

The immunological life cycle of tuberculosis

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Abstract | Immune responses to *Mycobacterium tuberculosis* are only partially effective; they drive the bacteria into a latent state, but rarely eliminate them. Unfortunately, the latent state of *M. tuberculosis* is reversible, and reactivation tuberculosis is the source of most transmission. Studies in animal models and in humans have not yet yielded a comprehensive picture of the mechanisms or correlates of immunity to *M. tuberculosis* infection, or of why immunity fails to eradicate the pathogen. This Review proposes that there are distinct stages in the immune response to *M. tuberculosis* that form an ‘immunological life cycle’. It is hoped that this thesis will provide a framework for investigation to understand immunity to *M. tuberculosis* and to guide tuberculosis vaccine discovery and development.

Protective immunity

The term protective immunity describes the immune responses of individuals who have been immunized or who have recovered from a primary infection and, following re-exposure to the pathogen, are protected from developing severe disease and chronic infection. Protective immunity can be sterilizing, if it protects against a productive infection.

Understanding immunity to *Mycobacterium tuberculosis* is a great scientific challenge directly applicable to the lives and health of a large fraction of the human population. Despite the availability of multiple anti-tuberculosis drugs for over 50 years, tuberculosis (TB) remains a common cause of morbidity and causes the death of over 1.5 million people per year, which is nearly as many as the number caused by HIV infection (see the [World Health Organization TB data](#)).

The scientific challenges in understanding immunity to *M. tuberculosis* arise from the observation that, although most humans and experimental animals develop apparently appropriate immune responses after infection, these immune responses do not reliably eradicate the bacteria. Instead, such responses cause *M. tuberculosis* to adopt a clinically silent, latent state of infection, from which the bacteria can be reactivated. Although broad outlines of the mechanisms of protective immunity have been discovered through studies in humans and experimental animals, the limitations of immunity to *M. tuberculosis* and the mechanisms used by the bacteria to impose these limitations are not well understood. As a practical problem, the limited understanding of immunity to *M. tuberculosis* deters rapid progress in developing TB vaccines that are superior to *Mycobacterium bovis* bacillus Calmette–Guérin (BCG), a vaccine developed in the early twentieth century that has limited efficacy in preventing active tuberculosis, despite having been administered to >3.5 billion people.

One potential limitation to understanding immunity to *M. tuberculosis* is that the conceptual framework underlying many studies may be oversimplified. In

experimental animal models, including in zebrafish, mice, guinea pigs, rabbits, cattle and non-human primates, end points such as mortality, bacterial burdens and tissue pathology allow for comparisons of the effects of bacterial and host variants on immunity to *M. tuberculosis*. Although these models have yielded considerable information on the mechanisms of pathogenesis and innate and adaptive immunity, a clear pathway for efficacious TB vaccine design and discovery remains to be defined¹. Likewise, studies of human immune responses to *M. tuberculosis* most often compare responses in healthy latently infected individuals with those in individuals with active TB disease. Despite the value of these studies, they have not yet identified clear mechanisms or correlates of effective immunity to human *M. tuberculosis* infection².

The purpose of this Review is to propose a framework for understanding and studying immunity to TB in animal models and humans. This framework is based on the assumption that there are multiple stages in the human immune response to *M. tuberculosis*, and that existing animal models mimic some, but not all, of these stages. Moreover, studies in humans may be designed and evaluated on the basis of this framework, with the anticipation that defining immune responses at distinct stages will provide a clearer understanding of the mechanisms and correlates of immunity. Although *M. tuberculosis* does not undergo the striking morphological changes in fixed time frames that are characteristic of eukaryotic parasites during their life cycles, there is a substantial basis for considering the

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distinct stages of *M. tuberculosis* infection as forming an 'immunological life cycle' (FIG. 1). Because pulmonary TB is the transmissible form of the infection, it is the focus of this Review.

As new approaches to studying human immune responses emerge and mature, a new framework for designing such studies for TB is timely. Opportunities and an increased need to understand the strengths and limitations of immunity to *M. tuberculosis* are provided by several developments. These include recent advances using systems biology approaches to analyse human responses to selected vaccines³, the development of new technologies (such as cytometry by time-of-flight) and the discovery of new markers and mediators of human immune responses, together with growing commitments by funding agencies, regulatory authorities and pharmaceutical companies to TB vaccine development. This understanding will inform the development and evaluation of TB vaccines and other new approaches for enhancing immunity to *M. tuberculosis*, with the hope of reducing the burden of this important and scientifically challenging infectious disease.

This Review considers selected aspects of the immune response to *M. tuberculosis* and concentrates on evidence that supports the model of an immunological life cycle for TB. It does not focus in depth on the mechanisms of *M. tuberculosis* virulence or pathogenicity and is not a comprehensive review of TB immunology (for more information on these topics, see REFS 4,5). Whenever possible, data from studies of human TB are featured; most of the other data cited are from studies in mice, because these animal models are well suited to mechanistic studies.

Stage 1: innate immune responses

Innate immune cells. *M. tuberculosis* is transmitted by aerosol, and largely, if not exclusively, inhabits professional phagocytic cells in the lungs, including macrophages, neutrophils, monocytes and dendritic cells (DCs)^{6–8}. In mice, the early innate immune response to *M. tuberculosis* is characterized by the progressive accumulation of neutrophils, inflammatory monocytes, interstitial macrophages and DCs in the lungs. As these cells are recruited, they become infected by the expanding population of mycobacteria and establish early granulomas. In other infectious diseases, the recruitment of phagocytic cells restricts and even eliminates invading pathogens, whereas the recruitment of phagocytes to sites of mycobacterial infection actually benefits the pathogen during the early stages of infection, by providing additional cellular niches for bacterial population expansion⁹.

