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Heterogeneity in tuberculosis

Anthony M. Cadena[1], **Sarah M. Fortune**[2], and **JoAnne L. Flynn**[1]

[1]Department of Microbiology and Molecular Genetics, 450 Technology Drive, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15261, USA.

[2]Department of Immunology and Infectious Diseases, Harvard T. H. Chan School of Public Health, 655 Huntington Avenue, Boston, Massachusetts 02115, USA.

Abstract

Infection with *Mycobacterium tuberculosis*, the causative agent of tuberculosis (TB), results in a range of clinical presentations in humans. Most infections manifest as a clinically asymptomatic, contained state that is termed latent TB infection (LTBI); a smaller subset of infected individuals present with symptomatic, active TB. Within these two seemingly binary states, there is a spectrum of host outcomes that have varying symptoms, microbiologies, immune responses and pathologies. Recently, it has become apparent that there is diversity of infection even within a single individual. A good understanding of the heterogeneity that is intrinsic to TB — at both the population level and the individual level — is crucial to inform the development of intervention strategies that account for and target the unique, complex and independent nature of the local host–pathogen interactions that occur in this infection. In this Review, we draw on model systems and human data to discuss multiple facets of TB biology and their relationship to the overall heterogeneity observed in the human disease.

Classically, *Mycobacterium tuberculosis* infection in humans is thought to result in one of two clinically defined states: latent infection (termed latent tuberculosis (TB) infection (LTBI)) or active disease. LTBI is characterized by the presence of immunological sensitivity to mycobacterial antigen (as determined by a tuberculin skin test or an interferon-γ (IFNγ) release assay) in the absence of the clinical symptoms of disease, which can be extremely varied but most often include cough, fever or weight loss. LTBI accounts for 90% of human infections and has an estimated burden of more than 2 billion individuals worldwide[1,2]. By contrast, active TB is diagnosed in patients who have clinical signs and symptoms of TB, and show microbiological evidence of *M. tuberculosis* infection. Pulmonary TB is typified by a chronic cough, fever, sustained weight loss, wasting and haemoptysis (that is, coughing up blood or blood-stained mucus)[3]. Microbiological confirmation — either by culturing *M. tuberculosis* from sputum or other relevant samples, or by identifying the organism through nucleic acid testing or acid-fast staining — is required for the unequivocal diagnosis of TB.

Correspondence to J.L.F. joanne@pitt.edu.

Competing interests statement

The authors declare no competing interests.

In 2015, there were 10.4 million new cases of TB infection and 1.8 million deaths due to TB[4]; the active disease state is estimated to occur in approximately 5% of initial infections in the first 18 months with a remaining risk of 5% (due to the reactivation of LTBI) throughout the lifetime of the individual[5]. Although these definitions of active TB and LTBI have been the basis of decades of clinical practice, the TB field has more recently embraced a newer paradigm that recognizes a spectrum of infection outcomes within these two states[6]. The biological and immunological under-pinnings of this variability are not well understood, and the clinical ramifications of this variability in human TB remain unknown.

This Review provides a framework for understanding the heterogeneity observed in *M. tuberculosis* infection by separating host, granuloma and bacterial features that contribute to the overall spectrum of TB. In examining each of these factors as they relate to the variability of TB, we highlight the intricate network of interactions that ultimately influences infection outcome and emphasize the need to consider all three domains for effective intervention.

Host heterogeneity

TB: a spectrum of clinical and pathological outcomes.

It is widely accepted that the severity of active TB can be highly varied and can show different patterns of lung involvement[7–9]. It is now appreciated that what is considered to be LTBI can also encompass a range of infection outcomes[6,10,11]. The large reservoir of individuals with asymptomatic LTBI represents a nuanced continuum of bacterial persistence and host containment, ranging from cleared infection to low-grade TB[6,10]. This concept of a spectrum of infection for LTBI extends the definition of subclinical, latent TB beyond a single status and enables us to better differentiate the risk of LTBI reactivation in an individual, prioritize preventive treatment and emphasize the heterogeneity of host responses to *M. tuberculosis* infection. Individuals who have a sterilized or extremely well-contained infection are the least likely to suffer reactivation of infection and presumably are the last to require intervention. By contrast, individuals with LTBI who are harbouring a low-grade, sub clinical infection are at a higher risk of reactivation and are more likely to require treatment.

Evidence supporting this concept has been observed in a human study using [^{18}F]fluorodeoxyglucose positron emission tomography and computed tomography (FDG PET–CT)[12]. The authors of this study found that among 35 antiretroviral therapy-naive, HIV-1-positive adults with LTBI, 10 patients with pulmonary irregularities indicative of subclinical TB disease had a significantly higher risk of developing active TB than did the remaining 25 patients who had no subclinical pathology as detected by FDG PET–CT. Specifically, 4 of the 10 patients with evidence of subclinical disease developed active disease, whereas none of the 25 patients in the second cohort developed active disease. The 10 patients who were at a higher risk of disease had radiological evidence of active nodules, infiltrates or fibrotic scars, whereas the 25 participants who did not develop disease had either normal lung parenchyma ($n = 10$) or only discrete nodules ($n = 15$). Importantly, although the group with pulmonary irregularities had an elevated risk relative to the group without subclinical pathology, there were six individuals in the higher-risk group who did

not develop active disease during the period of study, further emphasizing the variability in disease progression and host outcome. This study challenges the binary classification of active disease and LTBI, and instead emphasizes the importance of heterogeneity within LTBI in which a subset of individuals harbour a low-grade, subclinical and asymptomatic disease state that places them at increased risk of developing active TB.

These radiological findings in humans support our earlier observations of a spectrum of disease in the macaque model of TB[13,14] (BOX 1). A detailed study of experimentally infected cynomolgus macaques that quantitatively assessed parameters of infection among animals with clinically defined active infection or LTBI found marked heterogeneity in disease pathology, extrapulmonary dissemination and tissue involvement[14]. There were quantifiable differences between macaques with active TB and those with LTBI, including the degree of pathology and bacterial burden. However, there were also differences in both the lungs and the blood of monkeys in each of these two groups. Some monkeys with clinically active disease had bilateral lobe involvement, pulmonary cavitation, tuberculous pneumonia and extrapulmonary disease, whereas other monkeys with active disease had a pathology that was confined to the thoracic lymph nodes and a single lung lobe[14]. There was similar heterogeneity observed in the macaques that maintained LTBI; four animals appeared to only have lymph node involvement, whereas three animals had evidence of a Ghon complex. Of particular interest were five animals that were classified as having an intermediate disease state (between active disease and LTBI) and thus were analogous to the ten human patients described above[12]. Four of these animals had disease that was limited to the lungs and lymph nodes, but intermittently showed *M. tuberculosis* growth in cultures of bronchoalveolar lavage fluid samples or gastric aspirate samples (a surrogate for human sputum sampling), thereby precluding their inclusion in the LTBI cohort. These monkeys were deemed to have subclinical disease and were termed ‘percolators’ on the basis of occasional *M. tuberculosis*-positive samples[14]. Overall, these studies in humans and macaques support the idea of the biological heterogeneity of TB and suggest that there may be clinical benefit in appreciating the variability of host outcomes in TB by segregating patients according to disease risk.

Peripheral transcriptional signatures reveal dynamic and variable disease states.

Clinical heterogeneity has also been observed in the context of recent studies that have examined whole-blood transcriptional signatures related to host disease status[15–17]. In a landmark study published in 2010, Berry *et al.*[15] reported a 393-transcript signature that was unique to patients with active TB (from both intermediate-burden and high-burden areas) relative to subjects with LTBI and healthy controls. This signature reflected the upregulated transcription of IFN-inducible genes (that is, genes that are induced by both type I and type II IFNs) in blood neutrophils from patients with active TB that correlated with the extent of lung disease as assessed by radiography[15,18]. Notably, whereas the majority of individuals with LTBI clustered independently of the patients with active TB, 10–25% of the subjects with LTBI had similar transcriptional profiles to patients with active TB, and it was considered likely that these patients had subclinical, active disease[15]. These shared transcriptional profiles observed in a sub-set of patients with clinically defined LTBI

reiterate the heterogeneous radiological findings described above and further emphasize the varied nature of this disease.

Further studies have examined blood gene-expression profiles in patients with pulmonary TB at diagnosis and throughout TB treatment[19,20]. In the first study, Bloom *et al.*[19] found a 320-transcript signature in patients after 2 weeks of treatment that subsequently diminished by 6 months. Cliff *et al.*[20] reported similar changes in gene networks following antibiotic treatment. Within 1 week of TB treatment, the expression of more than 1,200 genes was markedly downregulated, including the expression of multiple inflammatory markers such as the complement proteins C1q and C2. This initial period of rapid transcriptional change was followed by a slower upregulation of genes involved in the humoral response[20]. Importantly, as was observed in the study by Berry *et al.*[15], several of the treated patients from both studies had notable differences in their gene-expression profiles relative to the majority of treated individuals. This host-specific variability once more highlights the heterogeneity that is intrinsic to TB and suggests an opportunity to stratify patients to different treatment strategies, particularly as the standard treatment regimen can be problematic owing to its length and its potential for toxicity and drug interactions[21,22].

A whole-blood gene expression analysis of patients with pulmonary and extrapulmonary TB found that transcriptional profiles are influenced by symptom status and the site of disease[23]. Individuals with the highest mean molecular distance to health[24] had the highest likelihood of presenting with one or more of the following symptoms: fever, night sweats, chest pain, cough or weight loss. These findings suggest that clinical illness is linked to the site of infection, bacterial burden and host response, which are reflected in the diversity of transcriptional profiles and host statuses. A recent study of adolescents with LTBI demonstrated that a whole-blood transcriptional signature could identify those who were at risk of developing active TB up to 12 months before clinical diagnosis; the signature was validated in a separate adult population[25]. These data further support the concept of a differential risk of reactivation in a population[25]; this differential risk is possibly due to a spectrum of disease states within asymptomatic infection. This study provides an opportunity to target drug treatment to those in whom LTBI reactivation is most likely to occur.

