Reconsidering the optimal immune response to *Mycobacterium tuberculosis*

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Abstract

Tuberculosis is unfortunately once again killing more people than any other infection. Despite global biomedical and public health efforts, standard interventions to control the pandemic have not advanced in most resource-poor settings, in contrast to other global diseases such as malaria and HIV infection. The authors propose that reconsidering concepts of the optimal host immune response to *Mycobacterium tuberculosis* is required to develop a paradigm that will inform novel interventions. The development of active tuberculosis associated with cancer treatment by immune checkpoint inhibition highlights that merely driving a stronger immune response may not improve control of the pathogen. Here, we present different models of the host-pathogen interaction that are consistent with the complexity of human infection. We consider both the protective and harmful components of the immune response in tuberculosis and develop a multiparameter framework that predicts disease risk. This novel conceptual model is important to inform emerging interventions to improve outcomes in tuberculosis by defining the ideal host response to target.

The global death toll from tuberculosis (TB) is an ongoing tragedy (1). Currently, clinicians in TB-prevalent resource poor settings attempt to control the pandemic with clearly inadequate tools: BCG vaccination, sputum smear microscopy and antibiotic regimes of a minimum of 6 months, each of which have remained essentially unchanged for many decades (2). Whilst initial trials of novel vaccinations have been disappointing (3, 4), two recent vaccine trials have generated more promising results (5, 6). The M72/AS01E vaccine reduced number of active cases by 50% in a phase 2b study (5), whilst a repeat BCG administration reduced sustained interferon gamma release assay (IGRA) conversion from 11.6% to 6.7% (6). Repeat BCG was not included in the first study, and so the relative efficacy cannot be defined. However, both studies demonstrate that a significant residual disease burden will persist, that can lead to ongoing transmission. Consequently, it is vital to consider what constitutes a protective immune response to *Mycobacterium tuberculosis* (Mtb), versus a pathological immune response that leads to cavitation and transmission (7). We review clinical and experimental observations that highlight the complexity of the host-pathogen interaction in human TB to develop an entirely new conceptual model that will inform future strategies.

The sequence of events in human TB infection

Humans and Mtb are thought to have co-evolved for an estimated 70,000 years (8) and therefore have developed a highly complex host-pathogen interaction. Mtb is transmitted by aerosol, generated by a patient with pulmonary disease affecting the lung apices (Figure 1A). The initial site of implantation is the lung base, as described by Ghon in 1916 (9). The early immunological events at the lung base are clearly distinct from the late immunological events at the lung apex, as cavitation almost never happens at the site of initial implantation (10). After inhalation, an early inflammatory response to Mtb results, which can be extensive with radiologically visible lung lesions and lymph node enlargement (the Ghon complex). However, in the vast majority of patients this self-resolves, only leaving post-inflammatory calcification (Figure 1B). Primary progressive disease is associated with immunocompromise, such as HIV co-infection, new-born infants or anti-TNF treatment (11).

Therefore, despite this extensive initial inflammation, the patient's host immune response controls infection and this individual is classified as having "latent" TB, with an approximately 1 in 10 lifetime risk of TB reactivation (2).

The distinction between the events at the lung base and the lung apex is often not made, but evidently the immunological process at the two sites are different. Mtb must transfer from the lung bases to the lung apices, probably transported within infected macrophages that traffic through the initial granulomas (12, 13), though the mechanism for implantation in the lung apex is unknown. Insight into dissemination is limited, in part due to the experimental challenges studying the process. Clinically, the development of miliary TB in immunocompromise would suggest that Mtb initially disseminates widely throughout the body (14), and then is controlled at the onset of adaptive immunity in the majority of the implantation sites, but not at the lung apices. Whether the hyperconservation of T cell epitopes in Mtb (15) is related to facilitating dissemination, or alternatively causing immunopathology to permit subsequent exit from the lung via airways, is an open question.