Considerable evidence indicates that *M. tuberculosis* and other pathogenic mycobacteria, such as *Mycobacterium marinum*, have evolved multiple mechanisms to manipulate their cellular niches for their own advantage. First, pathogenic mycobacteria modulate the trafficking and maturation of the phagosomes in which they reside^{10–12}, allowing them to evade lysosomal mechanisms of restriction, killing and degradation. Second, mycobacteria use several virulence mechanisms to optimize their spread from cell to cell. For example,

the ESX1 type VII secretion system — the absence of which attenuates the strain of *M. bovis* used in the BCG vaccine¹³ — promotes the necrotic death of infected cells and the recruitment of macrophages. This allows the intracellular bacteria to be released from the cell for uptake by the freshly recruited adjacent phagocytes, resulting in subsequent intracellular growth and bacterial population expansion⁹. Third, *M. tuberculosis* possesses multiple mechanisms for inhibiting host cell apoptosis^{14–17}; among other benefits to the bacteria, such inhibition allows for the prolonged survival of infected cells and for a larger number of bacteria to accumulate in a given cell before they are released by cell death¹². Although *M. tuberculosis* clearly possesses distinct mechanisms to regulate apoptotic and necrotic cell death, it remains to be determined how these mechanisms are regulated and how they are manifested during various stages of infection.

During the innate immune stage of *M. tuberculosis* infection, there appears to be little restriction of bacterial growth in the lungs, although this is a highly dynamic stage of infection. The expanding bacterial population spreads from cell to cell and increases the range of cell subsets that it infects to include DCs, which can subsequently initiate adaptive immune responses.

Mechanisms of innate immunity in TB. The innate immune stage is characterized by the recognition of *M. tuberculosis* components by multiple pattern-recognition receptors. Of the Toll-like receptors (TLRs), TLR2 has the largest number of identified mycobacterial agonists, including lipoproteins (as many as 99 of them), phosphatidylinositol mannans and lipomannan¹⁸. In addition, TLR9 senses mycobacterial DNA and contributes to the production of cytokines by macrophages and DCs in *M. tuberculosis*-infected mice¹⁹. Although deletion of *Tlr2* and *Tlr9*, singly or in combination, does not have a marked effect on the control of *M. tuberculosis* in mice, deletion of the gene encoding the shared TLR adaptor molecule MYD88 results in a rapidly lethal infection²⁰. This is probably due to defective signalling in response to interleukin-1 α (IL-1 α) and IL-1 β , as such signalling also depends on MYD88 (REF. 21). Additional recognition of *M. tuberculosis* is mediated by specific members of the C-type lectin receptor (CLR) family, including DC-SIGN^{22,23}, dectin 1 (REFS 24,25), the mannose receptor^{26,27} and mincle^{28,29}. Deletion of any one of these CLR genes has little or no effect on the course of infection, whereas deletion of the gene encoding the shared CLR adaptor molecule CARD9 is associated with accelerated mortality and excessive neutrophilic lung inflammation³⁰.

Of the cytosolic pattern-recognition receptors, nucleotide-binding oligomerization domain protein 2 (NOD2)^{31–33} and NOD-, LRR- and pyrin domain-containing 3 (NLRP3)³⁴ recognize the peptidoglycan subunit *N*-glycolyl muramyl dipeptide and one or more ESX1-secreted substrates (such as ESAT6), respectively. Therefore, stimulation of these pattern-recognition receptors, individually or collectively, induces the expression of pro-inflammatory cytokines,

Cytometry by time-of-flight

A recently described technology that uses time-of-flight mass spectrometry to detect cells bound by antibodies that have been labelled with stable isotopes of rare metals (rather than with the fluorescent compounds that are used in flow cytometry). The use and detection of these labelled antibodies allows the simultaneous detection of over 30 cell parameters, as there is no need for fluorescence compensation.

Granulomas

Aggregates of macrophages and dendritic cells and, at later stages of granuloma development, B and T cells. In areas of established granulomas, modified macrophages can resemble epithelial cells, and macrophages can also fuse to form multinucleated giant cells. Granuloma formation is a chronic inflammatory response that is initiated by various infectious and non-infectious agents.

Phagosomes

Intracellular vesicles that contain large particles (such as bacteria) that have been engulfed by phagocytosis. Phagosomes and endosomes undergo interconnected maturation and merge before fusion with lysosomes. *M. tuberculosis* can modify this pathway and prevent phagosomal maturation.

Type VII secretion system

A bacterial secretion system that uses the hydrolysis of ATP to drive the secretion of proteins and protein complexes across the mycobacterial membrane and cell wall to the extracellular space.

Pattern-recognition receptors

Host receptors (such as Toll-like receptors) that can sense pathogen-associated molecular patterns and initiate signalling cascades, leading to an innate immune response.

selected chemokines and cellular adhesion receptors that contribute to local and systemic immune cell mobilization and activation^{4,35}. However, the initial effects of these responses appear to provide additional cellular niches that favour bacterial growth. Nonetheless, they also provide the basis for the subsequent initiation of cellular adaptive immune responses by driving the recruitment and maturation of DCs⁴.

Despite the multiple molecular and cellular innate immune events that occur during stage 1 of infection (either primary or secondary infection), accelerating the availability of antigen-specific CD4⁺ effector T cells — through the adoptive transfer of these cells — has no effect on the survival or growth of *M. tuberculosis* during the first 7 days of infection³⁶. This suggests that the bacteria are in one or more niches where they either are not recognized by CD4⁺ T cells or are resistant to any anti-mycobacterial action of these cells. This finding indicates that characterizing the status and location of the bacteria during this first stage of infection and immunity is of paramount importance in designing CD4⁺ T cell-directed TB vaccines that block infection.

The innate immune stage of TB is clearly a dynamic one, although the current weight of evidence indicates that it is a stage of infection in which the pathogen dominates, and innate immune responses have little immediate antibacterial effect. Therefore, the significance of the innate immune stage may be its role in establishing an environment that allows an adaptive T cell response to follow. Consequently, understanding the variation of innate immune responses in individuals with differential outcomes of *M. tuberculosis* infection is likely to provide valuable insight into how best to design and choose vectors and adjuvants (which direct innate immune responses) for optimal TB vaccine development.

