

The Tuberculous Granuloma and Preexisting Immunity

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Abstract

Pulmonary granulomas are widely considered the epicenters of the immune response to *Mycobacterium tuberculosis* (Mtb), the causative agent of tuberculosis (TB). Recent animal studies have revealed factors that either promote or restrict TB immunity within granulomas. These models, however, typically ignore the impact of preexisting immunity on cellular organization and function, an important consideration because most TB probably occurs through reinfection of previously exposed individuals. Human postmortem research from the pre-antibiotic era showed that infections in Mtb-naïve individuals (primary TB) versus those with prior Mtb exposure (postprimary TB) have distinct pathologic features. We review recent animal findings in TB granuloma biology, which largely reflect primary TB. We also discuss our current understanding of postprimary TB lesions, about which much less is known. Many knowledge gaps remain, particularly regarding how preexisting immunity shapes granuloma structure and local immune responses at Mtb infection sites.

INTRODUCTION

During the nineteenth and early twentieth centuries, tuberculosis (TB) was a leading cause of death in the USA and Western Europe. Accordingly, TB was at the forefront of medical research. Autopsies were common, and the scientific literature from this era contains a treasure trove of meticulous descriptions of lung pathology across the spectrum of human *Mycobacterium tuberculosis* (Mtb) infection, from asymptomatic infection to TB disease (1–6). Because these studies were performed before the advent of antibiotics to treat TB, the natural history of infection could be painstakingly detailed. A central paradigm was that primary TB, which occurs in individuals (usually children) after their first Mtb exposure, and postprimary TB, which develops several years after first exposure, are starkly different diseases. The pulmonary lesions associated with these two forms of TB are quite distinct, differing in location, composition, and pathogenesis.

Today, autopsies are rare, and assessments of Mtb-infected human lung tissue are usually restricted to therapeutically resected lobes from individuals with advanced disease and long-term antibiotic exposure. Our recent understanding of TB granuloma biology is based largely on animal models, which have revealed a complex dichotomy: While individual granulomas can vary in their ability to control Mtb replication, and some can even eradicate the pathogen, the overall result is often a standoff between Mtb and the immune system, a compromise that permits Mtb persistence but keeps the pathogen relatively contained. Although these recent studies have revealed important insights, an important caveat is that they are usually performed in animals without prior Mtb exposure. They reflect models of primary TB granulomas, and the distinctions between primary and postprimary lesions are rarely considered. This is problematic because most cases of human TB occur in individuals previously exposed to the bacteria.

In this review, we attempt to bridge insights gained from historical studies of human TB pathology with those from more recent studies in animal models. Our understanding of the older human research has been greatly advanced by Hunter (6, 7) and Hunter & Actor (8), who, by combining a careful review of the literature with their own assessment of human Mtb-infected lung tissue, have expressed concern that current animal models do not adequately reflect postprimary TB. We summarize these arguments and use them to try to contextualize recent findings in TB granuloma biology from animals, which provide good models for primary TB. We review immune pathways and microenvironments within primary TB granulomas that promote and restrict Mtb immunity. We discuss how immunologic memory associated with prior Mtb exposure may alter these factors at tissue infection sites and highlight knowledge gaps that need further investigation. In addition to adaptive immunity, prior exposure may shape innate immune function, a phenomenon termed trained innate immunity, but because excellent reviews of these topics in TB are available elsewhere (9–11), we do not review them here. We hope our review will spur future investigations using human postmortem tissues and improved animal models to understand how preexisting immunity shapes TB pathogenesis.