In non-human primates, similar studies have investigated gene-expression changes specific to lung granulomas[26] and longitudinal changes in gene-expression profiles in the blood[27]. In the first study[26], granulomas from rhesus macaques were found to undergo transcriptional reprogramming from early (4 weeks) to late (13 weeks) time points in infection[26]. In the second study[27], an analysis of peripheral blood from cynomolgus macaques revealed that the greatest transcriptional change occurred 3–8 weeks after infection[27]. There was a positive correlation between the transcriptional signature and inflammation as assessed by FDG PET–CT. One of the most important findings was that even before infection, stronger type I IFN signatures were observed in the macaques that went on to develop active TB, which suggests that there is an innate response linked to host susceptibility to progressive disease. These pre-existing differences in immune status may be due to host genetics; several genetic polymorphisms have been linked to TB risk (BOX 2). Collectively, these studies suggest a

model in which the dynamic regulation of inflammation shapes the trajectory of infection to create a continuum of outcomes.

Granuloma heterogeneity

TB granulomas have varied structures and cellular compositions that influence host outcome.

The pathological hallmark of human TB is the granuloma, which is an organized and localized aggregate of immune cells that consists of macrophages, lymphocytes and other host immune cells, and forms in response to persistent stimuli[28]. These structures arise at the sites of *M. tuberculosis* infection, and are the locations in which there is active interchange between the host and the pathogen. The formation of granulomas is crucial for controlling and containing infection[29,30], but granulomas may also contribute to early *M. tuberculosis* proliferation and dissemination[31–33]. Studies of human autopsy specimens from more than 50 years ago have revealed that in active disease and LTBI, granulomas exhibit morphological heterogeneity[34,35]. In addition to the classic caseous granuloma, granulomas can be non-necrotizing, neutrophil-rich, mineralized, completely fibrotic or cavitary[30]. In most of these types, the basic granuloma architecture exhibits the following structure: a central acellular necrotic core, termed the caseum, surrounded by a diverse population of macrophages that is itself circumscribed by a lymphocytic cuff of $CD4^+$ and $CD8^+$ T cells and B cells, and may have a peripheral fibrotic edge[29,32]. Granulomas mainly contain macrophages that are at various stages of activation, and T cells and B cells, but they can also contain neutrophils, dendritic cells and fibroblasts[30,32] (FIG. 1).

The important lesson learned from appreciating the heterogeneity of human TB granulomas is that each separate granuloma represents a localized micro environment that can be independently influenced by the quality of the localized immune response; the pathogenicity, state and number of bacteria; the extent of immunopathology; and the overall host disease status[36,37]. Recent studies using both animal models of TB[14,37] and surgically resected tissue from humans with TB[38,39] support these findings and reiterate the inherent complexity of the disease. Importantly, this granuloma-specific heterogeneity is crucial in determining host outcome, as only one or a few granulomas that poorly contain their bacteria are probably responsible for allowing bacterial dissemination, worsening pathology and driving the onset of active disease[37,40–42]. In a macaque model of TB, Lin *et al.*[37] demonstrated that animals with clinically active disease had both sterile granulomas and regions of severe pathology (that is, consolidations and TB-associated pneumonia) that had very different profiles of bacterial killing. These findings shift the view of host control from a systemic response to a localized response within individual granulomas. The mechanisms that establish diverging granuloma fates are unclear, but involve complex interactions between tissue inflammation, host immune responses and bacterial processes. We discuss these factors in greater detail below.

Inflammation: a delicate and dynamic balance.

Following successful infection, *M. tuberculosis* initiates a clash of pro-inflammatory and anti-inflammatory signals within the lungs that is vital in establishing the granuloma and in

influencing its eventual trajectory[30,41,43–47]. The resulting 'tug-of-war' between these mediators of inflammation can both promote and limit bacterial dissemination[6,30,48]. Skewing towards a robust pro-inflammatory state can lead to remodelling within the granuloma, liquefaction (or softening) of caseum[49] and the destruction of the surrounding lung parenchyma. Such processes are linked to the onset of active disease[40], and are necessary for cavitation into neighbouring airways and successful transmission of *M. tuberculosis*[49,50]. By comparison, the resolution of inflammation within the granuloma and the lungs is associated with better host outcome[40], a reduced risk of reactivation51 and a better long-term prognosis after treatment[52].

In a seminal study published in 2016, Malherbe *et al.*[52] observed that residual inflammation detected by FDG PET–CT in patients with seemingly cured pulmonary TB was associated with the presence of *M.tuber culosis* mRNA in both sputum and bronchoalveolar lavage fluid samples. Patients from two separate cohorts in South Africa and South Korea displayed a range of radiographic responses, with the majority exhibiting pulmonary inflammation that persisted a year after treatment despite receiving a curative regimen. This study highlights the spectrum of host outcomes, even following treatment, as well as the heterogeneous and potentially predictive role of inflammation in TB outcome.

A second study published in 2016 examined lipid inflammatory pathways within human and rabbit granulomas[46]. Marakalala *et al.*[46] showed that granulomas have highly organized regions of inflammatory signalling that are closely linked to function: specifically, pro-inflammatory, antibacterial lipid mediators are concentrated in the centre of the granulomas, and anti-inflammatory, tissue-preserving mediators are in the periphery[46]. These observations are consistent with a previous study of human and macaque granulomas that found similar spatial compartmentalization of inflammatory programmes organized around differential populations of macrophages[53]. Marakalala *et al.*[46] proposed that the precise localization and balance of these inflammatory boundaries influences individual granuloma fate, which collectively affects host outcome. This model is broadly consistent with earlier studies from several groups that have highlighted the importance of balance in the eicosanoid inflammatory axis in TB[54–60]. Importantly, this hypothesis helps to explain the variation observed in TB granulomas, as slight differences in inflammatory pathways probably contribute to diverse granuloma architectures and functions, and have different consequences for bacterial control. This large area of work ultimately suggests that beneficial inflammatory intervention at the local granuloma level has the possibility to skew granuloma responses in favour of host resolution.

The heterogeneity of immune responses in TB granulomas.

Closely linked to the inflammatory axis are the innate and adaptive immune responses that occur within the TB granuloma. These responses are an integral component of host defence and bacterial containment[34,43,44,61], and they are intimately linked with granuloma outcome[37,62–64]. The particular immune cells involved in TB immunity[34,65–67], and their localization and kinetics[32,41,61,68], have been extensively reviewed in the past few years. Here, we discuss how the heterogeneity of the immune response in TB influences the local granuloma microenvironment. In a recent study in macaques, we demonstrated that the vast

majority of granulomas begin with a single bacterium[37]. The bacteria grow within the nascent granuloma up to 4 weeks following infection, at which time the adaptive immune response is engaged. After 4 weeks, bacterial killing is seen in the majority of granulomas, and approximately 10% of granulomas are sterilized by 11 weeks; sterilization increased even in the macaques that were developing active TB. However, there was substantial variability in the frequency of granulomas that were sterilized in each monkey, again supporting the variability of granuloma outcomes between individuals.

So, what is the variability of the host adaptive immune response in granulomas? In a pilot study that examined lung granulomas from patients with chronic pulmonary TB, Subbian *et al.*[38] found significant variability in the density of T cells and in the extent of fibrosis, which varied in accordance with immune activation and bacterial load. The authors suggested that the observed heterogeneity was at least partly driven by differential immune profiles in individual granuloma microenvironments that reflected the maturation state of the granuloma. A similar study[62] that carefully measured T cell cytokine responses in discrete granulomas from infected macaques noted considerable heterogeneity across and within animals. As was observed by Subbian *et al.*38 in the resected human tissue, Gideon *et al.*[62] found extensive variability in the total numbers and phenotypes of T cells, as well as a wide range of cytokine profiles and bacterial burdens within individual granulomas, even within the same macaque. Fewer than 10% of granuloma T cells were found to produce cytokines following *M. tuberculosis* antigen stimulation, and the majority of these cells produced only a single cytokine[62]; the dominant cytokines included IFNγ, interleukin-2 (IL-2), tumour necrosis factor (TNF), IL-10 and IL-17. However, when viewed as a whole, granulomas were found to be multifunctional cytokine environments with different T cells contributing a broader cytokine repertoire.

Subsequent comparisons of differential T cell responses with granuloma bacterial burden have shown that a combination of pro-inflammatory and anti-inflammatory cytokines produced by different T cells — for example, IL-10 with IL-17 — best associated with granuloma sterility, reiterating a necessity for balanced cytokine responses in achieving bacterial clearance[30,61]. However, we do not understand the mechanisms that lead to the heterogeneity of adaptive immune responses in granulomas or the extent to which these differences are driven by the described variability in innate immune responses. The relatively small population of granuloma T cells that express cytokines raises questions about the remainder of the T cells. For example, are they non-TB-specific T cells that are just recruited in response to granulomatous inflammation? Does the initial interaction of *M. tuberculosis* with a specific type of phagocyte skew the T cell responses in the granuloma that emerges? Is the local adaptive immune response modulated by the local initial innate immune response? Is there exhaustion of T cells in the granuloma, resulting in few responding T cells? There are many potential mechanisms for the regulation of T cell responses in granulomas, and further careful analysis of individual granulomas may provide answers to these questions. Nonetheless, the available data on granuloma T cells parallel the observations and data above, which indicate that individual granulomas have unique and distinct inflammatory signatures[46] and killing potentials[37], and this suggests that there is an overall relationship between local host immune responses, the subsequent skewing of inflammation and differential bacterial containment that governs granuloma fate (FIG. 2).

There are recent studies[40,51,69] that support this paradigm. In one study, Coleman *et al.*[40] used serial FDG PET–CT imaging to evaluate granuloma dynamics in macaques that subsequently developed active disease or LTBI following a low-dose *M. tuberculosis* infection[40]. This revealed two features observed in the first 3–6 weeks post infection that were associated with the eventual onset of active TB (months later): first, the development of new pulmonary granulomas (that is, dissemination); and second, increased inflammation in pulmonary granulomas, as measured by FDG avidity. These two observations not only emphasize the importance of the early interactions between the host and pathogen in determining host outcome[41], but also indicate that the early cellular response of a granuloma is linked to inflammation that then influences dissemination risk. Very recent work in our laboratories has confirmed these findings, demonstrating that only a subset of granulomas disseminate to form new, productive granulomas and showing that granuloma size at 4–5 weeks post infection correlates with dissemination[69]. Recent work in the zebrafish–*Mycobacterium marinum* model has identified similar crucial events in granuloma formation and organization that depend on the macrophage-specific reprogramming of adhesion pathways that are dependent on epithelial cadherin (E-cadherin; also known as cadherin 1)[70]. Disruption of this axis resulted in disordered granuloma formation and increased host clearance following increased neutrophil access. These observations once more link cellular responses with granuloma trajectory, and further suggest that variability may be encoded early in infection and influenced by multiple immune components.