After implantation, following a delay between months or many years (16), Mtb must generate a pro-inflammatory lesion that leads to tissue breakdown, access to the airway, exponential bacterial growth and transmission (17). However, even after an extensive lesion develops, this should not be regarded as an inevitable sequence of events: in the pre-antibiotic era, one third of "consumptives" would self-heal (18), and with surgery to collapse a cavity this rises to about 70% cure even in the absence of antibiotics (19). In high incidence areas, areas of extensive pulmonary scarring from TB that have self-healed without diagnosis or treatment are frequently observed (Figure 1C). A radiologically visible lesion 0.5cm in diameter would have contained approximately 65 million inflammatory cells, and yet this infected aggregate can often self-resolve. In addition, this flux between the host and pathogen has been confirmed by recent radionucleotide imaging studies demonstrating that in the same individual, some lesions may progress while others regress (20). Therefore, a very fine balance exists between protection and progression in TB, determined at the local lesion level, and often the host immune response can bring extensive pulmonary infection under control.

Immunological determinants of protection versus pathology

The host immune response is clearly needed to control Mtb infection. A deficiency of CD4-positive T cells, TNF-α or IFN-γ/IL-12 signalling leads to disseminated TB in both human and animal studies (11), and the list of specific immunodeficiencies that can increase the risk of active TB continues to expand (21, 22). However, the fact that complete absence of a component of the immune response causes disease does not prove that an excess will increase protection, and these cases of specific immunodeficiencies can in fact be considered biological outliers (23). An alternative perspective is that a strong immune response can be as harmful as a weak immune response in TB. For this discussion, we characterise a strong immune response as increased cytokine secretion, augmented cellular infiltration, increased cell death and high matrix metalloproteinase (MMP) production, while a weak immune response lacks these features.

The concept that an excessive host immune response can be harmful has been identified as long as the disease itself, and was the subject of bitter disputes between Koch and Virchov (24). Koch's treatment of patients with tuberculin to augment the host immune response to Mtb worsened pathology in more patients than those who benefitted (24). Cavitary pulmonary TB, which transmits infection, occurs most commonly between the age of 20 – 25 (25) , which could be regarded as the immunological prime, and is certainly not a time of immunocompromise. In these seminal epidemiological studies, Comstock demonstrated that the stronger the delayed type hypersensitivity (DTH) response to Mtb antigens as a child, the more likely individuals are to develop pulmonary disease as a young adult (25). The only data available for analysis were response to Mtb antigens and subsequent development of TB, and so the study indicates that a strong response associates with disease many years later.

Recently, further evidence that an excessive response increases the risk of TB has emerged from the cancer immunotherapy field. Treating cancer patients with immune checkpoint inhibitors to globally activate the immune response improves outcomes in diverse malignancies (26), but also leads to active TB in a growing number of patients (27, 28). This phenomenon is entirely consistent with the

hyper-susceptibility of programmed death (PD)-1 knockout mice to Mtb infection, which die even more rapidly than IFN-γ deficient mice (29, 30). The divergence is not due to increased bacterial load, as cyclophilin D-deficient mice with increased T cell responses to Mtb and no increase in bacterial load also have worse outcome (31). These emerging lessons from novel medical interventions, which are providing unique insight into the function of the immune system in health and disease, further re-inforce that an excessive response to Mtb is harmful.

Taken alongside the concept that an either insufficient or excessive response may be harmful, the Mtb exposure intensity and strain virulence needs to be considered (32, 33). Higher Mtb exposure levels are well documented to lead to active TB (34). The observation that Mtb T cell epitopes are hyper-conserved supports the notion that Mtb specifically benefits from the host immune response (15). Therefore, any conceptual model about the TB host-pathogen interaction needs to consider both the protective and harmful effects of host immunity, and the exposure level to Mtb.

Conceptual models of human immunity to Mtb

The simplest established model of the host-pathogen interaction is a linear one, whereby greater immunity leads to reduced disease incidence (Figure 2A). However, this model is inconsistent with epidemiological, clinical and experimental observations outlined above that demonstrates a strong immune response associates with increased incidence of cavitary pulmonary disease, and should be discarded.

Therefore, the concept of a U shaped curve is emerging within the field (Figure 2B). Here, both an inadequate or excessive immune response leads to TB. This model is improved by discriminating between different clinical presentations of TB, which are usually grouped together. A very weak response, such as advanced HIV infection, newborn infants or IFN-γ deficiency, typically leads to disseminated disease, such as miliary TB or TB meningitis (35), whereas individuals with a strong immune response develop cavitary pulmonary TB (25). This distinction has significant implications. First, simply driving a greater immune response in the entire population may protect some individuals

from disseminated disease but worsen disease in others, as it risks shifting a proportion of people with a controlling response into a cavitation response. Additionally, the proposal that interventions which improve outcome for immunocompromise-associated TB will then also improve outcome in pulmonary disease (36) runs counter to clinical observations that pulmonary disease is caused by immune excess. In fact, interventions which improve immunodeficient TB may worsen immune-excess TB. Therefore, defining the characteristics of the immunological controllers, versus the two types of progressors, is a very pressing question to inform optimal strategies.