GRANULOMAS IN PRIMARY TUBERCULOSIS

In its simplest terms, a granuloma can be defined as an aggregate of macrophages, yet the organization of TB granulomas is remarkably heterogeneous and usually involves numerous immune cell types. Murine studies have shown that granulomas arise after alveolar macrophages phagocytize inhaled Mtb bacilli and migrate into the lung interstitium (12). Other immune cells, including neutrophils and monocyte-derived macrophages, are recruited, take up Mtb, and begin to organize into the nascent granuloma. Given this sequence of events, granulomas in primary TB tend to localize where inhaled Mtb-laden droplets are mostly likely to implant, the well-ventilated bases of the human lung and within 1 cm of the pleural surface (13), as airways that supply alveoli in the

lung periphery are larger and straighter than those that supply the lung interior (14). This location is quite distinct from the site of postprimary TB infection, which occurs in the lung apices, and is discussed in more detail in the section titled Postprimary Tuberculosis: A Distinct Pathogenesis.

Several animal models have been used to study the pathologic features and host-pathogen interactions within the pulmonary TB granuloma. Nonhuman primates (NHP) are generally accepted as having clinical disease that is most similar to that in humans, and they form all the stereotypical features of human granulomas: a central core of *Mtb*-infected and uninfected macrophages with or without a nutrient-deprived region of caseous necrosis (an area of cellular debris resulting from cell death, so named by pathologists because it was thought to resemble a cheese-like substance), a surrounding rim of epithelioid macrophages, a T cell-rich lymphocytic cuff that contains tertiary lymphoid structures (TLS), and often fibrosis (15–17). Rabbits and guinea pigs also form caseating TB granulomas and have been used extensively for TB research (18, 19), but these models are limited by a relative paucity of experimental tools. Zebrafish are useful because of their optical transparency, allowing visualization of the early events of granuloma formation (20). Murine tuberculosis is the most tractable model, although infection of the most common mouse strain (C57BL/6) with common experimental *Mtb* strains has been criticized for failing to reproduce key features of human tuberculosis granulomas, particularly central necrosis and epithelioid macrophages (15). Modulation of host and pathogen genetics can mitigate this limitation and result in a wide range of granuloma features. The C3HeB/FeJ (i.e., C3H or Kramnik) strain can form granulomas with central necrosis when infected with common experimental strains (21) and can even develop large caseating lesions when infected with highly transmissible W Beijing strains (22). The gene responsible for preventing the phenotype seen in C3H mice was recently identified as *Sp140*, which suppresses type I interferon transcription, revealing new insight into the underlying mechanisms of necrotic granuloma formation (23). Additionally, recent research using the Beijing lineage HN878 *Mtb* strain in C57BL/6 mice revealed the formation of TLS in the granuloma cuff, a feature of human granulomas (24). Furthermore, most C57BL/6 or C3H mice infected with a physiologic ultralow dose (ULD; 1–3 CFU) of aerosolized H37Rv exhibit a single, discrete granuloma, which gains hallmark features of human granulomas, chiefly spatial segregation of infected macrophages and T cells, as well as TLS located on the granuloma cuff (25). Together, these studies suggest that granuloma structure may be modified upon alterations of *Mtb* genetics, host genetics, and infectious dose, enhancing the mouse model's preclinical relevance.

GRANULOMA STRUCTURE AND IMMUNE CELL TRAFFICKING

Historically, the TB granuloma was thought to benefit the host primarily by “walling off” *Mtb* bacilli from the rest of the lung. This idea arose from the observation of structural changes surrounding infected macrophages, which were thought to result in physical barriers that prevent bacterial escape. More recently, however, there has been growing appreciation that granulomas can also provide a permissive environment for *Mtb*, in part by limiting the trafficking and function of immune cells (26–28). While the scarcity of T cells at *Mtb*-infected sites within the granuloma is well described, few studies have directly evaluated what governs access into the granuloma core. Here, we discuss key structural components of the granuloma and the factors dictating entry and function of immune cells within them (Figure 1).