In a separate macaque study, we evaluated reactivation risk in a large number ($n = 26$) of clinically latent cynomolgus macaques using FDG PET–CT imaging before and during the administration of antibody specific for TNF[51], which is a trigger for the reactivation of infection in humans[71–73] and macaques. We previously showed that not all macaques undergo infection reactivation during 8 weeks of TNF neutralization[74], and this allowed us to study which factors correlated with the risk of reactivation. FDG PET–CT scans performed before TNF neutralization identified increased lung inflammation and the presence of an extrapulmonary site of infection as features that predict reactivation risk with 92% sensitivity and specificity[51]. Using this metric, we then classified a separate set of 25 clinically latent macaques as being at high or low risk of reactivation, but necropsied them without TNF neutralization. The macaques that were at high risk of infection had at least one granuloma with a relatively high bacterial burden, suggesting that, first, the robust control of infection at all sites is essential for preventing reactivation, and second, the reactivation risk is granuloma specific, once more returning our focus to the individual granuloma and its unique immune response. Further support for this idea came from data indicating that only a subset of granulomas in each macaque had dynamic changes in FDG avidity or size during TNF neutralization; this suggests that some granulomas are more TNF dependent than others in the same animal. It is important to note that the stable granulomas during TNF neutralization were not necessarily sterile, yet they did not seem to be affected by the loss of TNF.

Thus, our current understanding of granuloma-specific host responses can be considered as a set of mathematical equations that can be solved in many different ways by each granuloma to reach the result of infection control (FIG. 3). In this scenario, modulating one aspect of the immune response could cause one or a few granulomas to lose control of the infection,

leading to dissemination and disease, while other granulomas are unaffected. Indeed, computational modelling of TB granulomas has demonstrated that there are many pathways to containment or loss of control, and the ultimate outcome depends on the particular combination of different factors[75–78]. Again, it seems from these data that only one or a few granulomas need to fail — either during chronic infection or LTBI, or even in early infection — to result in active TB. Collectively, these findings provide a basis to explain the heterogeneity among infected hosts, as we believe variable granuloma contexts — particularly early in infection[41] — initiate heterogeneous disease states (FIG. 4).

Bacterial heterogeneity

The heterogeneity in granuloma course raises questions both about the contribution of bacterial processes to granuloma fate and about the impact of the heterogeneous granuloma state on the bacterial population. For the purpose of this Review, we define ‘deterministic’ bacterial heterogeneity as the ways in which bacterial variability might influence host–pathogen interactions. We then briefly review the extensive literature on the ways in which differences in granuloma course can also affect bacterial cell state — here termed ‘reactive’ bacterial variability — and consequently the efficacy of antibiotic treatment.

Is there deterministic bacterial variation?

In considering how differences in the course of infection or granuloma fate emerge, one possibility is that variation in the bacterial population helps to shape granuloma trajectory. It is clear from studies of fixed genetic variants that differences between bacteria can influence host responses. For example, compared with the *M. tuberculosis* H37Rv strain, *M. tuberculosis* strains from the East Asian lineage induce a distinct immunopathological response in mouse models, with larger areas of pneumonia and relatively less granulomatous lung tissue[79,80]. These differences in immunopathology have been linked, at least partially, to differences in the production of distinct cell wall glycolipids[81], which result in the differential activation of Toll-like receptors and resulting inflammatory responses[82–85].

Although the importance of differences between *M. tuberculosis* strains has become clear, it is less clear whether bacterial variation contributes to differences in granuloma trajectory in a given individual. Bacterial barcoding analyses have demonstrated that the majority of TB granulomas are founded by a single bacterium[37]. From studies in macaques, there seems to be an early wave of dissemination during the first 6–8 weeks of infection in which most granulomas are established, although later dissemination is not uncommon[69]. Thus, there is a very tight bottleneck on the bacterial population early in infection at the time of secondary granuloma formation. However, this is only likely to result in a significant bacterial founder effect if there is biologically meaningful variation in the bacterial population.

In many infections, the infecting pathogen population is large and already diverse, or there is sufficient genomic instability that within a short period of time there is tremendous opportunity for genetic diversification of the pathogen population[86,87]. However, *M. tuberculosis* is comparatively genetically monomorphic, and the infectious dose is very low[88]. Whole-genome sequencing of macaque granulomas and human samples indicates that even after extended periods of infection, little genetic diversity accumulates in the bacterial

population[36,89,90]. The epidemiological guide is that fewer than five single-nucleotide polymorphisms separate bacteria within a given individual[36,89,90], making it unlikely that genetic diversification of the *M. tuberculosis* population followed by sharp bottlenecking creates sufficiently distinct pathogen populations to drive differences in granuloma fate.

By contrast, there is a surprising amount of phenotypic heterogeneity within *M. tuberculosis* populations. More than 50 years ago, Bigger[91] described the existence of a small, phenotypically distinct subpopulation of bacteria (in his studies of *Staphylococcus pyogenes*) that were recognizable as non-growing, highly antibiotic-tolerant cells; he termed these cells 'persisters'. More recently, considerable efforts to identify phenotypically distinct subpopulations of *M. tuberculosis* cells have led to the recognition that there are likely to be many phenotypic variants in any given *M. tuberculosis* population and that these variants arise through a variety of mechanisms[92,93]. This variability has typically been described in terms of cell-to-cell differences in antibiotic susceptibility; it is unclear to what extent this variation results in differences between bacterial cells in terms of their pathogenicity. It is also unclear whether there are mechanisms to generate variation in bacterial pathogenicity at a sufficiently high frequency but also of sufficiently long duration to plausibly drive granuloma fate. Asymmetric growth and division generates high-frequency variants, but their phenotypes change rapidly over successive cycles of cell division[94]. Studies describing subpopulations of drug-resistant cells that arise at a relatively high frequency and show semi-stable drug resistance across several generations suggest that epigenetic mechanisms may exist and fill this gap[95]. However, the relative importance of any of these populations has been hard to address experimentally because genetic mechanisms to perturb these distinct cell states have not been identified.

Reactive bacterial variation.

The concept of functionally important bacterial heterogeneity that arises in distinct granuloma environments is much better established in the TB field than is the concept of deterministic bacterial variation. Mitchison and colleagues[96] were among the first proponents of this model, which they invoked to explain the results of the early trials of combination drug treatment for TB that were conducted in the 1950s and 1960s. In the earliest studies of various antimicrobial regimens, investigators recognized that treatment failure due to the emergence of drug resistance occurred frequently even when patients were treated with two fully effective antibiotics. Given the limited capacity of the bacterium for genetic diversification due to its low mutation rate and relatively small population size, these data were difficult to explain unless there were subpopulations of organisms that were not exposed to or functionally susceptible to both drugs[96]. From these data and experimental studies of drug efficacy, Mitchison[93] proposed that in a given individual there are different subpopulations of organisms that have distinct drug susceptibilities based on growth rate differences or granuloma pH.

The Mitchison model did not claim that these functionally distinct bacterial subpopulations were spatially segregated in distinct granulomas. However, the model was consistent with pioneering work by Medlar *et al.*[97] who undertook histopathological and microbiological examination of thousands of TB granulomas obtained at autopsy, and noted that some

'closed' granulomas (that were not accessible to an airway) contained microscopically visible but unculturable bacteria, whereas other 'open' granulomas were teeming with readily culturable organisms. Subsequent work confirmed these findings[98,99], and led investigators to further suggest that at least a proportion of the bacterial population in closed granulomas was not dead but instead viable and unculturable[100]. These findings are early evidence of the spectrum of granulomas in a given individual as reflected in a variety of bacterial states; closed granulomas were first described in samples from patients who died of active TB and who also had open granulomas characterized by high numbers of readily culturable organisms. This and other work led investigators to postulate that under certain types of environmental stress — most notably hypoxia — the bacterium enters a state of so-called 'non-replicating persistence', which is characterized by metabolic remodelling and multidrug tolerance[101]. Importantly, these changes in bacterial cell state correlate with changes in cell wall composition that alter the acid fastness of the organism and thus its ability to be detected via Ziehl–Neelsen staining[102]. Acid-fast staining-negative TB bacilli have been associated with stages of infection in which the bacterial population is not actively replicating[103], although this association is imperfect as large replicating populations of weakly acid-fast bacteria have been described in some animal models[104].

As these discrepancies suggest, there is likely to be a range of bacterial states *in vivo* that are only crudely probed by metrics such as aggregate growth rate and acid fastness. Studies suggest that even in sputum — so presumably coming from open granulomas — there are subpopulations of 'differentially culturable' *M. tuberculosis* bacilli[105,106]. However, it has been surprisingly challenging to develop molecular definitions for the state of *M. tuberculosis* in TB granulomas in humans or non-human primates, and even more difficult to establish the molecular mechanisms by which bacterial subpopulations arise and are maintained. In part, this question has become hard to address because of the success of antibiotic therapy.

Unlike Medlar *et al.*[97], researchers today do not have access to thousands of human TB granulomas. Even in macaque granulomas, it has proved technically difficult to probe *M. tuberculosis* state, for example, by transcriptional profiling, in individual granulomas, because many granulomas have relatively few organisms, from which it is difficult to obtain transcripts. Several studies have successfully probed bacterial gene expression and phenotypic state of *M. tuberculosis* isolated from sputum. Although these studies capture bacteria only from the granulomas that progress, even in *M. tuberculosis* isolated from sputum there is evidence of metabolic reprogramming[105–109]. These results are broadly consistent with studies in mice in which bacterial reporter strains have provided single-cell measures of pathogen state that suggest that the adaptive immune response drives the emergence of a subpopulation of metabolically active but non-growing bacterial cells[110,111]. It remains to be determined whether this is more nuanced: that is, whether specific immune environments drive distinct bacterial responses or whether there is a feedback loop such that the emergent bacterial populations then promote different immune reactions.

Variable responses to drug treatment.