Stage 2: immunological equilibrium

Delayed initiation of adaptive immunity. A prominent characteristic of adaptive immune responses to *M. tuberculosis* is a long delay in onset. Based on currently available data from tuberculosis skin tests, measurable adaptive immune responses emerge in humans approximately 42 days after *M. tuberculosis* exposure and infection^{7,37}. Interestingly, a similar delay is observed in hepatitis C virus infection, which is another persistent infection of humans^{38,39}. A delayed onset (after 11–14 days) of *M. tuberculosis* antigen-specific T cell responses is also observed in mice following aerosol infection^{10,40,41}. In mice, the activation of *M. tuberculosis* antigen-specific CD4⁺ T cells occurs earliest in lymph nodes that drain the lungs^{10,40,41} and requires the transport of live bacteria from the lungs to the draining lymph nodes by myeloid DCs^{11,41}. After aerosol infection of mice, this transport takes 8–10 days (compared with the ~20 hours required for the transport of influenza virus⁴²), implying that this delayed transport is the rate-limiting step in initiating adaptive immune responses to *M. tuberculosis*. It is currently unclear why this step is so prolonged, although there is evidence that *M. tuberculosis* infection of myeloid DCs inhibits their migration in response to ligands for CC-chemokine receptor 7 (CCR7)⁴³.

In addition, the inhibition of neutrophil apoptosis by *M. tuberculosis* contributes to the delayed kinetics of adaptive immune response induction¹². Aerosol infection of mice with a pro-apoptotic mutant ($\Delta nuoG$) strain of *M. tuberculosis* is associated with accelerated DC-mediated transport of bacteria to the lymph nodes and accelerated activation of naive CD4⁺ T cells; this effect is abrogated by the specific depletion of neutrophils¹². Once bacteria are transported to the draining lymph nodes and produce antigens for presentation to naive CD4⁺ T cells, the proliferation, differentiation and trafficking to the lungs of effector CD4⁺ T cells occurs with kinetics similar to those observed with soluble protein antigens⁴¹. However, *M. tuberculosis* antigen-specific regulatory T cells also develop during the course of infection and contribute to the delayed priming of CD4⁺ and CD8⁺ T cells in the lung-draining lymph nodes⁴⁴.

Arrest but not execution of bacteria. The onset of adaptive immune responses in TB results in the arrest of the progressive growth of the bacterial population and may result in transient disease symptoms, including fever and an unusual skin rash termed erythema nodosum³⁷. After the onset of adaptive immunity, most humans become asymptomatic, do not shed bacteria and are considered to have latent TB infection. It is important to note that the size of the bacterial burden in human latent TB infection is unknown, owing to the current lack of available methods to determine it.

The progressive growth of the bacteria in immunocompetent mice infected with virulent strains of *M. tuberculosis* is also arrested concurrently with the accumulation of effector CD4⁺ and CD8⁺ T cells in the lungs, and these cells maintain a plateau population size of approximately 10⁶ bacteria until the mice die 12–20 months later⁴⁵. These data indicate that, although adaptive immune responses in mice are sufficient to arrest the growth of *M. tuberculosis*, their ability to eliminate *M. tuberculosis* is limited. Multiple mechanisms probably contribute to the limited ability of adaptive immune responses to kill *M. tuberculosis*. Such mechanisms include: impaired MHC class II-mediated antigen presentation^{46,47}; induction of the anti-inflammatory mediator lipoxin A4 (REF. 17); restriction by regulatory T cells⁴⁸; downregulation of bacterial antigen gene expression and, therefore, failure to induce antigen-specific CD4⁺ T cells^{49,50}; and resistance to the macrophage-activating effects of interferon- γ (IFN γ)^{51–53}.

It is noteworthy that, although the size of the bacterial population remains stable, a subpopulation of bacteria continues to replicate during this chronic, clinically silent stage of infection in mice⁵⁴. Moreover, a recent study in non-human primates revealed that *M. tuberculosis* also accumulates mutations during latency⁵⁵. Taken together, these data provide convincing evidence that latent TB is not simply a state of bacterial stasis, but a state of dynamic bacterial and immunological equilibrium.

Lipoxin

A member of a family of leukocyte-derived eicosanoids that are generated during the inflammatory response and function as downregulatory signals.