Epithelioid Macrophages

Epithelioid macrophages form at the granuloma periphery and are characterized by a high cytoplasm-to-nucleus ratio and interdigititation with neighboring cells, akin to the structural epithelial cells from which their name is derived (29, 30). Despite their common association

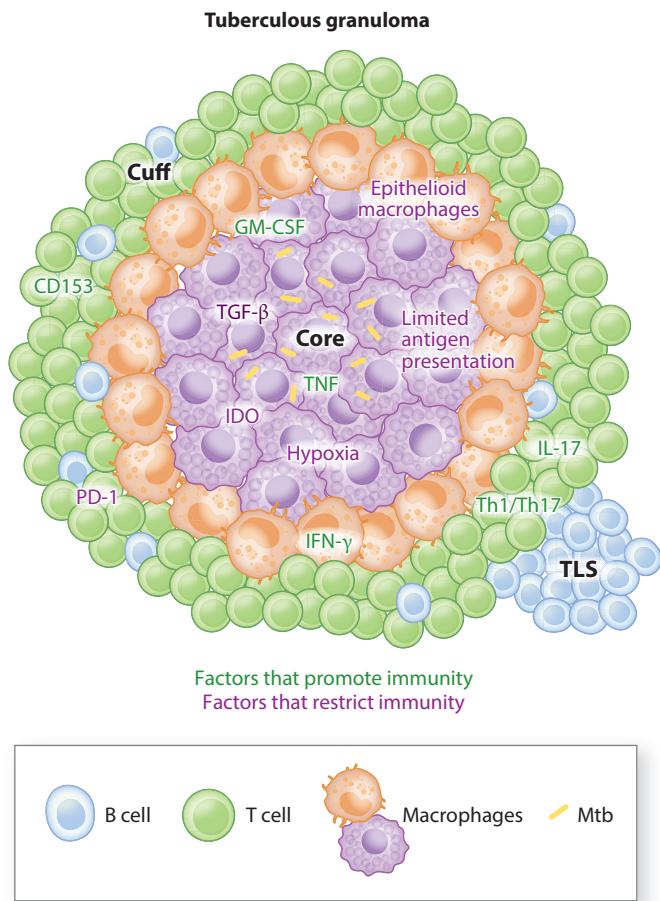


Figure 1

Host factors can both promote and restrict antimycobacterial immunity within the tuberculous granuloma. The granuloma is a prototypic structure that forms in response to *Mycobacterium tuberculosis* (Mtb) infection and comprises a heterogeneous population of cells, including a central core of infected macrophages surrounded by a cuff of lymphocytes and associated tertiary lymphoid structures (TLS). The dichotomous nature of the granuloma has led to questions regarding its benefit in controlling infection. Research primarily in animal models has shown that it can restrict bacterial growth and dissemination by forming an oxygen-limiting environment encapsulated within a rim of epithelioid macrophages. Other mechanisms that promote bacterial clearance include production of proinflammatory cytokines, such as IFN- γ , TNF- α , IL-17, and granulocyte-macrophage colony-stimulating factor (GM-CSF), and expression of protective factors, such as CD153. However, the granuloma is a double-edged sword, as the same structure that facilitates bacterial containment can also impede access of immune effectors to the site of infection, leading to a paucity of protective cytokines directly in association with infected cells. Further contributing to the success of Mtb, the granuloma is enriched for anti-inflammatory mediators, such as TGF- β , indoleamine 2,3-dioxygenase (IDO), and PD-1, and the bacteria have evolved ways to prevent effective presentation of bacterial antigens to immune cells. The combination of these factors within the granuloma results in a highly complicated structure that contains a mixture of pro- and antimycobacterial features and likely has contributed to the long-standing success of Mtb as a human pathogen.