Although the contribution of specific bacterial populations — in distinct metabolic states — to the rate of progression to disease remains unclear, there is strong evidence that non-replicating or differentially culturable bacteria are more tolerant of antibiotics, which makes granuloma state an important determinant of treatment response. Current treatment for drug-sensitive, active TB remains the standard 6-month regimen of isoniazid, rifampin, pyrazinamide and ethambutol. This regimen provides cure rates of 90–95% in trial TB control programmes[21], but several attempts to shorten the treatment period have failed[112]. It is thought that the prolonged duration of TB treatment is dictated by the fact that at any given time some bacteria are functionally tolerant to the administered drugs, and this is fundamentally a result of granuloma heterogeneity. The search for antibiotics that are active against bacteria across a range of granuloma environments has led to the identification of new classes of antibiotics such as the nitroimidazoles[113–115] and may explain the clinical potency of drugs that target energy metabolism such as bedaquiline[116,117].

Granuloma state also influences the activity and concentrations of different antibiotics independent of bacterial state. This was first recognized for the first-line antibiotic pyrazinamide, which is a crucial agent for bringing the duration of TB treatment down to 6 months (a reduction compared with the very lengthy treatments of earlier times[118]). Pyrazinamide is only active under acidic conditions and thus is thought to be particularly useful against bacteria in inflammatory environments. More recently, it has become clear that different drugs do not access all TB granulomas equally. Using matrix-assisted laser desorption ionization (MALDI) mass spectrometry, Prideaux *et al.*[119] demonstrated that some drugs — for example, the second-line agent moxifloxacin — diffuse poorly into caseum, whereas other drugs, such as rifampicin, efficiently penetrate all granulomas. Thus, differences in granuloma fate and anatomy can result in uneven drug delivery, and at a minimum provide a framework for constructing drug regimens that are both more effective and better protected against the emergence of drug resistance.

Conclusions

In this Review, we have developed a model for the biological heterogeneity of TB infection, which manifests at the level of individual granulomas through variability in inflammation, the local adaptive immune response and bacterial state. Granuloma heterogeneity has implications for how we study TB, and highlights the importance of tissue-level analyses rather than analyses of circulating immune cells or even tissue samples that contain multiple granulomas. It also indicates a great opportunity to define the features of successful and unsuccessful immune responses within a given individual. We anticipate that these types of study will be most useful as a foundation for rational vaccine design, and potentially help investigators to determine whether it is necessary to target one or multiple paths to achieve bacterial containment or sterility. Although there remains much work to be done, we are optimistic that embracing this paradigm of granuloma heterogeneity is central to skewing the host–pathogen ‘arms race’ in favour of humans.

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References

1. Getahun H, Matteelli A, Chaisson RE & Raviglione M Latent Mycobacterium tuberculosis infection. N. Engl. J. Med 372, 2127–2135 (2015). [PubMed: 26017823]
2. Corbett EL et al. The growing burden of tuberculosis: global trends and interactions with the HIV epidemic. Arch. Intern. Med 163, 1009–1021 (2003). [PubMed: 12742798]
3. Lawn SD & Zumla AI Tuberculosis. Lancet 378, 57–72 (2011). [PubMed: 21420161]
4. World Health Organization. Global Tuberculosis Report 2015 (World Health Organization, 2015).
5. Andrews JR et al. Risk of progression to active tuberculosis following reinfection with Mycobacterium tuberculosis. Clin. Infect. Dis 54, 784–791 (2012). [PubMed: 22267721]
6. Lin PL & Flynn JL Understanding latent tuberculosis: a moving target. J. Immunol 185, 15–22 (2010). [PubMed: 20562268]
7. Chen RY et al. PET/CT imaging correlates with treatment outcome in patients with multidrug-resistant tuberculosis. Sci. Transl Med 6, 265ra166 (2014).
8. Lenzini L, Rottoli P & Rottoli L The spectrum of human tuberculosis. Clin. Exp. Immunol 27, 230–237 (1977). [PubMed: 849655]
9. Poulsen A Some clinical features of tuberculosis. Acta Tuberc. Scand 33, 37–92 (1957). [PubMed: 13424392]
10. Barry CE, 3rd et al. The spectrum of latent tuberculosis: rethinking the biology and intervention strategies. Nat. Rev. Microbiol 7, 845–855 (2009). [PubMed: 19855401]
11. Young DB, Gideon HP & Wilkinson RJ Eliminating latent tuberculosis. Trends Microbiol 17, 183–188 (2009). [PubMed: 19375916]
12. Esmail H et al. Characterization of progressive HIV-associated tuberculosis using 2-deoxy-2-[18F]fluoro- D-glucose positron emission and computed tomography. Nat. Med 22, 1090–1093 (2016). [PubMed: 27595321] This study provides evidence of subclinical, active disease in a subset of adults infected with HIV-1 and latent TB, reiterating the spectrum of disease seen in human TB.
13. Capuano SV et al. Experimental Mycobacterium tuberculosis infection of cynomolgus macaques closely resembles the various manifestations of human M. tuberculosis infection. Infect. Immun 71, 5831–5844 (2003). [PubMed: 14500505]
14. Lin PL et al. Quantitative comparison of active and latent tuberculosis in the cynomolgus macaque model. Infect. Immun 77, 4631–4642 (2009). [PubMed: 19620341]
15. Berry MP et al. An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. Nature 466, 973–977 (2010). [PubMed: 20725040] Using transcriptional profiling of whole blood, this study identifies a discriminatory, IFN-inducible, neutrophil-mediated signature of active TB. This work also provides new insights into the spectrum of TB by revealing a subset of patients with latent TB who have signatures that overlap closely with the signature of active disease.
16. Maertzdorf J et al. Functional correlations of pathogenesis-driven gene expression signatures in tuberculosis. PLoS ONE 6, e26938 (2011). [PubMed: 22046420]
17. Ottenhoff TH et al. Genome-wide expression profiling identifies type 1 interferon response pathways in active tuberculosis. PLoS ONE 7, e45839 (2012). [PubMed: 23029268]
18. Cliff JM, Kaufmann SH, McShane H, van Helden P & O'Garra A The human immune response to tuberculosis and its treatment: a view from the blood. Immunol. Rev 264, 88–102 (2015). [PubMed: 25703554]

19. Bloom CI et al. Detectable changes in the blood transcriptome are present after two weeks of antituberculosis therapy. PLoS ONE 7, e46191 (2012). [PubMed: 23056259]
20. Cliff JM et al. Distinct phases of blood gene expression pattern through tuberculosis treatment reflect modulation of the humoral immune response. J. Infect. Dis 207, 18–29 (2013). [PubMed: 22872737]
21. Zumla A, Nahid P & Cole ST Advances in the development of new tuberculosis drugs and treatment regimens. Nat. Rev. Drug Discov 12, 388–404 (2013). [PubMed: 23629506]
22. Koul A, Arnoult E, Lounis N, Guillemont J & Andries K The challenge of new drug discovery for tuberculosis. Nature 469, 483–490 (2011). [PubMed: 21270886]
23. Blankley S et al. The transcriptional signature of active tuberculosis reflects symptom status in extra-pulmonary and pulmonary tuberculosis. PLoS ONE 11, e0162220 (2016). [PubMed: 27706152]
24. Banchereau R et al. Host immune transcriptional profiles reflect the variability in clinical disease manifestations in patients with Staphylococcus aureus infections. PLoS ONE 7, e34390 (2012). [PubMed: 22496797]
25. Zak DE et al. A blood RNA signature for tuberculosis disease risk: a prospective cohort study. Lancet 387, 2312–2322 (2016). [PubMed: 27017310]
26. Mehra S et al. Transcriptional reprogramming in nonhuman primate (rhesus macaque) tuberculosis granulomas. PLoS ONE 5, e12266 (2010). [PubMed: 20824205]
27. Gideon HP, Skinner JA, Baldwin N, Flynn JL & Lin PL Early whole blood transcriptional signatures are associated with severity of lung inflammation in cynomolgus macaques with Mycobacterium tuberculosis infection. J. Immunol 197, 4817–4828 (2016). [PubMed: 27837110]
28. Williams GT & Williams WJ Granulomatous inflammation — a review. J. Clin. Pathol 36, 723–733 (1983). [PubMed: 6345591]
29. Russell DG, Cardona PJ, Kim MJ, Allain S & Altare F Foamy macrophages and the progression of the human tuberculosis granuloma. Nat. Immunol 10, 943–948 (2009). [PubMed: 19692995]
30. Flynn JL, Chan J & Lin PL Macrophages and control of granulomatous inflammation in tuberculosis. Mucosal Immunol 4, 271–278 (2011). [PubMed: 21430653]
31. Davis JM & Ramakrishnan L The role of the granuloma in expansion and dissemination of early tuberculous infection. Cell 136, 37–49 (2009). [PubMed: 19135887]
32. Ramakrishnan L Revisiting the role of the granuloma in tuberculosis. Nat. Rev. Immunol 12, 352–366 (2012). [PubMed: 22517424]
33. Flynn JL Mutual attraction: does it benefit the host or the bug? Nat. Immunol 5, 778–779 (2004). [PubMed: 15282559]
34. O'Garra A et al. The immune response in tuberculosis. Annu. Rev. Immunol 31, 475–527 (2013). [PubMed: 23516984]
35. Canetti G The Tubercle Bacillus in the Pulmonary Lesion of Man; Histobacteriology and its Bearing on the Therapy of Pulmonary Tuberculosis (Springer, 1955).
36. Ford CB et al. Use of whole genome sequencing to estimate the mutation rate of Mycobacterium tuberculosis during latent infection. Nat. Genet 43, 482–486 (2011). [PubMed: 21516081]
37. Lin PL et al. Sterilization of granulomas is common in active and latent tuberculosis despite within-host variability in bacterial killing. Nat. Med 20, 75–79 (2014). [PubMed: 24336248] This study is the first to demonstrate that the majority of granulomas are seeded by a single bacillus and that there is differential bacterial killing of granulomas within the host that is independent of host status. These findings shift the focus to the local granuloma level and suggest that the individual trajectories of granulomas influence the clinical outcome of infection.
38. Subbian S et al. Lesion-specific immune response in granulomas of patients with pulmonary tuberculosis: a pilot study. PLoS ONE 10, e0132249 (2015). [PubMed: 26133981]
39. Lenaerts A, Barry CE, III & Dartois V Heterogeneity in tuberculosis pathology, microenvironments and therapeutic responses. Immunol. Rev 264, 288–307 (2015). [PubMed: 25703567]
40. Coleman MT et al. Early changes by 18fluorodeoxyglucose positron emission tomography coregistered with computed tomography predict outcome after Mycobacterium tuberculosis infection in cynomolgus macaques. Infect. Immun 82, 2400–2404 (2014). [PubMed: 24664509]

This study reveals that early granuloma dissemination and inflammation influence the clinical outcome of infection in infected cynomolgus macaques.