A third approach would be a binary model (Figure 2C). In this, some aspects of the host immune response to the pathogen are protective, whilst different responses drive pathology, and a central overlap occurs between the two processes. If these specific components could be determined, it would greatly advance vaccine development as augmenting the protective response whilst suppressing the pathological response would then terminate the pandemic. However, since many of the effector molecules of the immune response, such as inflammatory cytokines, may be communal, it seems more likely that significant overlap exists and separating processes into exclusively protective and pathological may be challenging.

A 3-dimensional model considering host and pathogen factors

The models presented above do not consider intensity of Mtb exposure, which clearly determines risk (33, 34), nor do they differentiate between the host's innate and adaptive immune response. An emerging concept is that innate memory may be just as important as adaptive memory (37-39), and so the two should be considered separately. A balance between innate and adaptive immunity is likely required, with either a gross deficit or excess of either harmful, and similarly a relatively strong innate response can compensate for a weak adaptive, and vice-versa. Plotting the host immunological and mycobacterial elements into a model generates a more complex 3-dimensional picture of optimal immunity to Mtb (Figure 3). Furthermore, this characterises the likelihood of different forms of TB, such as disseminated TB associated with immunodeficiency (red), control (white) or cavitary

pulmonary TB due to an excessive immune response (purple). The optimal immune response can be predicted to be an asymmetrical pyramid, whereby the disease likelihood rises as the Mtb exposure increases and the area of an optimal immune response to control infection declines.

Within this model, each individual will start from a different position on the matrix, determined by diverse factors such as their genetic make-up and BCG vaccination. Age is an important determinant: new born infants have poor immunity, and have highest risk of disseminated TB (red zone), then children have a relative low risk (white zone), but after puberty enter the period of highest risk of pulmonary TB as young adults (purple zone). Other factors will also contribute to movement in the matrix, such as environmental mycobacterial exposure, nutritional status, co-morbidities, medication and intensity of Mtb exposure. The primary implication of this more advanced model is that a novel intervention for TB may improve the chances of Mtb control for some individuals but conversely worsen it for others. A key consideration is that for Mtb to spread, it must cause extensive lung inflammation, tissue destruction and cavitation from excessive immune responses.

Whilst this model appears to suggest a high risk of TB, it essential to note that approximately90% of Mtb-exposed individuals end life in the central white control zone (2), while only around 6% end in the purple zone, developing pulmonary TB and transmitting infection to continue the pandemic. A vaccination strategy that prevents pulmonary disease would break the cycle of transmission and end the TB pandemic. As many cases of disease are due to relatively recent transmission, such a vaccine could have a rapid impact (16).

Potential mechanisms driving excessive inflammation

The characterisation of the immune response between protective and pathological elements opens the question about what events propels the immune response over the optimal "control" zone and into the "cavitary" zone. Hunter has argued that current models of TB are inconsistent with histological analysis of human pulmonary TB cases, and has proposed that a progressive build-up of Mtb antigens leads to a sudden excessive inflammatory event (40). We have hypothesised that Mtb may drive an

autoimmune / autoinflammatory response to exacerbate inflammation (41), and this suggestion has since been supported by bioinformatic analyses of gene expression signatures in TB, infectious and autoimmune disease (42). Tangential evidence also arises from the association of immune checkpoint inhibition and active TB (27, 28), since the predominant side effects of these agents are autoimmune in nature (26).

