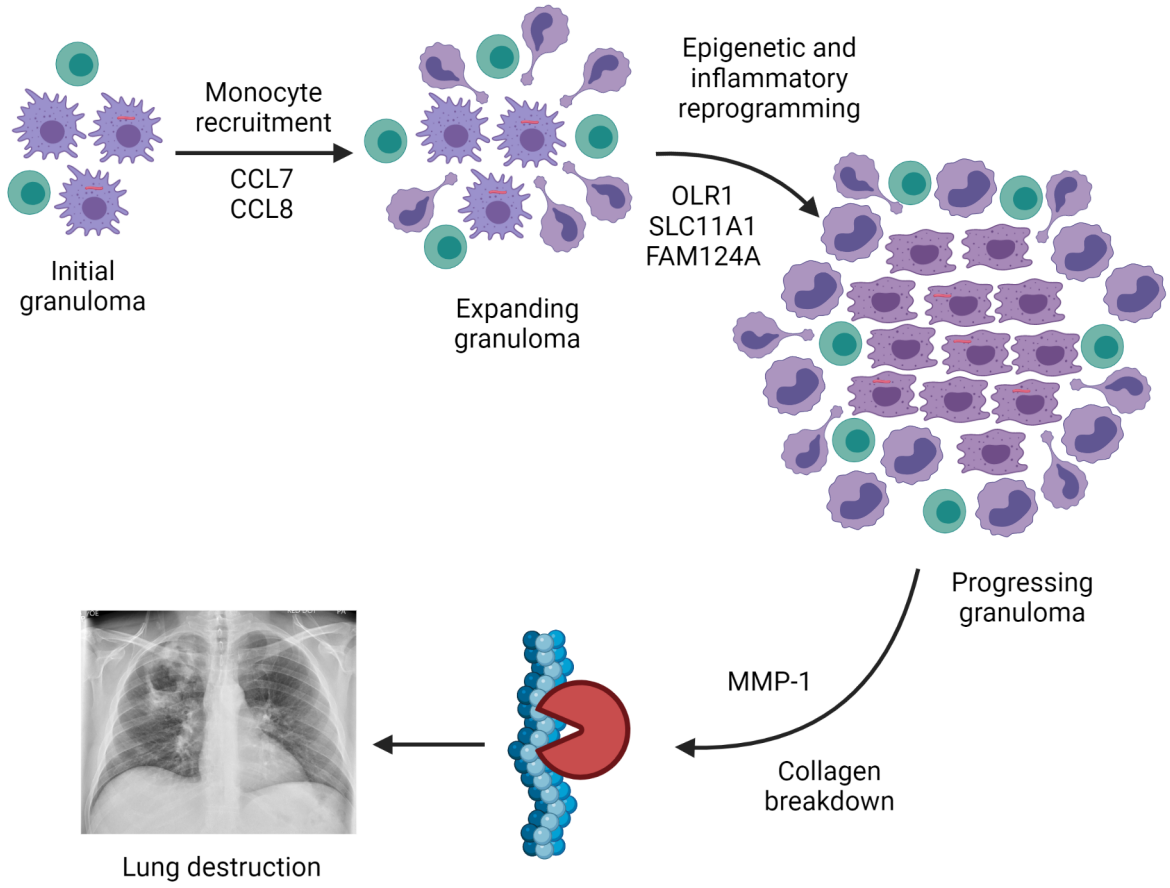
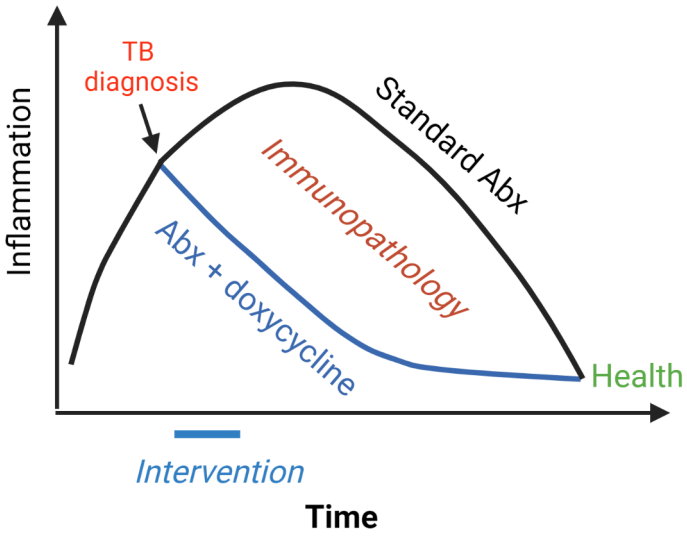

Aerosol transmission
Implantation at lung base
Dissemination
Failed immunity: Miliary TB
STOP
Extrapulmonary TB
STOP
Optimal immunity: Latent TB
STOP
Early apical lesion
Progressing and regressing foci
Excessive immunity: Cavitary TB




Monocyte
recruitment
CCL7
CCL8
Initial
granuloma
Expanding
granuloma
Epigenetic and
inflammatory
reprogramming
OLR1
SLC11A1
FAM124A
Progressing
granuloma
MMP-1
Collagen
breakdown
Lung destruction


TB
diagnosis
Standard Abx
Immunopathology
Abx + doxycycline
Health
Inflammation
Intervention
Time