41. Cadena AM, Flynn JL & Fortune SM The importance of first impressions: early events in Mycobacterium tuberculosis infection influence outcome. mBio 7, e00342–16 (2016). [PubMed: 27048801]
42. Lieberman TD et al. Genomic diversity in autopsy samples reveals within-host dissemination of HIV-associated Mycobacterium tuberculosis. Nat. Med 22, 1470–1474 (2016). [PubMed: 27798613]
43. Cooper AM, Mayer-Barber KD & Sher A Role of innate cytokines in mycobacterial infection. Mucosal Immunol 4, 252–260 (2011). [PubMed: 21430655]
44. Lerner TR, Borel S & Gutierrez MG The innate immune response in human tuberculosis. Cell. Microbiol 17, 1277–1285 (2015). [PubMed: 26135005]
45. Lin PL et al. Early events in Mycobacterium tuberculosis infection in cynomolgus macaques. Infect. Immun 74, 3790–3803 (2006). [PubMed: 16790751]
46. Marakalala MJ et al. Inflammatory signaling in human tuberculosis granulomas is spatially organized. Nat. Med 22, 531–538 (2016). [PubMed: 27043495] By analysing the proteomes of human and rabbit granulomas, this study reveals that pro-inflammatory and anti-inflammatory programmes occur simultaneously, but in physically distinct compartments.
47. Fallahi-Sichani M, El-Kebir M, Marino S, Kirschner DE & Linderman JJ Multiscale computational modeling reveals a critical role for TNF-α receptor 1 dynamics in tuberculosis granuloma formation. J. Immunol 186, 3472–3483 (2011). [PubMed: 21321109]
48. Guirado E & Schlesinger LS Modeling the Mycobacterium tuberculosis granuloma — the critical battlefield in host immunity and disease. Front. Immunol 4, 98 (2013). [PubMed: 23626591]
49. Kaplan G et al. Mycobacterium tuberculosis growth at the cavity surface: a microenvironment with failed immunity. Infect. Immun 71, 7099–7108 (2003). [PubMed: 14638800]
50. Ernst JD The immunological life cycle of tuberculosis. Nat. Rev. Immunol 12, 581–591 (2012). [PubMed: 22790178]
51. Lin PL et al. PET CT identifies reactivation risk in cynomolgus macaques with latent M. tuberculosis. PLoS Pathog 12, e1005739 (2016). [PubMed: 27379816]
52. Malherbe ST et al. Persisting positron emission tomography lesion activity and Mycobacterium tuberculosis mRNA after tuberculosis cure. Nat. Med 22, 1094–1100 (2016). [PubMed: 27595324] The authors of this paper show that in spite of a standard curative 6-month regimen, there were multiple patients with persistent pulmonary inflammation that was coincident with the detection of *M. tuberculosis* mRNA. These observations highlight the variability of treatment outcome in individual granulomas, even after a year of successful treatment, and implicate a complementary, ongoing requirement for immunity in maintaining sterility.
53. Mattila JT et al. Microenvironments in tuberculous granulomas are delineated by distinct populations of macrophage subsets and expression of nitric oxide synthase and arginase isoforms. J. Immunol 191, 773–784 (2013). [PubMed: 23749634]
54. Tobin DM et al. Host genotype-specific therapies can optimize the inflammatory response to mycobacterial infections. Cell 148, 434–446 (2012). [PubMed: 22304914]
55. Chen M et al. Lipid mediators in innate immunity against tuberculosis: opposing roles of PGE2 and LXA4 in the induction of macrophage death. J. Exp. Med 205, 2791–2801 (2008). [PubMed: 18955568]
56. Divangahi M et al. Mycobacterium tuberculosis evades macrophage defenses by inhibiting plasma membrane repair. Nat. Immunol 10, 899–906 (2009). [PubMed: 19561612]
57. Divangahi M, Desjardins D, Nunes-Alves C, Remold HG & Behar SM Eicosanoid pathways regulate adaptive immunity to Mycobacterium tuberculosis. Nat. Immunol 11, 751–758 (2010). [PubMed: 20622882]
58. Divangahi M, Behar SM & Remold H Dying to live: how the death modality of the infected macrophage affects immunity to tuberculosis. Adv. Exp. Med. Biol 783, 103–120 (2013). [PubMed: 23468106]
59. Mayer-Barber KD et al. Host-directed therapy of tuberculosis based on interleukin-1 and type I interferon crosstalk. Nature 511, 99–103 (2014). [PubMed: 24990750] This study establishes a

link between IL-1 and type I IFNs that is mediated by eicosanoids. It also confirms the role of host-directed manipulation of the eicosanoid balance in favour of prostaglandin E^2 in resolving disease exacerbations *in vivo*.

60. Wallis RS & Hafner R Advancing host-directed therapy for tuberculosis. Nat. Rev. Immunol 15, 255–263 (2015). [PubMed: 25765201]
61. Orme IM, Robinson RT & Cooper AM The balance between protective and pathogenic immune responses in the TB-infected lung. Nat. Immunol 16, 57–63 (2015). [PubMed: 25521685]
62. Gideon HP et al. Variability in tuberculosis granuloma T cell responses exists, but a balance of pro- and anti-inflammatory cytokines is associated with sterilization. PLoS Pathog 11, e1004603 (2015). [PubMed: 25611466]
63. Cilfone NA, Perry CR, Kirschner DE & Linderman JJ Multi-scale modeling predicts a balance of tumor necrosis factor-α and interleukin-10 controls the granuloma environment during Mycobacterium tuberculosis infection. PLoS ONE 8, e68680 (2013).
64. Cilfone NA et al. Computational modeling predicts IL-10 control of lesion sterilization by balancing early host immunity-mediated antimicrobial responses with caseation during Mycobacterium tuberculosis infection. J. Immunol 194, 664–677 (2015). [PubMed: 25512604]
65. Srivastava S, Ernst JD & Desvignes L Beyond macrophages: the diversity of mononuclear cells in tuberculosis. Immunol. Rev 262, 179–192 (2014). [PubMed: 25319335]
66. Behar SM, Carpenter SM, Booty MG, Barber DL & Jayaraman P Orchestration of pulmonary T cell immunity during Mycobacterium tuberculosis infection: immunity interruptus. Semin. Immunol 26, 559–577 (2014). [PubMed: 25311810]
67. Guirado E, Schlesinger LS & Kaplan G Macrophages in tuberculosis: friend or foe. Semin. Immunopathol 35, 563–583 (2013). [PubMed: 23864058]
68. Sia JK, Georgieva M & Rengarajan J Innate immune defenses in human tuberculosis: an overview of the interactions between Mycobacterium tuberculosis and innate immune cells. J. Immunol. Res 2015, 747543 (2015). [PubMed: 26258152]
69. Martin CJ et al. Digitally barcoding Mycobacterium tuberculosis reveals in vivo infection dynamics in the macaque model of tuberculosis. mBio 8, e00312–17 (2017). [PubMed: 28487426]
70. Cronan MR et al. Macrophage epithelial reprogramming underlies mycobacterial granuloma formation and promotes infection. Immunity 45, 861–876 (2016). [PubMed: 27760340]
71. Keane J et al. Tuberculosis associated with infliximab, a tumor necrosis factor α-neutralizing agent. N. Engl. J. Med 345, 1098–1104 (2001). [PubMed: 11596589]
72. Bruns H et al. Anti-TNF immunotherapy reduces CD8+ T cell-mediated antimicrobial activity against Mycobacterium tuberculosis in humans. J. Clin. Invest 119, 1167–1177 (2009). [PubMed: 19381021]
73. Maini R et al. Infliximab (chimeric anti-tumour necrosis factor α monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial. ATTRACT Study Group. Lancet 354, 1932–1939 (1999). [PubMed: 10622295]
74. Lin PL et al. Tumor necrosis factor neutralization results in disseminated disease in acute and latent Mycobacterium tuberculosis infection with normal granuloma structure in a cynomolgus macaque model. Arthritis Rheum 62, 340–350 (2010). [PubMed: 20112395]
75. Marino S et al. Computational and empirical studies predict Mycobacterium tuberculosis-specific T cells as a biomarker for infection outcome. PLoS Comput. Biol 12, e1004804 (2016). [PubMed: 27065304]
76. Marino S et al. Macrophage polarization drives granuloma outcome during Mycobacterium tuberculosis infection. Infect. Immun 83, 324–338 (2015). [PubMed: 25368116]
77. Kirschner DE & Linderman JJ Mathematical and computational approaches can complement experimental studies of host–pathogen interactions. Cell. Microbiol 11, 531–539 (2009). [PubMed: 19134115]
78. Kirschner DE, Hunt CA, Marino S, Fallahi-Sichani M & Linderman JJ Tuneable resolution as a systems biology approach for multi-scale, multi-compartment computational models. Wiley Interdiscip. Rev. Syst. Biol. Med 6, 289–309 (2014). [PubMed: 24810243]