If Mtb is driving an autoimmune process, the likely mechanisms and antigens involved are totally unknown. We suggest that one candidate mechanism is the presentation of lipids by CD1 molecules to tissue resident innate-like T cells (43). Phospholipids are major components of mammalian and bacterial membranes including those of Mtb. Although the lipid moieties of phospholipids derived from bacteria are structurally different to their mammalian counterparts, both mammalian and bacterial phospholipids can activate CD1d- and CD1b-restricted T cells, thus providing a mechanism for CD1-mediated T cell activation by shared phospholipids (44, 45). Furthermore, stress related lipids, such as host derived cholesteryl-esters that accumulate in TB lesions, are CD1c ligands that activate T cells (46). Mtb may induce an inflammatory milieu through upregulation of host-derived stress molecules, including CD1, provoking pro-inflammatory T cell responses. Examples include rapid IL-17 production in response to mycobacteria by innate γδ cells (47, 48), and activation of pro-pro-inflammatory tissue resident innate-like T cells such as γδ, mucosal associated invariant T cells, natural killer cells, and innate lymphoid cells (49).

Thus, Mtb may drive local inflammation by upregulating host derived stress ligands that provoke innate T cell activation and the resulting autoimmune inflammatory response may exacerbate pathology, a proposal reminiscent of recent findings in lung cancer (50). Furthermore, emerging evidence suggests an important role for B7-like members such as butyrophilin (BTN) and butyrophilin-like (BTNL) molecules as self-ligands that regulate innate like T cells such as tissue resident γδ cells (43, 51). Interestingly, the expression of BTN and BTNL molecules are significantly down-regulated in inflammatory disorders and cancer (52). Hence, the inflammatory process in TB may cause further local T cell dysregulation and perpetuate excessive pathology.

Along similar lines to this autoimmunity hypothesis, Divangahi and Sassetti have independently proposed that the development of active TB reflects primarily a loss of tolerance (53-55). Whether the inflammation is driven by autoimmunity or loss of tolerance is perhaps more a matter of semantics, as they could be regarded as the same fundamental process. Consequently, diverse research groups have reached a similar hypothesis from very different experimental approaches. One irrefutable conclusion is the urgent necessity to address the knowledge gap of what drives pathology versus protection in TB to inform new therapies (54).

Clues to human TB pathogenesis from *Mycobacterium bovis*

A significant unanswered question within the TB field is why Mtb and *M bovis* are so similar genetically, with 99.7% shared sequence identity, and yet the host tropism is very different (56). This suggests that comparison may shed light on Mtb pathogenicity. Only Mtb can cause lung cavitation and transmission in humans, while *M bovis* can cause lymph node disease in humans but does not cause sufficient lung disease to spread to new hosts apart from in very occasional cases (57). Therefore, the key difference does not seem to be the ability to survive within the host macrophage, but instead the ability to cause sufficient pulmonary inflammation to result in lung cavitation and transmission. *M bovis* has multiple genetic regions of deletion (RD) relative to Mtb, and the primary difference when compared to Mtb is the loss of lipoprotein-related genes (56). Lipoproteins facilitate transport of hydrophobic lipids in solution, such as cholesterol and triglycerides. Consequently, one hypothesis would be that these lipoproteins are necessary to shuttle lipids from infected cells to uninfected cells, thereby amplifying the proinflammatory host immune response driven by CD1-responsive T cells as proposed above. These lipids could be pathogen derived, host stress lipids or common to both. Alternatively, the divergence in lipid-related genes may be related to metabolism, as nutritional restriction by macrophages is emerging as a critical control mechanism of Mtb growth (58).

Investigating the optimal response to TB

The models of the host-pathogen interaction that we present suggest that a more detailed dissection of events is required to inform novel interventions. As Mtb and humans have co-evolved for so long, correlation between experimental systems and human TB is essential. Furthermore, studying the correct compartment is key, as events in the periphery may not reflect those in the lung apex. The investigation of individuals who are recurrently exposed to Mtb, but do not develop infection, and individuals who have spontaneously controlled disease, are two patient groups that may provide unique insight into protective responses (59), if sufficient individuals can be identified. This approach may characterise innate profiles associated with resistance. However, these studies are inherently challenging, as it is difficult to differentiate individuals who have been infected and eradicated Mtb very early from the alternative groups with identical test results; never exposed or Mtb-infected but PPD false-negative. Furthermore, multiple mechanisms of innate resistance may exist, such as macrophage- and CD1-mediated early eradication processes (59), and trained innate immunity (60). Individuals who are IGRA positive and then revert to negative may provide another group who may have successfully controlled Mtb (61). We propose that integration of clinical studies with advanced cellular models may be critical to dissect underlying processes (62). While on one hand they can be dismissed as not reflecting the complexity of events *in vivo*, their primary advantage is their tractability to perform mechanistic studies in a human-Mtb system to confirm observations from clinical and animal studies.