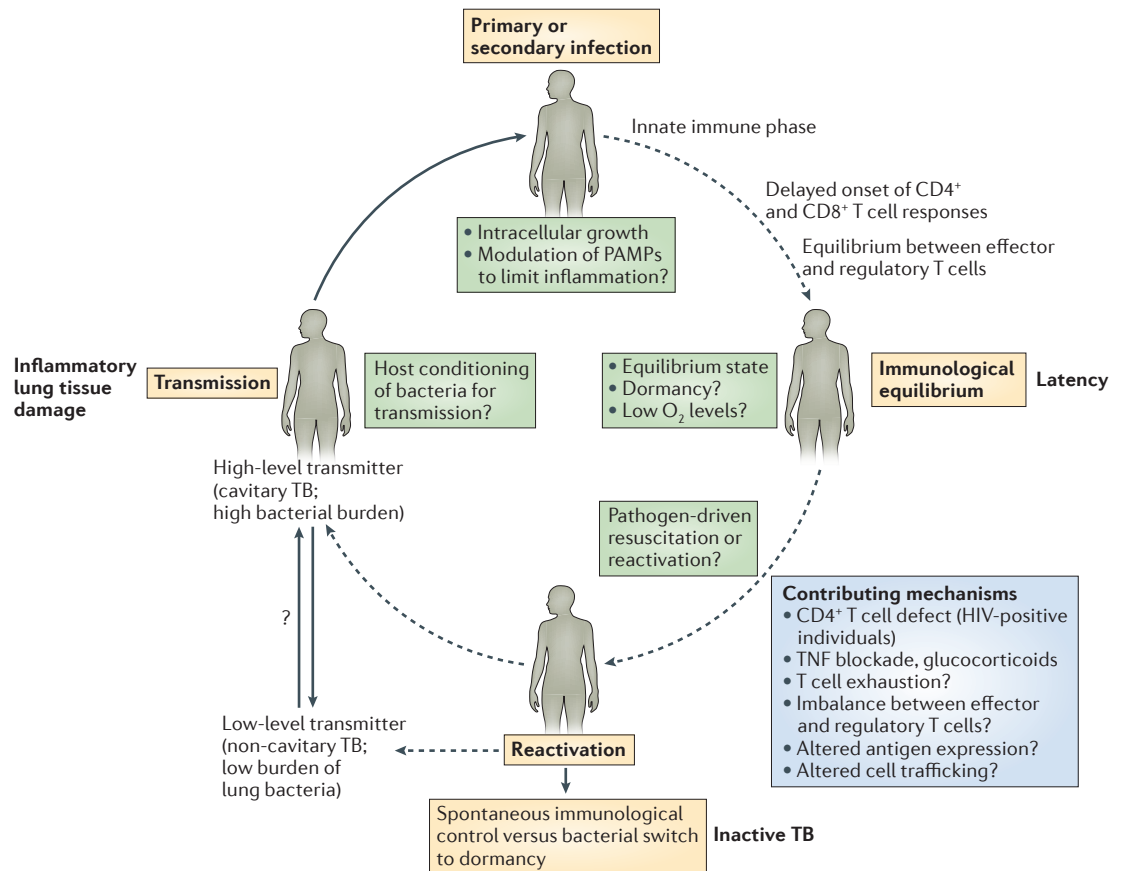


Figure 1 | **The stages in the immunological life cycle of tuberculosis.** The framework for the life cycle is based on clinical, epidemiological and immunological studies in humans. Included are examples of some of the immunological mechanisms and functions that characterize each stage, in cases where they are known. Examples of mechanisms with question marks are hypothetical and are discussed in the text. Shown in the centre are examples of the known or experimentally supported states of the bacteria at distinct stages of the immunological life cycle. PAMP, pathogen-associated molecular pattern; TB, tuberculosis; TNF, tumour necrosis factor.

Immunological mechanisms that contribute to equilibrium. Adaptive immunity to *M. tuberculosis* in humans⁵⁶, mice⁴⁵, cattle⁵⁷ and non-human primates⁵⁸ depends on CD4⁺ T cells; additional contributions of CD8⁺ T cells are well established in mice⁴⁵ and non-human primates⁵⁹. In addition to responses by classical MHC class I- or class II-restricted $\alpha\beta$ T cells that recognize bacterial peptide epitopes, responses by other T cell subsets are observed. Such cells include CD1-restricted, mycobacterial lipid-specific T cells (which are predominantly CD4⁺)^{60,61}, HLA-E-restricted CD8⁺ T cells^{62,63} and mucosa-associated innate-like T cells⁶⁴. Although these other T cell subsets are under active investigation, their roles in immunity to TB have not yet been determined.

Among the mediators of immunity to *M. tuberculosis*, tumour necrosis factor (TNF) and IFN γ are the best described in humans^{65–67}, owing to the use of TNF-blocking therapeutic agents and the characterization of mutations in the IFN γ receptor gene. Additional molecules that contribute to the immune control of *M. tuberculosis* in mice, but that have not yet been shown to be significant in humans, include IL-17, cytolytic T cell-expressed

perforin and the IFN γ -induced molecules nitric oxide synthase 2 (NOS2) and LRG47 (also known as IRGM1)⁴. Furthermore, several mediators have been characterized for their specific roles in the human immune response to *M. tuberculosis*. Granulysin is a cytolytic T cell granule protein that has direct anti-mycobacterial activity *in vitro*⁶⁸, although its role in controlling *M. tuberculosis in vivo* remains unknown. Vitamin D also has broad functions *in vitro* that contribute to immune-mediated control of *M. tuberculosis*; for example, it is an essential cofactor for the IFN γ -mediated induction of the anti-mycobacterial peptide cathelicidin⁶⁹. Furthermore, vitamin D levels in humans are closely associated with susceptibility to active TB⁷⁰.

Despite extensive investigation, a clear, reproducible correlate of human immunity to *M. tuberculosis* infection has not yet been identified. There are several potential reasons for this. First, our knowledge of the full repertoire of T cell subsets and molecular mediators of protective immunity is still emerging, implying that one or more crucial determinants have not yet been examined. Second, it seems increasingly likely that no single parameter will mediate or correlate with protective

immunity in tuberculosis, implying that increasing use of systems biology, bioinformatics and biostatistics will be needed to formulate optimal models and test them in expanded studies. Third, it is possible that using healthy subjects with latent TB infection as the 'gold standard' of protective immunity may lead to erroneous conclusions, as latent TB does not equate with sterile immunity, and latent TB progresses to reactivation TB in a substantial fraction of individuals. Therefore, there is clearly a great need for methods to reliably identify distinct states of infection and the corresponding immune responses after exposure to *M. tuberculosis*².

The bacterial contribution to equilibrium. Strong evidence exists that the mycobacteria are also active contributors to the immunological equilibrium state in latent TB. First, a well-characterized bacterial regulon that is controlled by DosR–DosS — a two-component signal transduction system in mycobacteria — is induced by several stimuli thought to prevail during latent TB, including local hypoxia^{71,72}, nitric oxide⁷³ and carbon monoxide^{74,75}. This 'dormancy' regulon controls the expression of genes that allow the bacteria to use alternative energy sources, especially lipids, and genes encoding factors that are selectively recognized by T cells from humans with latent TB (but not active TB)^{76,77}. The expression of this gene network — as well as of other genes involved in using alternative energy sources (such as genes encoding isocitrate lyases⁷⁸) — implies that *M. tuberculosis* has evolved specific mechanisms to adopt a state of latency, and that latency is not merely the suppressive effect of the host immune response on bacterial replication.

In addition, *M. tuberculosis* encodes five proteins that resemble the well-characterized *Micrococcus luteus* resuscitation-promoting factor (Rpf), which is a secreted protein that has the ability to 'resuscitate' bacteria from a nutrient-starved dormant state (reviewed in REF. 79). Deletion of one or more of the *M. tuberculosis* Rpf genes generates bacteria that have an impaired recovery from dormancy, indicating that these genes may participate in the progression from latency to reactivation^{80,81}. Finally, *M. tuberculosis* encodes 88 toxin–antitoxin gene pairs, the expression balance of which regulates multiple phenomena, including whether the bacteria replicate or remain static⁸². Thus, *M. tuberculosis* possesses at least three systems (the dormancy regulon, resuscitation-promoting factors and toxin–antitoxin gene pairs) that regulate its metabolic and growth state. Further investigation is likely to provide insights into the host and bacterial mechanisms that regulate these systems and that determine whether the bacteria remain in an equilibrium state with the host or resume growth and reactivate to cause active TB disease.