79. Lopez B et al. A marked difference in pathogenesis and immune response induced by different Mycobacterium tuberculosis genotypes. Clin. Exp. Immunol 133, 30–37 (2003). [PubMed: 12823275]
80. Ribeiro SC et al. Mycobacterium tuberculosis strains of the modern sublineage of the Beijing family are more likely to display increased virulence than strains of the ancient sublineage. J. Clin. Microbiol 52, 2615–2624 (2014). [PubMed: 24829250]
81. Reed MB et al. A glycolipid of hypervirulent tuberculosis strains that inhibits the innate immune response. Nature 431, 84–87 (2004). [PubMed: 15343336]
82. Dormans J et al. Correlation of virulence, lung pathology, bacterial load and delayed type hypersensitivity responses after infection with different Mycobacterium tuberculosis genotypes in a BALB/c mouse model. Clin. Exp. Immunol 137, 460–468 (2004). [PubMed: 15320894]
83. Manca C et al. Differential monocyte activation underlies strain-specific Mycobacterium tuberculosis pathogenesis. Infect. Immun 72, 5511–5514 (2004). [PubMed: 15322056]
84. Portevin D, Gagneux S, Comas I & Young D Human macrophage responses to clinical isolates from the Mycobacterium tuberculosis complex discriminate between ancient and modern lineages. PLoS Pathog 7, e1001307 (2011). [PubMed: 21408618]
85. Carmona J et al. Mycobacterium tuberculosis strains are differentially recognized by TLRs with an impact on the immune response. PLoS ONE 8, e67277 (2013). [PubMed: 23840651]
86. Grant AJ et al. Modelling within-host spatiotemporal dynamics of invasive bacterial disease. PLoS Biol 6, e74 (2008). [PubMed: 18399718]
87. Joseph SB, Swanstrom R, Kashuba AD & Cohen MS Bottlenecks in HIV-1 transmission: insights from the study of founder viruses. Nat. Rev. Microbiol 13, 414–425 (2015). [PubMed: 26052661]
88. Jacobs AL Infective dose in pulmonary tuberculosis. Tubercle 22, 266–271 (1941).
89. Walker TM et al. Whole-genome sequencing to delineate Mycobacterium tuberculosis outbreaks: a retrospective observational study. Lancet Infect. Dis 13, 137–146 (2013). [PubMed: 23158499]
90. Bryant JM et al. Inferring patient to patient transmission of Mycobacterium tuberculosis from whole genome sequencing data. BMC Infect. Dis 13, 110 (2013). [PubMed: 23446317]
91. Bigger JW Treatment of staphylococcal infections with penicillin by intermittent sterilisation. Lancet 244, 497–500 (1944).
92. Kester JC & Fortune SM Persisters and beyond: mechanisms of phenotypic drug resistance and drug tolerance in bacteria. Crit. Rev. Biochem. Mol. Biol 49, 91–101 (2014). [PubMed: 24328927]
93. Mitchison DA How drug resistance emerges as a result of poor compliance during short course chemotherapy for tuberculosis. Int. J. Tuberc. Lung Dis 2, 10–15 (1998). [PubMed: 9562106]
94. Kieser KJ & Rubin EJ How sisters grow apart: mycobacterial growth and division. Nat. Rev. Microbiol 12, 550–562 (2014). [PubMed: 24998739]
95. Wakamoto Y et al. Dynamic persistence of antibiotic-stressed mycobacteria. Science 339, 91–95 (2013). [PubMed: 23288538]
96. Fox W, Ellard GA & Mitchison DA Studies on the treatment of tuberculosis undertaken by the British Medical Research Council tuberculosis units, 1946–1986, with relevant subsequent publications. Int. J. Tuberc. Lung Dis 3, S231–S279 (1999). [PubMed: 10529902]
97. Medlar EM, Bernstein S & Steward DM A bacteriologic study of resected tuberculous lesions. Am. Rev. Tuberc 66, 36–43 (1952). [PubMed: 14933745]
98. Beck F & Yegian D A study of the tubercle bacillus in resected pulmonary lesions. Am. Rev. Tuberc 66, 44–51 (1952). [PubMed: 14933746]
99. Canetti G Anatomical and bacteriological changes in tuberculous lesions under the influence of antibiotics and chemotherapy. Bull. Int. Union Tuberc 24, 144–240 (1954).
100. Salkin D & Wayne LG The bacteriology of resected tuberculous pulmonary lesions. I. The effect of interval between reversal of infectiousness and subsequent surgery. Am. Rev. Tuberc 74, 376–387 (1956). [PubMed: 13354921]
101. Wayne LG & Sohaskey CD Nonreplicating persistence of Mycobacterium tuberculosis. Annu. Rev. Microbiol 55, 139–163 (2001). [PubMed: 11544352]

102. Vilcheze C & Kremer L Acid-fast positive and acid-fast negative Mycobacterium tuberculosis: the Koch paradox. Microbiol. Spectr http://dx.doi.org/10.1128/microbiolspec.TBTB2-0003-2015 (2017).
103. Seiler P et al. Cell-wall alterations as an attribute of Mycobacterium tuberculosis in latent infection. J. Infect. Dis 188, 1326–1331 (2003). [PubMed: 14593589]
104. Obregon-Henao A et al. Cortisone-forced reactivation of weakly acid fast positive Mycobacterium tuberculosis in guinea pigs previously treated with chemotherapy. Mycobact. Dis 2, 1000116 (2012).
105. Mukamolova GV, Turapov O, Malkin J, Woltmann G & Barer MR Resuscitation-promoting factors reveal an occult population of tubercle bacilli in sputum. Am. J. Respir. Crit. Care Med 181, 174–180 (2010). [PubMed: 19875686]
106. Chengalroyen MD et al. Detection and quantification of differentially culturable tubercle bacteria in sputum from patients with tuberculosis. Am. J. Respir. Crit. Care Med 194, 1532–1540 (2016). [PubMed: 27387272]
107. Garton NJ et al. Cytological and transcript analyses reveal fat and lazy persister-like bacilli in tuberculous sputum. PLoS Med 5, e75 (2008). [PubMed: 18384229]
108. Walter ND et al. Transcriptional adaptation of drug-tolerant Mycobacterium tuberculosis during treatment of human tuberculosis. J. Infect. Dis 212, 990–998 (2015). [PubMed: 25762787]
109. Honeyborne I et al. Profiling persistent tubercule bacilli from patient sputa during therapy predicts early drug efficacy. BMC Med 14, 68 (2016). [PubMed: 27055815]
110. Sukumar N, Tan S, Aldridge BB & Russell DG Exploitation of Mycobacterium tuberculosis reporter strains to probe the impact of vaccination at sites of infection. PLoS Pathog 10, e1004394 (2014). [PubMed: 25233380]
111. Manina G, Dhar N & McKinney JD Stress and host immunity amplify Mycobacterium tuberculosis phenotypic heterogeneity and induce nongrowing metabolically active forms. Cell Host Microbe 17, 32–46 (2015). [PubMed: 25543231]
112. Gillespie SH et al. Four-month moxifloxacin-based regimens for drug-sensitive tuberculosis. N. Engl. J. Med 371, 1577–1587 (2014). [PubMed: 25196020]
113. Mukherjee T & Boshoff H Nitroimidazoles for the treatment of TB: past, present and future. Future Med. Chem 3, 1427–1454 (2011). [PubMed: 21879846]
114. Matsumoto M et al. OPC-67683, a nitro-dihydro-imidazooxazole derivative with promising action against tuberculosis in vitro and in mice. PLoS Med 3, e466 (2006). [PubMed: 17132069]
115. Stover CK et al. A small-molecule nitroimidazopyran drug candidate for the treatment of tuberculosis. Nature 405, 962–966 (2000). [PubMed: 10879539]
116. Koul A et al. Diarylquinolines target subunit c of mycobacterial ATP synthase. Nat. Chem. Biol 3, 323–324 (2007). [PubMed: 17496888]
117. Cole ST Inhibiting Mycobacterium tuberculosis within and without. Phil. Trans. R. Soc. B Biol. Sci http://dx.doi.org/10.1098/rstb.2015.0506 (2016).
118. Zhang Y & Mitchison D The curious characteristics of pyrazinamide: a review. Int. J. Tuberc. Lung Dis 7, 6–21 (2003). [PubMed: 12701830]
119. Prideaux B et al. The association between sterilizing activity and drug distribution into tuberculosis lesions. Nat. Med 21, 1223–1227 (2015). [PubMed: 26343800] This study reveals that different drugs demonstrate variable penetration, accumulation and spatial distribution within tuberculous granulomas, thus setting up separate niches for the development of drug resistance and bacterial persistence.
120. Myllymaki H, Bauerlein CA & Ramet M The zebrafish breathes new life into the study of tuberculosis. Front. Immunol 7, 196 (2016). [PubMed: 27242801]
121. Kramnik I & Beamer G Mouse models of human TB pathology: roles in the analysis of necrosis and the development of host-directed therapies. Semin. Immunopathol 38, 221–237 (2016). [PubMed: 26542392]
122. Scanga CA & Flynn JL Modeling tuberculosis in nonhuman primates. Cold Spring Harb. Perspect. Med 4, a018564 (2014). [PubMed: 25213189]
123. Flynn JL, Gideon HP, Mattila JT & Lin PL Immunology studies in non-human primate models of tuberculosis. Immunol. Rev 264, 60–73 (2015). [PubMed: 25703552]

124. Pena JC & Ho WZ Monkey models of tuberculosis: lessons learned. Infect. Immun 83, 852–862 (2015). [PubMed: 25547788]

125. Diedrich CR et al. Reactivation of latent tuberculosis in cynomolgus macaques infected with SIV is associated with early peripheral T cell depletion and not virus load. PLoS ONE 5, e9611 (2010). [PubMed: 20224771]

126. Lin PL et al. CD4 T cell depletion exacerbates acute Mycobacterium tuberculosis while reactivation of latent infection is dependent on severity of tissue depletion in cynomolgus macaques. AIDS Res. Hum. Retroviruses 28, 1693–1702 (2012). [PubMed: 22480184]

127. Shah JA et al. Human TOLLIP regulates TLR2 and TLR4 signaling and its polymorphisms are associated with susceptibility to tuberculosis. J. Immunol 189, 1737–1746 (2012). [PubMed: 22778396]

128. Shah JA et al. A functional TOLLIP variant is associated with BCG-specific immune responses and tuberculosis. Am. J. Respir. Crit. Care Med http://dx.doi.org/10.1164/rccm.201611-2346OC (2017).

129. Graustein AD et al. The SIGLEC14 null allele is associated with Mycobacterium tuberculosis- and BCG-induced clinical and immunologic outcomes. Tuberculosis (Edinb.) 104, 38–45 (2017). [PubMed: 28454648]

130. Smith CM et al. Tuberculosis susceptibility and vaccine protection are independently controlled by host genotype. mBio 7, e01516–16 (2016).