Conclusion

The ongoing TB pandemic emphasises the importance of re-considering fundamental concepts of disease in light of historical and emerging data. Both clinical and experimental observations suggest that simple linear or U-shaped models of the optimal host immune response to Mtb are unlikely to be adequate, and a more nuanced multi-dimensional model is required. The 3-parameter stratification of

TB risk presented here is likely still an over-simplification, but provides a framework within which to consider the optimal immune response to control Mtb infection. This permits dissection of the protective and pathological elements of the host immune response to inform strategies to reduce transmission in future studies. In addition, for previous studies, infants who have had a long-lasting increase to their immune response to Mtb (63) should be followed with the same intensity for adverse events after puberty as for potential early beneficial effects.

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290 **Figure legends**

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292 **Figure 1**: **(A)** Apical lung destruction in TB. Extensive right upper zone lung inflammation and
293 cavitation in a patient with pulmonary tuberculosis. These patients are highly infectious and drive the
294 pandemic. **(B)** Self-limiting initial infection. Mtb has been inhaled to the lung base. The initial
295 granulomatous response has left a calcified Ghon focus (broad arrow) and calcification in a
296 mediastinal lymph node (narrow arrow). This initial infection has resolved and the individual would
297 be classified as having "latent" TB. **(C)** Apical lung scarring from self-resolved TB. A calcified area
298 of fibrosis (arrow) demonstrates an area of TB infection that has progressed and then regressed
299 without treatment.

300 **Figure 2**: **(A)** Linear model of the host-pathogen interaction, proposing that the stronger the immune
301 response, the greater the protection from TB. This seems inconsistent with historical and emerging
302 clinical observations. **(B)** U-shaped curve. Both an insufficient and excessive immune response
303 increases the risk of TB, with a central region of optimal protection. Whilst an insufficient response
304 tends to lead to disseminated disease, an excessive response is required to drive cavitation and
305 transmission. **(C)** Binary model. Some host immune response elements control Mtb growth, while
306 others cause pathology and transmission, and some are shared. The degree of overlap between
307 pathology and protection is unknown.

308 **Figure 3**: **(A)** Multidimensional model of TB risk. If the strength of the immune response is broken
309 into innate and adaptive elements, and then plotted against Mtb exposure intensity, a more complex
310 picture of risk of active TB emerges. Red represents disseminated TB, grading through progressively
311 reduced risk to white indicating protection. An over-exuberant immune response leads to lung
312 inflammation, cavitation and transmission (purple). **(B)** External appearance of TB risk matrix cube.