Stage 3: reactivation TB

Reactivation of latent TB reflects progression to active, symptomatic disease, which is usually characterized by the shedding of *M. tuberculosis* in respiratory secretions, especially during coughing. Reactivation TB must be distinguished from re-infection with a second strain of

bacteria, which can occur even in immunocompetent individuals⁸³. However, most cases of TB in adults are attributable to reactivation, except in geographical regions with an extremely high prevalence of TB. One study clearly showed, through the genotyping of strains, that reactivation TB can occur decades after initial infection⁸⁴. Reactivation TB is widely attributed to 'weakened' immunity, although only a minority of cases are attributable to well-characterized defects in immunity.

Established mechanisms underlying TB reactivation. In humans, only two mechanisms have been identified that explain reactivation TB, both of which have become relevant only in the recent past.

The first mechanism involves the quantitative and qualitative CD4⁺ T cell defects that occur in people infected with HIV⁵⁶. In addition to the extensive depletion of CD4⁺ T cells, there is strong experimental evidence from human studies to suggest that, before this profound CD4⁺ T cell depletion, HIV targets and depletes *M. tuberculosis* antigen-specific CD4⁺ T cells at a greater frequency than CD4⁺ T cells specific for other antigens^{85,86}. This finding may account for the increased risk of active TB early after HIV infection, before there is a measurable depletion of circulating CD4⁺ T cells⁵⁶. In addition, the depletion of CD4⁺ T cells by simian immunodeficiency virus (SIV) in non-human primates causes the reactivation and progression of TB⁵⁸, and depletion of CD4⁺ T cells during the chronic stage of *M. tuberculosis* infection in mice allows for the resumption of net bacterial growth in the lungs⁴⁹. Despite the abundant evidence that deficiencies of CD4⁺ T cells cause reactivation of *M. tuberculosis*, the precise mechanisms that these cells use to establish and maintain immune control of *M. tuberculosis* in the latent state remain to be identified.

The second well-characterized mechanism that is clearly associated with reactivation TB is the therapeutic neutralization of TNF⁶⁵, especially by monoclonal antibodies⁸⁷. Despite the strength of the association, the effects of TNF blockade that account for reactivation TB are not fully characterized, but they include: decreased macrophage-mediated anti-mycobacterial activity and the subsequent death of macrophages⁸⁸; the induction of a higher frequency of regulatory T cells⁸⁹; and the depletion of a subset of CD45RA⁺ effector memory CD8⁺ T cells that contain granzyme and have been shown to contribute to *M. tuberculosis* killing *in vitro*⁹⁰.

Together, the increased frequency of TB in people infected with HIV or treated with TNF-blocking agents establish CD4⁺ T cells and TNF as two of the major elements that mediate protective immunity in TB and that prevent reactivation, although the underlying mechanisms are incompletely understood.

Established associations with other medical conditions.

In addition to the mechanisms known to promote TB reactivation, other medical conditions have been found to be associated with an increased risk of reactivation, although the underlying mechanisms are not well understood. These conditions include diabetes mellitus,

Toxin–antitoxin gene pairs

Paired loci found in the chromosomes of almost all free-living bacteria, as well as in many plasmids and phage genomes. They encode a toxin and its antidote, which have been shown to contribute to plasmid stability through a mechanism known as post-segregational killing. They are also proposed to function in bacterial programmed cell death or stress physiology.

the increasing prevalence of which in developing countries is leading to the convergence of its geographical distribution with that of TB to increase the severity of the TB epidemic⁹¹. There is recent intriguing evidence that mice with diabetes have a longer delay in the onset of adaptive immune responses to *M. tuberculosis* than mice without diabetes, owing to delayed trafficking of DCs from the lungs to the lymph nodes⁹². However, the mechanism underlying this delay has not yet been determined, and little is known regarding the mechanisms by which diabetes predisposes to TB in humans.

Treatment with glucocorticoids is also a well-known risk factor for reactivation TB⁹³, although the pleiotropic primary and secondary effects of glucocorticoids on immunity and inflammation make it difficult to determine which have the most potent impact on the reactivation of *M. tuberculosis*. Furthermore, a thin body habitus (with or without malnutrition) has long been linked to TB reactivation⁹⁴. This relationship might be partially explained by the effects of leptin, which is best characterized for its regulation of energy expenditure and appetite, as the circulating levels of leptin are low in thin and malnourished people⁹⁵. Leptin also modulates the development and function of T helper 1 (T_H1) cells⁹⁶, suggesting a mechanism for the enhanced susceptibility to TB in thin people. Indeed, leptin-unresponsive mice poorly control *M. tuberculosis* infection⁹⁷. Other conditions that have been epidemiologically linked to an increased likelihood of *M. tuberculosis* reactivation include silicosis, haematological malignancies, cancer chemotherapy, uraemia, gastrectomy and advanced age⁹⁸, but none of these have been studied with respect to their effects on specific immune mechanisms.

Although the aforementioned mechanisms and associations are notable, they account for a small minority of cases of TB reactivation. This suggests that the widely held model that 'weakened' or waning immunity accounts for TB reactivation requires reconsideration. In particular, advances in basic immunology suggest several alternative models that warrant attention. The hypothetical models described below were selected because they can be tested, and because they are plausible. However, it is highly likely that additional mechanisms and models exist and are worthy of investigation.

Possible mechanism: T cell exhaustion. One increasingly well-characterized mechanism of failed immunity in chronic infections is T cell exhaustion, in which pathogen-specific T cells are present but express inhibitory receptors that prevent their proliferation and their ability to mediate effector functions⁹⁹. T cell exhaustion is best described in chronic viral infections, such as lymphocytic choriomeningitis virus (LCMV) infection in mice, and hepatitis C virus and HIV infection in humans⁹⁹. So far, it is not clear whether T cell exhaustion occurs in TB, although a recent study revealed an inverse relationship between the numbers of polyfunctional *M. tuberculosis* antigen-specific CD4⁺ T cells in the blood and the apparent bacterial burden in the lungs¹⁰⁰. This finding is consistent with CD4⁺ T cell exhaustion and warrants

further investigation. By contrast, a recent study in mice revealed that programmed cell death protein 1 (PD1) — an inhibitory receptor expressed by exhausted T cells — is expressed on *M. tuberculosis* antigen-specific CD4⁺ T cells in the lungs, but that these cells retain the ability to proliferate and can differentiate into cytokine-producing effector CD4⁺ T cells¹⁰¹. Moreover, PD1-deficient mice — which can clear infection with an otherwise persistent strain of LCMV — succumb to overwhelming pulmonary inflammation when infected with *M. tuberculosis*¹⁰²; this effect of PD1 deficiency is attenuated by the depletion of CD4⁺ T cells. Together, these data indicate that pathways that operate in exhausted CD8⁺ T cells in chronic viral infections have different functions in CD4⁺ T cells in TB. In addition, these data suggest that a complex pathogen containing multiple antigens, such as *M. tuberculosis*, may use mechanisms other than T cell exhaustion to prevent its elimination.