131. Berrington WR & Hawn TR Mycobacterium tuberculosis, macrophages, and the innate immune response: does common variation matter? Immunol. Rev 219, 167–186 (2007). [PubMed: 17850489]

132. Casanova JL & Abel L Genetic dissection of immunity to mycobacteria: the human model. Annu. Rev. Immunol 20, 581–620 (2002). [PubMed: 11861613]

133. Misch EA & Hawn TR Toll-like receptor polymorphisms and susceptibility to human disease. Clin. Sci. (Lond.) 114, 347–360 (2008). [PubMed: 18230059]

134. Churchill GA et al. The Collaborative Cross, a community resource for the genetic analysis of complex traits. Nat. Genet 36, 1133–1137 (2004). [PubMed: 15514660]

135. Collaborative Cross Consortium. The genome architecture of the Collaborative Cross mouse genetic reference population. Genetics 190, 389–401 (2012). [PubMed: 22345608]

Box 1 |

Modelling heterogeneity in animal models of *Mycobacterium tuberculosis* infection

Animal models of *Mycobacterium tuberculosis* infection are crucial to dissect the features of infection pathogenesis, tuberculosis (TB) pathology and immunology, and bacterial virulence. Several animal models have been used throughout the past few decades to study TB, including zebrafish[120], mice[121] and non-human primates[122–124]. Each system has both benefits and limitations as a model of TB, and although none perfectly recapitulates human *M. tuberculosis* infection, they all contribute to our understanding of this disease. The model recognized to perhaps best recapitulate the multiple facets of variability in TB is the cynomolgus macaque. Although ethical considerations, limited reagents and cost may detract from the general use of cynomolgus macaques, the range of outcomes both clinically and pathologically is remarkably similar to those observed in humans[13,14]. There is a 50:50 ratio of latent infection and active disease in adult cynomolgus macaques infected with low-dose (<25 colony-forming units) virulent *M. tuberculosis* Erdman when using criteria identical to those used for human diagnosis, including immunological sensitivity, culture positivity of gastric aspirates and/or bronchoalveolar lavage fluid samples, signs of disease and increased erythrocyte sedimentation rates[13,14]. Importantly, within these binary definitions, infected macaques recapitulate the entire spectrum of human disease at both the local (lung) level and the overall host level, including the ability to reactivate latent infection following immune suppression with simian immunodeficiency virus[125], or treatment with antibodies against tumour necrosis factor[74] or CD4 (REF. 126).

Box 2 |

Host genetics and tuberculosis

Host genotype is increasingly being associated with variation in host susceptibility and outcome in *Mycobacterium tuberculosis* infection. Recent studies have pinpointed several genetic factors that correlate with protection, susceptibility to disease and vaccine responses[54,127–133]. Toll-interacting protein (TOLLIP) is an example of a protein for which variation in its transcriptional expression was linked with susceptibility to tuberculosis (TB)[127] and the efficacy of bacillus Calmette–Guérin (BCG) vaccination[128]. In the first study[127], TOLLIP deficiency was associated with an increased risk for TB that was mediated by a loss of negative regulation of Toll-like receptor 2 (TLR2) and TLR4 signalling. The subsequent related study[128] found that a deficiency of this protein was correlated with decreased interleukin-2 production by BCG-specific $CD4^+$ T cells. Similar studies have determined an important link between host genetic factors, immune responses and TB outcome. Using an inbred recombinant mouse panel known as the ‘Collaborative Cross’ (REFS 134,135), Smith *et al.*[130] found that host susceptibility to TB and BCG vaccine efficacy were remarkably variable and genetically uncoupled from one another, such that protection to vaccination correlated with the intrinsic quality of the immune response.

Tuberculin skin test

A test that involves the induction of a delayed-type hypersensitivity reaction by an intradermal injection of purified protein derivative, which is a mixture of *Mycobacterium tuberculosis*- derived proteins. The tuberculin skin test is also known as the Mantoux test and is used as a diagnostic tool for *M. tuberculosis* infection, but it does not distinguish latent infection from active tuberculosis.

Acid-fast staining

A method for staining mycobacteria for microscopic visualization, as the Gram stain is not useful for mycobacteria. Acid-fast staining relies on phenolic compounds that interact with the lipid-rich cell walls of mycobacteria, and the resistance of this interaction to acid alcohol is the basis of the term ‘acid-fast’.

Positron emission tomography

An imaging method that depends on the 3D detection of radiation (positrons) from a probe that is typically localized by uptake and retention in a specific cell or by a specific process *in vivo*. This uptake provides functional information about the organ of interest.

Computed tomography

An imaging method that uses computer-processed combinations of many X-ray images taken from different angles to produce cross-sectional (tomographic) images (virtual ‘slices’) of specific areas of a scanned object, resulting in a 3D representation of an organ in a living subject. This provides structural information about the organ of interest.

Pulmonary cavitation

The formation of a cavity in the lung. A cavity is an abnormal, gas-filled space with a lining wall that has developed within and replaced the normal lung architecture. In tuberculous disease, these cavities are formed when necrosis invades through the wall of an airway, dilating and distorting the structure, and leading to the discharge of necrotic debris into the bronchial tree.

Ghon complex

A term for pathological lesions in latent tuberculosis infection that consist of an often-calcified granuloma and an associated lymph node.

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Molecular distance to health

A numerical score that measures the global transcriptional difference of each patient relative to the median in healthy controls.

Caseum

A hallmark feature of human tuberculous granulomas that results from a distinctive type of central necrotic breakdown known as caseous necrosis. The term caseum derives from the ‘cheese-like’ appearance of the necrotic area.

Consolidations

Pathological processes by which the pulmonary infiltration of cells, fluid or other material leads to the loss of aeration and of the normal spongy consistency, causing parenchymal tissue to have a more firm, solid texture. Such a change is most commonly associated with infection-induced inflammatory infiltrates and leads to pneumonia.

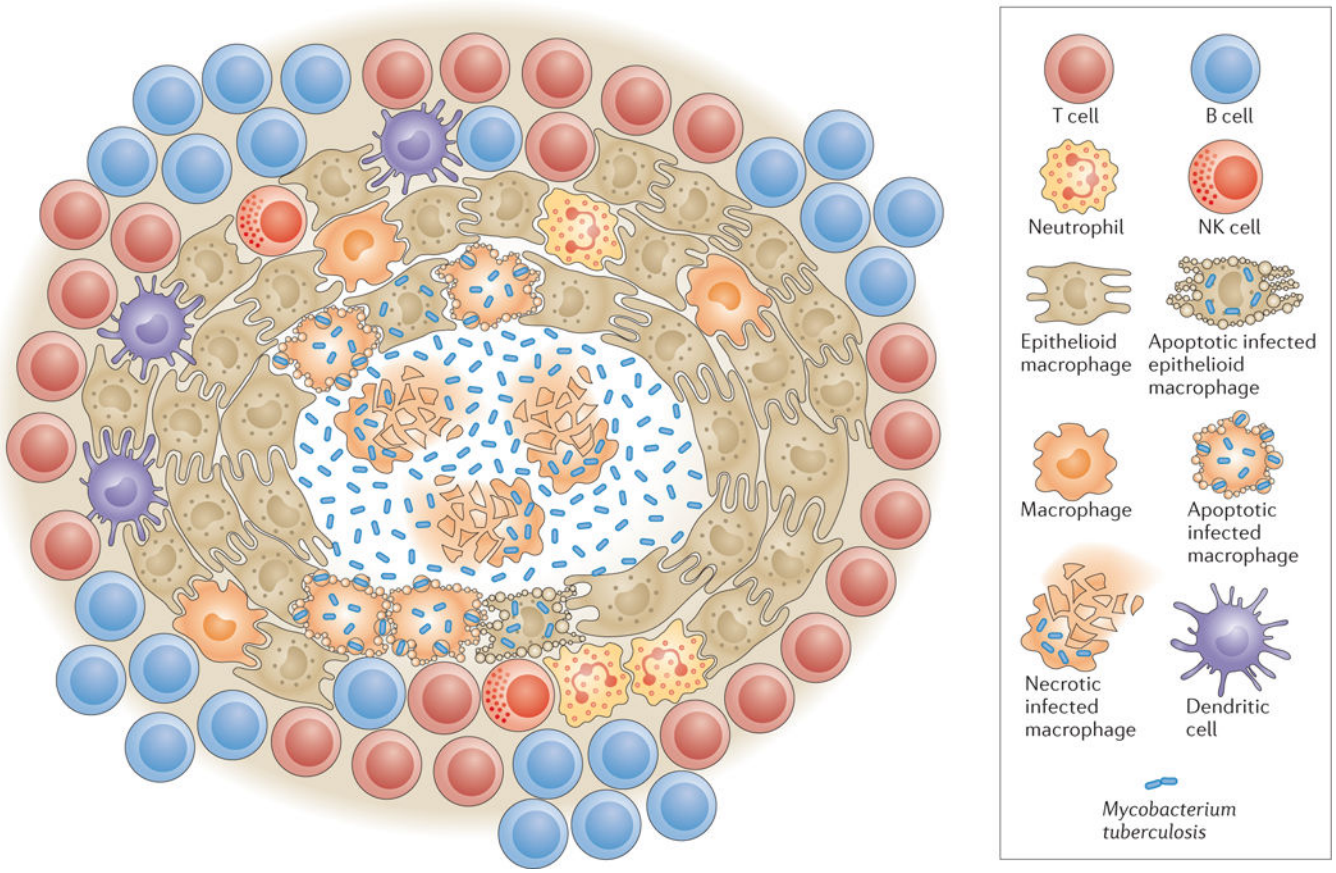

Figure 1 |. A classical tuberculosis granuloma.
The hallmark tuberculosis granuloma is a highly organized collection of immune cells that aggregate around a central necrotic core. Reproduced from REF. 32 © Macmillan Publishers Limited. NK, natural killer.