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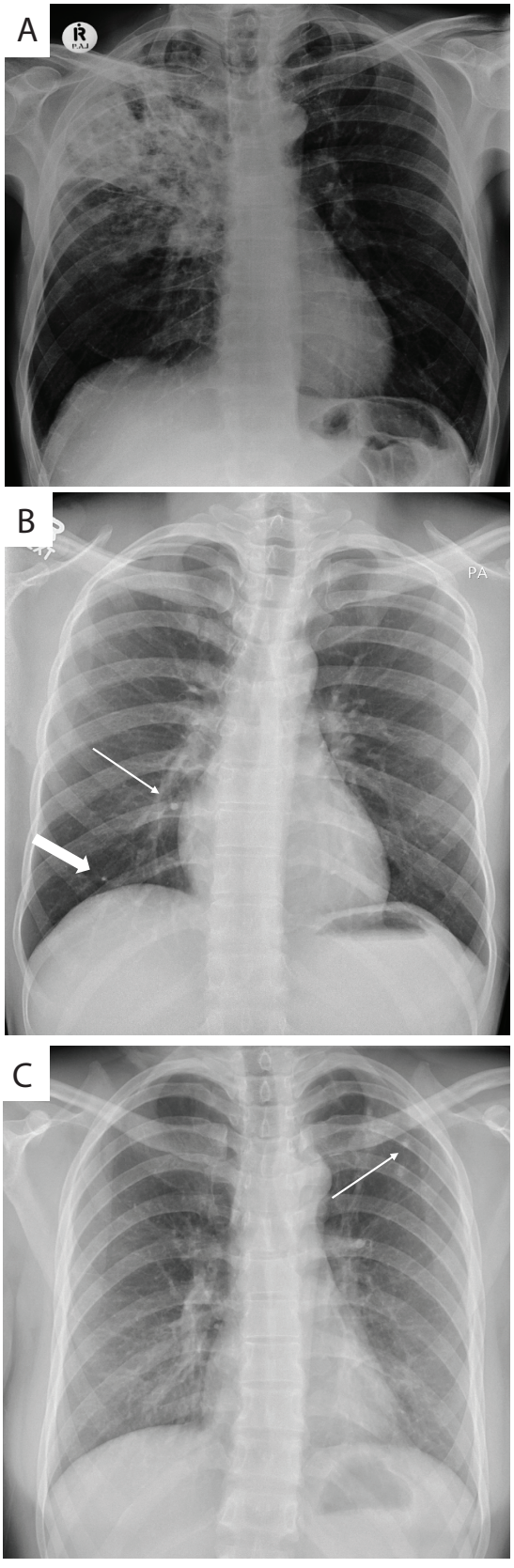
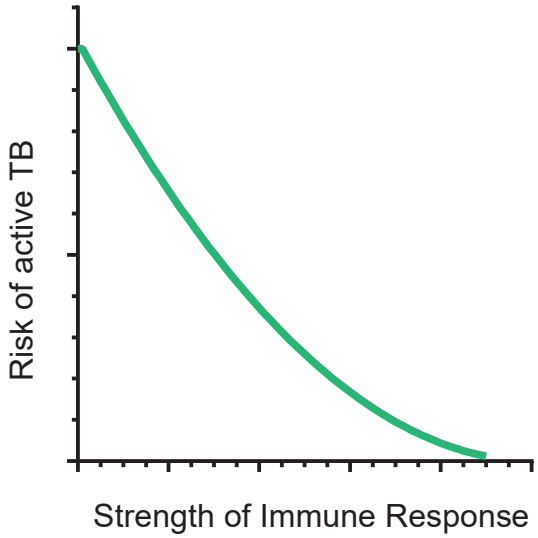
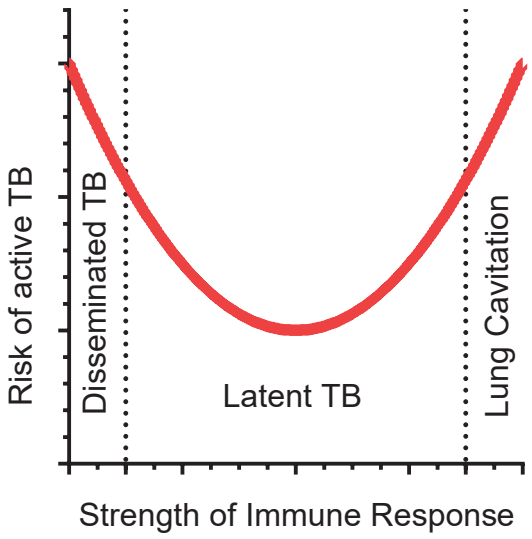


Figure 1: **(A)** Apical lung destruction in TB. Extensive right upper zone lung inflammation and cavitation in a patient with pulmonary tuberculosis. These patients are highly infectious and drive the pandemic. **(B)** Self-limiting initial infection. Mtb has been inhaled to the lung base. The initial granulomatous response has left a calcified Ghon focus (broad arrow) and calcification in a mediastinal lymph node (narrow arrow). This initial infection has resolved and the individual would be classified as having "latent" TB. **(C)** Apical lung scarring from self-resolved TB. A calcified area of fibrosis (arrow) demonstrates an area of TB infection that has progressed and then regressed without treatment.