Possible mechanism: altered antigen expression. Unlike viruses, which have a programmed pattern of gene expression, *M. tuberculosis* and other bacteria and parasites respond to signals from their environment to regulate their gene expression. In addition to allowing bacterial survival and growth under diverse conditions, this ability to regulate gene expression contributes to the alteration of antigen gene expression profiles at distinct stages of infection, allowing the bacteria to evade recognition by T cells specific for certain antigens. In particular, the expression of at least two antigens that are immunodominant in humans and mice — ESAT6 and Ag85B — is downregulated after the appearance of CD4⁺ and CD8⁺ T cells in the lungs of infected mice^{103,104}. In the case of Ag85B, which is contained in several of the lead candidate TB vaccines, downregulation of gene expression contributes to a marked reduction in the frequency of activated Ag85B-specific effector CD4⁺ T cells during the chronic stage of infection, and this contributes to the persistence of bacteria in the lungs⁴⁹. It is likely that *M. tuberculosis* responds similarly to environmental cues in humans; whether this results in reduced activation of effector T cells and contributes to TB reactivation remains to be determined. However, the magnitude of the reduction in gene expression is more marked for Ag85B (and the closely related antigen Ag85A) than for ESAT6 (REF. 104), and the expression of genes encoding other antigens (such as HspX and Rv2660c) is maintained or increased during chronic infection. This indicates that, although the profile of antigen expression may change during infection, a distinct repertoire of antigens and T cells may contribute to the maintenance of host–pathogen equilibrium during latency.

Given the implications of these results for choosing antigens for new TB vaccines, the characterization of antigen gene expression during distinct stages of infection should be a high priority. Indeed, a protein-subunit TB vaccine incorporating a 'latency' antigen that is expressed predominantly during the chronic stage of infection has shown a greater efficacy than the same vaccine containing antigens expressed exclusively or predominantly in the initial stages of infection¹⁰⁵.

Glucocorticoids

A group of compounds that belong to the corticosteroid family. These compounds can be either naturally produced (hormones) or synthetic. They affect metabolism and have anti-inflammatory and immunosuppressive effects. Many synthetic glucocorticoids (for example, dexamethasone) are used in clinical medicine as anti-inflammatory drugs.

Thin body habitus

Thin body build. As defined in the studies of tuberculosis risk, the deviation of the weight of an individual from the median weight for height of the group as a whole was ascertained and used to stratify individuals. The incidence of tuberculosis was highest in the group whose weight deviated from the median by 15% or more.

Possible mechanism: altered cell trafficking. Maintaining an efficacious immune response at the site of *M. tuberculosis* infection is likely to require the continuous recruitment of effector immune cells, although little is known about the kinetics of cell turnover in granulomas. If cell trafficking to granulomas needs to be maintained for decades to maintain local immunity in latent TB, it stands to reason that defective cell trafficking, even if slight or intermittent, could allow for TB reactivation. In mice, transgenic overexpression of CC-chemokine ligand 2 (CCL2; also known as MCP1)¹⁰⁶ or the absence of CCR2 (REFS 107,108) decreases the recruitment of monocytes and DCs to the site of *M. tuberculosis* infection and is associated with poorer immune control of infection. By contrast, CXC-chemokine receptor 3 (CXCR3)-deficient mice are more resistant to infection and can control chronic *M. tuberculosis* infection in the lungs more effectively than wild-type mice¹⁰⁹.

In humans, several polymorphisms in genes encoding chemokines and chemokine receptors — such as functional variants of *CCL2*, *CCL3L1* and *CCR5* — have been associated with active *M. tuberculosis* infection^{110,111}. Because the effects of these polymorphisms have been described in adults, it is likely that their association is with reactivation TB, suggesting that maintaining optimal recruitment of specific myeloid and lymphoid subsets is required for durable control of *M. tuberculosis*, and that suboptimal cell trafficking may permit reactivation.

Can the bacteria be the primary drivers of reactivation?

As noted above, *M. tuberculosis* has specific programmes for initiating a state of dormancy in response to certain environmental signals (some of which are imposed by adaptive immune responses), and this state manifests as clinical latency. In turn, *M. tuberculosis* also has specific programmes for recovering from dormancy, suggesting that the bacteria may assume a primary role in some cases of reactivation TB that are not explained by immune defects or deficiencies. Therefore, this is an area that should be investigated in more detail.

Spontaneous deactivation. One variation of reactivation that occurs in a substantial proportion of actively infected humans is the spontaneous resolution or deactivation of the infection, that is, progression from active to inactive disease without anti-tuberculous chemotherapy. Inactive TB differs from latent TB in that in the former there are often abnormalities detected on chest X-rays, whereas such findings are absent in latent TB. Although spontaneous resolution of active TB to inactive TB is reported to have occurred in as many as 50% of individuals in the pre-chemotherapy era (reviewed in REF. 112), its mechanisms are not understood. A recent study of long-term survivors of untreated TB revealed that approximately 70% of these individuals had CD4⁺ effector memory T cell responses to *M. tuberculosis* antigens, suggesting that they were persistently infected. By contrast, a substantial fraction of the remaining individuals had CD4⁺ central memory T cell

responses, consistent with clearance of infection¹¹³. These observations further highlight the spectrum of host–pathogen interactions in TB, and suggest that future studies of immune responses in humans with active TB should be designed to account for the possibility that a substantial fraction of the subjects might have immune responses that allow them to regain control of their infection.