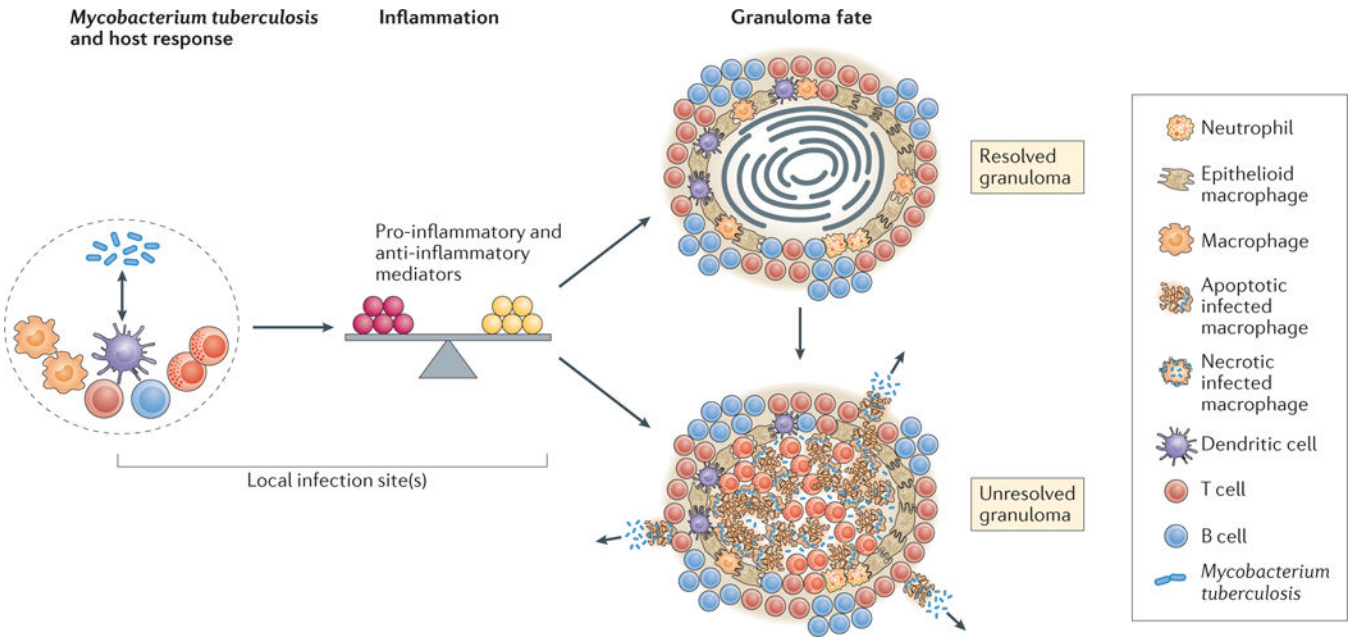

Figure 2 |. Granuloma fate is influenced by a complex and dynamic exchange of host and bacterial features.

In the lungs, a crucial interplay between the bacteria and host immune cells influences inflammatory programmes that contribute to granuloma outcome. This process is highly dynamic and iterative, with multiple components having pleiotropic, knock-on and feedback effects on inflammation and the host–pathogen interaction.

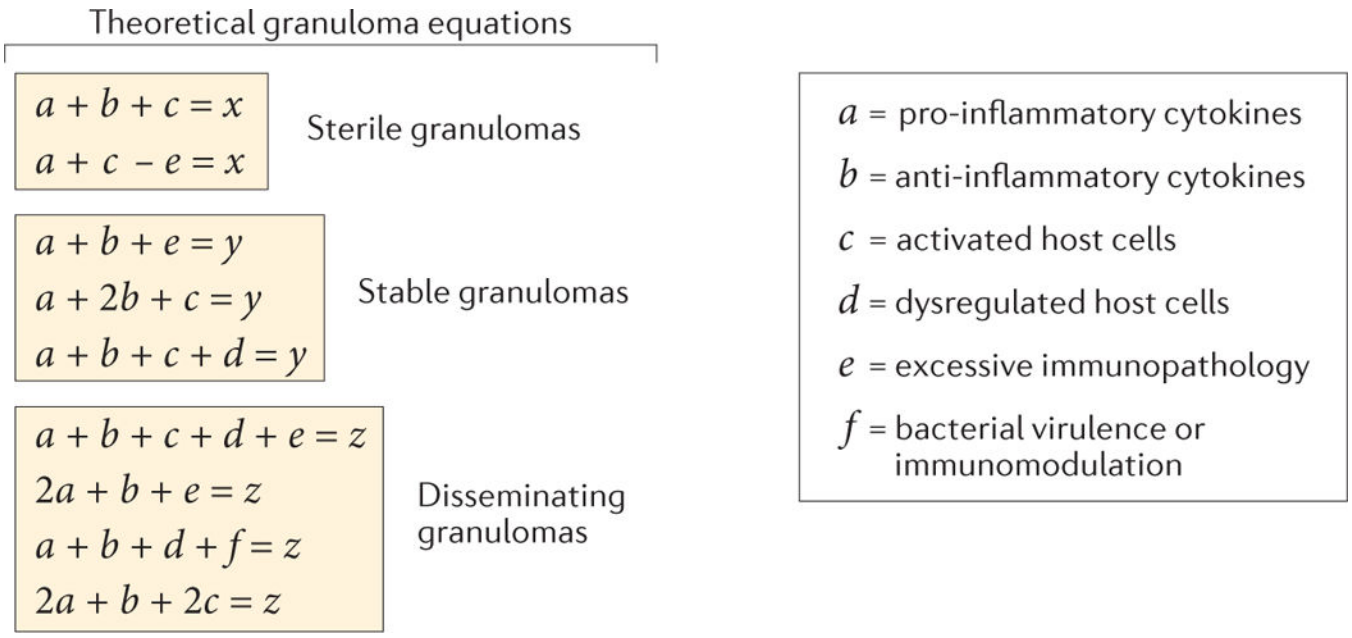

Figure 3 |. Multiple 'equations' can determine granuloma fate.

There are multiple pathways that lead to both resolved and unresolved granuloma outcomes in human tuberculosis. These are fluid and dynamic processes that change as the structures encounter different contexts of immune responses, inflammation and bacterial persistence.

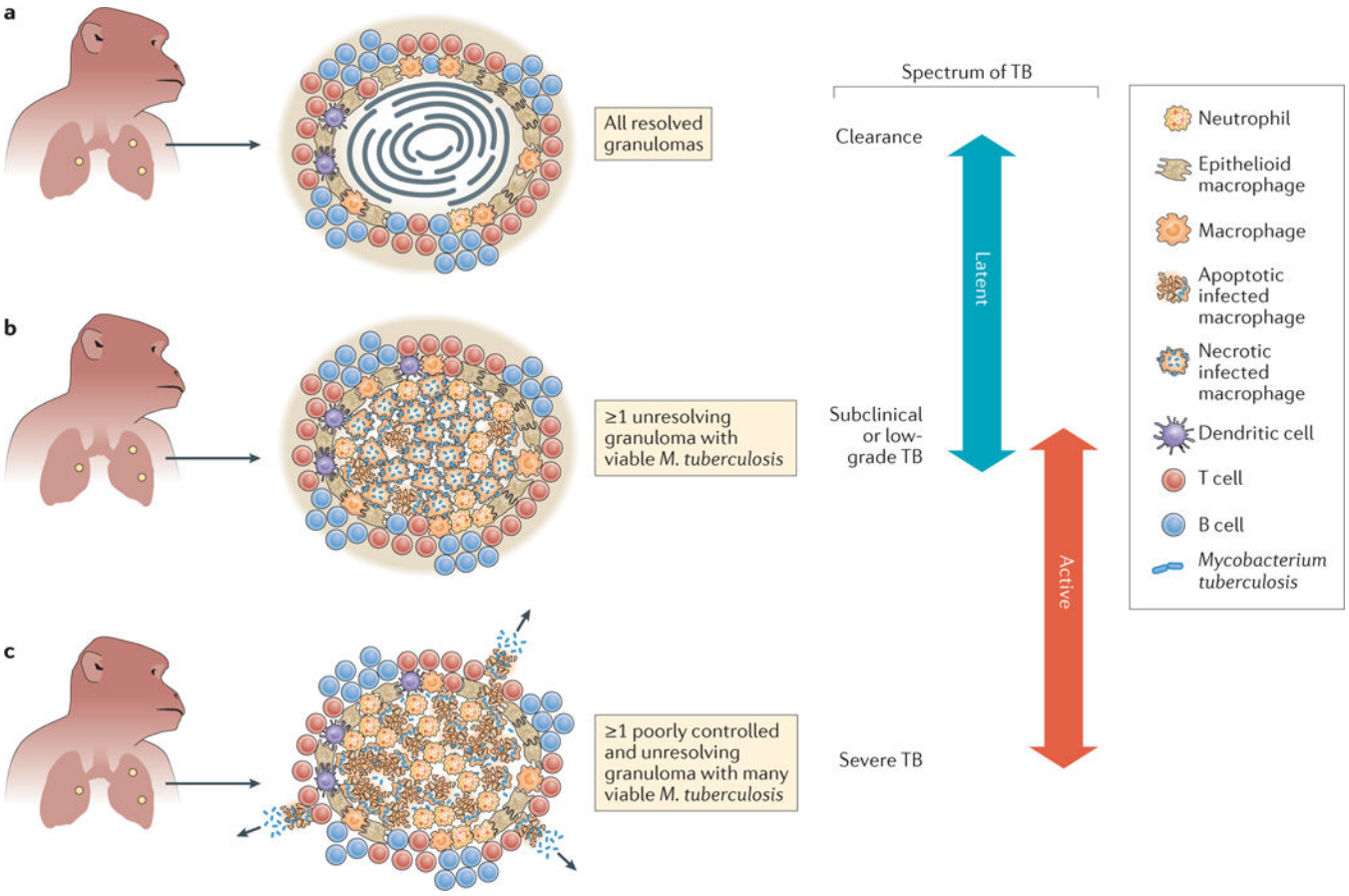

Figure 4 |. Individual granulomas establish variable host outcomes and contribute to the overall spectrum of tuberculosis.

a | An individual cynomolgus macaque in which all granulomas have resolved, thus leading to an asymptomatic, contained outcome, has the lowest risk of reactivation of infection, and has effectively cleared most or all of the *Mycobacterium tuberculosis* infection. **b** | An individual macaque that has one or more granulomas that contain viable *M. tuberculosis* but can be asymptomatic for clinical tuberculosis (TB). These granulomas are not actively disseminating or progressing to worse pathologies, but they may have a persistent low level of inflammation. **c** | An individual macaque that has one or more very poorly controlled granulomas that are actively disseminating bacteria to other sites (indicated by outward-pointing arrows) can develop progressive disease and potentially worse forms of active TB.