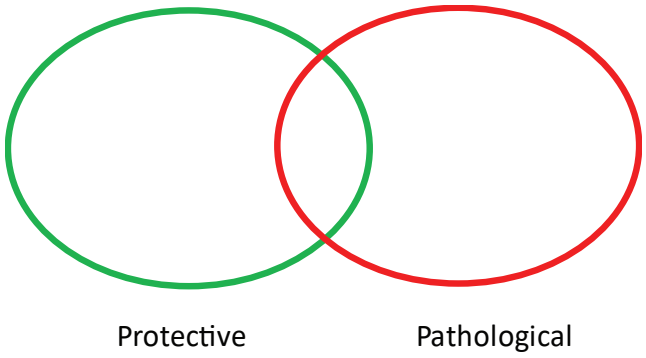
A

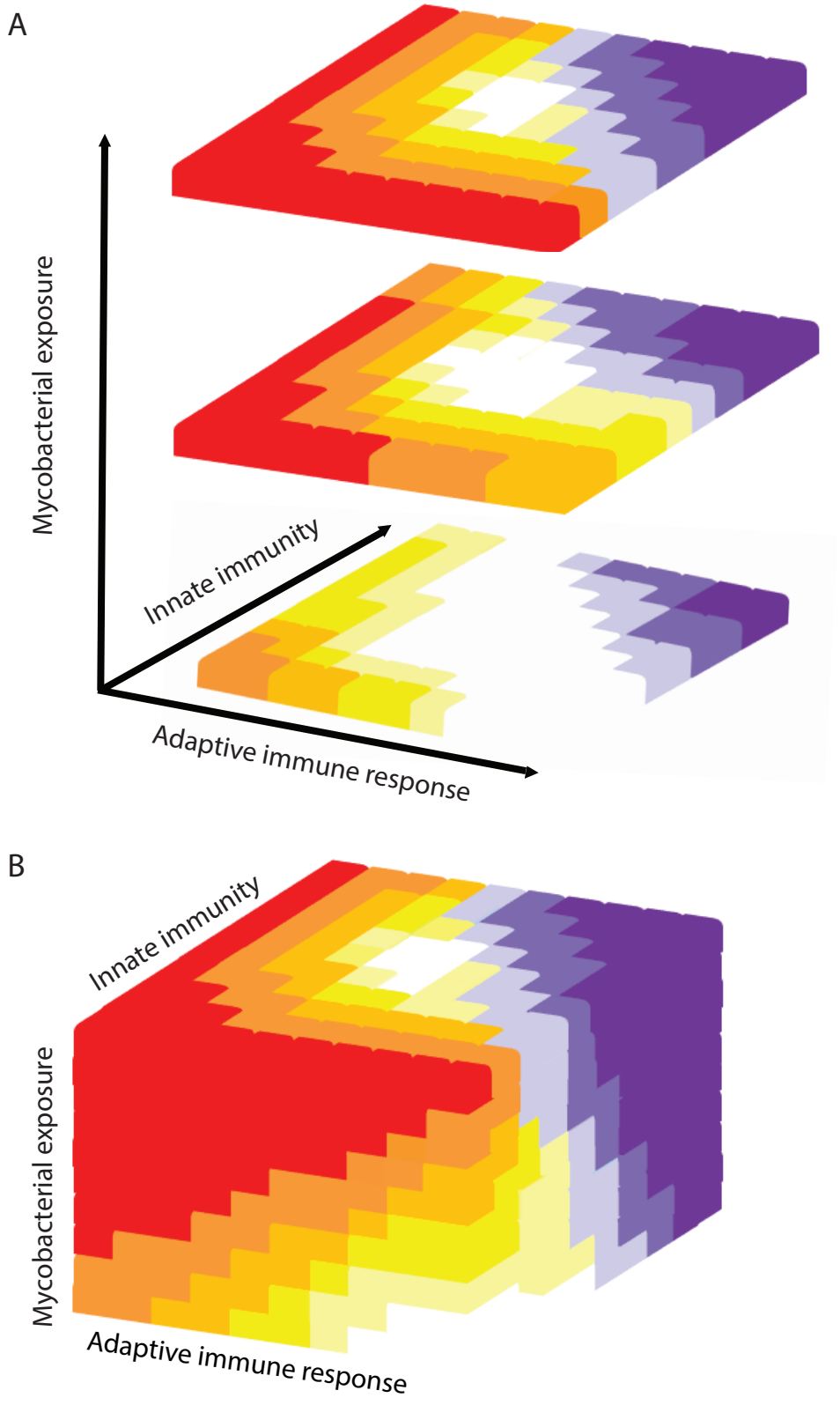

Risk of active TB
Strength of Immune Response

B


Risk of active TB
Disseminated TB
Latent TB
Lung Cavitation
Strength of Immune Response

C


Protective
Pathological


A
Mycobacterial exposure
Innate immunity
Adaptive immune response
B
Innate immunity
Mycobacterial exposure
Adaptive immune response