Overall, reactivation TB is poorly understood, especially from the perspective of the mechanisms that allow progression from latency to reactivation. As studies to understand this important phenomenon in the context of ‘weakened’ immunity have revealed only two clearly established mechanisms (CD4⁺ T cell depletion and TNF blockade), and these apply to a minority of cases of reactivation TB, there is considerable opportunity for the discovery of additional mechanisms that account for a greater number of cases. As one especially promising example, transcriptional profiling of peripheral blood cells from humans with latent and active TB has revealed the previously unsuspected association of active TB with a type I IFN signature and with the expression of neutrophil-specific genes¹¹⁴. In this study, a range of transcriptional signatures was observed among individuals, and some of these may be attributable to distinct stages of the immunological life cycle of TB. It is likely that additional prospective analyses — particularly of people recently exposed to active TB cases and presumably newly infected¹¹⁵ — will clarify the roles of the type I IFN and neutrophil signatures in the pathogenesis of and immunity to TB.

Stage 4: transmission

An obligate step in all infectious diseases is transmission to new hosts. In the case of TB, this occurs through the airborne route, in which bacteria are expelled (usually by coughing) from an individual with active disease and then inhaled by susceptible hosts. As in many other infectious diseases, the transmission of TB is not uniform, and certain individuals cause far more secondary cases than do others^{116,117}. In particular, individuals with a form of TB termed cavitory TB are especially infectious¹¹⁸. Cavitory TB is the consequence of lung tissue destruction and the formation of macroscopic open spaces that contain numerous *M. tuberculosis* bacilli¹¹⁹ and connect to large airways, which facilitates efficient expectoration of the bacteria.

Evidence that immune responses promote transmission. Several lines of evidence indicate that — in addition to their widely known roles in protecting an infected individual from rapidly lethal TB — human T cell responses contribute to the lung tissue destruction underlying cavitory TB, and thereby may contribute to host-to-host TB transmission. In particular, multiple studies have revealed that individuals with TB who are co-infected with HIV have a lower frequency of cavitory TB, and a recent systematic review revealed a linear correlation between the number of circulating CD4⁺ T cells and the frequency of cavitory TB⁵⁶. Indeed,

Effector memory T cell

A terminally differentiated T cell that lacks lymph-node-homing receptors but expresses receptors that enable it to home to inflamed tissues. Effector memory T cells can exert immediate effector functions without the need for further differentiation.

Central memory T cell

An antigen-experienced T cell that expresses cell-surface receptors required for homing to secondary lymphoid organs. These cells are generally thought to be long-lived and can serve as the precursors for effector T cells in recall responses.

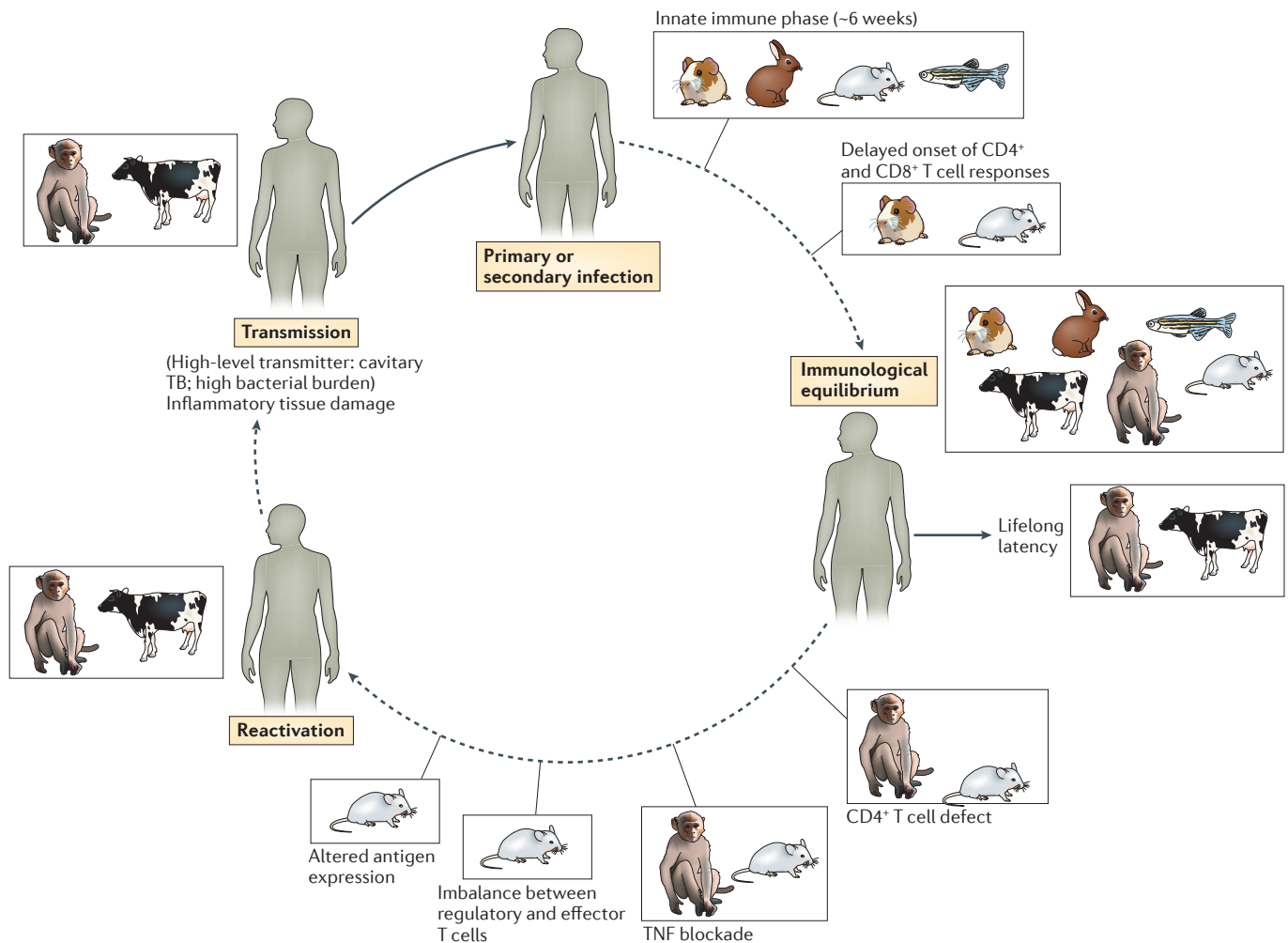


Figure 2 | Stages of the immunological life cycle of human tuberculosis that can currently be modelled in experimental animals. Shown are the animal models (zebrafish, mouse, guinea pig, rabbit, cattle and non-human primate) that have been used to study individual stages of the immunological life cycle. In some cases, infection of specific animals may reproduce stages that are not included, but the models for these stages, using these animals, have not yet been fully explored. TB, tuberculosis; TNF, tumour necrosis factor.

this study showed that the likelihood of cavitory TB was fourfold higher in subjects who had more than 200 CD4⁺ T cells per μ l of blood than in individuals with fewer than 200 CD4⁺ T cells per μ l of blood. In addition, HIV-infected people transmit TB less efficiently than do HIV-uninfected people (reviewed in REF. 56). This is in contrast to observations of other infections, such as influenza virus infections, in which immunodeficient people shed higher levels of virus and for longer periods of time than those with intact immunity¹²⁰. It is unclear whether the effect of CD4⁺ T cells on the promotion of cavitory TB is direct or indirect, and the mechanisms by which CD4⁺ T cells contribute to lung tissue damage and cavitory TB are not well characterized. Although the collagen-degrading metalloproteinase MMP1 has been implicated as a mediator¹²⁰, its relationship to the contributions of CD4⁺ T cells has not yet been established. Precedents provided by studies of tissue damage in T cell-dependent autoimmune diseases may guide studies to determine whether specific functions

of effector CD4⁺ T cells are involved, and whether the antigen specificity of CD4⁺ T cells is distinct in humans with cavitory TB compared with those with non-cavitory TB.

A recent study of genetic diversity in human T cell epitopes of *M. tuberculosis* contributed evidence consistent with a role for T cell responses in TB transmission¹²¹. The study of nearly 500 experimentally verified human T cell epitopes in a collection of geographically and genetically diverse strains of *M. tuberculosis* (the ancestors of which had diverged at least 15,000 years ago) revealed that the known human T cell epitopes of *M. tuberculosis* are the most conserved elements of the *M. tuberculosis* genome¹²¹. These results are consistent with a model in which human T cell responses, although providing (partial) protection to individual infected hosts, provide a net evolutionary benefit to *M. tuberculosis*. T cell responses probably mediate this effect by contributing to inflammatory tissue damage and lung cavitation, which promotes the transmission of the bacteria to new hosts.

Non-human models of TB transmission are needed.

Although the results in humans demonstrate an association between CD4⁺ T cells, cavitary TB and TB transmission, the discovery of the underlying direct and indirect mechanisms is likely to require studies in a non-human animal model. However, a small-animal model that can be used to study TB transmission has yet to be developed. Although mice, guinea pigs and rabbits all provide models for certain stages of TB infection and immunity (FIG. 2), none of these animals transmit TB efficiently. By contrast, bovine strains and other animal-adapted strains (such as *Mycobacterium microti* in voles¹²²) in the *M. tuberculosis* complex and closely-related species (such as *M. marinum* in fish¹²³) are transmitted in the wild, and these strains represent opportunities for the study of determinants of transmission in naturally co-evolved host–pathogen pairs. For the optimal design and execution of such studies, as well as of studies on the effects of vaccination and other immunological interventions in preventing and promoting TB transmission, an investment in generating and validating immunological and genetic tools for use in these animals is necessary.

Conclusion and perspective

The development of efficacious vaccines against TB presents unique challenges that demand a better understanding of protective and pathological immune responses in TB. First, clear correlates of protective immunity have not yet been identified, especially in humans, making surrogate end points inadequate for evaluating TB vaccine efficacy. Second, even though systematic study and selection of vaccine antigens has led to the development of promising candidate vaccines^{105,124}, the efficacy of these vaccines in preventing TB in a human population remains to be determined. Third, TB may be unique in its exploitation of immune responses to promote transmission.

A much better understanding of the mechanisms and targets of pathogenic immune responses in TB is needed to minimize the likelihood of vaccine-induced pathogenic immune responses and the risk of serious adverse effects of new TB vaccines. Thus, although vaccine development for TB needs to proceed as rapidly as possible, these efforts

need to be matched by a more extensive understanding of beneficial and detrimental immune responses to natural infection. These studies will benefit from consideration of the stage of the TB immunological life cycle that is represented in the subjects, as distinct immune responses may have different roles at distinct stages of the life cycle. For example, studies in mice indicate that IL-17 is beneficial to the host during early *M. tuberculosis* infection¹²⁵, whereas it appears to be detrimental, especially in high concentrations, later in infection¹²⁶. Likewise, neutrophils promote the development of adaptive immune responses to *M. tuberculosis* early after infection^{12,43}, whereas they can be detrimental in later stages¹²⁷. These examples in a simplified system illustrate how the failure to consider the stage of infection and the immunological life cycle would lead to conflicting conclusions regarding the roles of IL-17 and neutrophils in mice with tuberculosis. Considering the immunological life cycle stage in studies of human subjects with TB may allow for better distinction of beneficial and detrimental innate and adaptive immune responses and mechanisms.

This Review proposes and presents evidence that immunity to *M. tuberculosis* develops and proceeds in a manner consistent with four distinct stages of a life cycle, in which the state of the bacteria and the nature of immune responses exhibit specific features. In some cases, the distinctions between the stages of the immunological life cycle are clear, as exemplified by the stage limited to innate immune responses and the stage of immunological equilibrium after adaptive immune responses develop and latency is established. However, much more knowledge is needed to fully understand the differences that occur in T cell phenotypes and functions, and in the targets of T cell responses, between the stage of immunological equilibrium and the reactivation and transmission stages. Although our understanding of the mechanisms and targets of immunity to *M. tuberculosis* has advanced considerably, a higher resolution understanding is needed, and some of this will require the development of new experimental models. The overall goal of this Review is to provide a conceptual framework for prioritizing, designing and interpreting the results of future studies, in order to derive the maximum benefit from efforts to decrease the global burden of tuberculosis.

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FURTHER INFORMATION

Joel D. Ernst's homepage: <http://pathology.med.nyu.edu/people/faculty/ernst-joel-d>

World Health Organization TB data:

<http://www.who.int/tb/country/en/index.html>

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