with granulomas, their origin and function remain largely unclear. Historically, epithelioid macrophages have been referred to as protein synthesizing rather than phagocytic (31), leading to confusion about their role in tuberculosis. More recently, evidence has emerged that they likely can be both. In NHP, HAM56⁺ cells at the epithelioid macrophage/caseum interface can engulf neutrophils (32) and bacteria (33). Longitudinal studies from zebrafish have shown that both synthetic and phagocytic cell types coexist, perhaps because of constant recruitment of new macrophages that undergo epithelialization (34). More recently, zebrafish have been used to identify the molecular basis of epithelialization, whereby epithelioid macrophages upregulate a gene module driven by Stat6-dependent E-cadherin expression, reminiscent of the epithelial–mesenchymal transition (35, 36). This epithelial program is essential for adherens junction formation, which serves as a scaffold for the granuloma. The finding that Stat6-driven type 2 immunity controls this process is surprising given the classical role for type 1 immunity in TB. However, it aligns with roles for type 2 responses in other granulomatous diseases, such as schistosomiasis (37), as well as E-cadherin regulation (38), suggesting that these processes are highly conserved. As our understanding of the granuloma structure improves, research groups have begun to untangle whether they are ultimately beneficial or detrimental to the host. Studies using macrophage-specific E-cadherin dominant-negative zebrafish showed that partial disruption of macrophage epithelialization led to diminished bacterial burdens and improved zebrafish survival, suggesting that the epithelioid macrophage plays a bacterial-protective role, perhaps by excluding protective immune factors from the site of infection (36, 39). However, complete disruption of the epithelialization program upon Stat6 deletion paradoxically led to increased bacterial growth, indicating that some level of bacterial containment within granulomas may be required for host protection (35). Combined, these findings open an opportunity for host-directed therapies targeting macrophage epithelialization, such as protein kinase C (40), to improve immune infiltration into the granuloma.

Chemokine Receptors

Mtb-specific T cells must interact directly, and thus colocalize, with infected cells to confer optimal immunity (41). T cell trafficking into and within tissues is determined mainly by the chemokine receptors (CCRs) that they express, but which CCRs promote this colocalization is incompletely understood (42). CXCR3 is highly expressed on Mtb-specific T cells within the lung parenchyma, while those within the vasculature express CX3CR1. Adoptive transfers of T cells lacking individual CCRs into Mtb-infected mice recently identified mechanistic roles for these CCRs in trafficking into the lung parenchyma (43). In these studies, cells lacking CXCR3 trafficked to the lung parenchyma at a rate ~75% that of wild type over 35 h, while cells lacking CX3CR1 entered at nearly twice the wild-type speed, suggesting that CXCR3 promotes rapid trafficking of Mtb-specific T cells while CX3CR1 holds cells within blood vessels. Minor roles were also uncovered for CCR2, CCR5, CCR6, CXCR5, and CXCR6, though the largest effect was a rate decrease of only ~10% in CCR5 deficiency (43). Given the relatively short time frame studied, it is uncertain what effects these CCRs may have over the course of infection. Indeed, Potter et al. (44) recently used serial IV labeling to track the localization of T cells over time, finding that most IFN- γ -producing T cells within granulomas had entered the parenchyma more than 24 h prior, highlighting the importance of longer-term studies to interrogate CCR-driven localization. Little is known about which CCRs drive T cells to different microenvironments within the infected lung after exiting the vasculature. A recent study in NHP showed robust expression of CXCR3 on CD4⁺ T cells in granulomas, suggesting that CXCR3 has a role in directing cells toward infected regions (45). However, the presence of CXCR3⁺ T cells in lung regions distal to the granuloma, together with the finding that multiple CCRs contribute to trafficking to the Mtb-infected lung, suggests that a single CCR may be insufficient for optimal trafficking (43).

TERTIARY LYMPHOID STRUCTURES

TB granuloma-associated TLS form across species (17, 46). These structures share many features with secondary lymphoid organs (SLOs), including B cell aggregates, T follicular helper (Tfh) cells, follicular dendritic cells (fDCs), and high endothelial venules (HEVs). Similar to SLOs, TLS serve as sites of local antibody production and facilitate antigen presentation to T cells (47). However, TLS differ from SLOs on the basis of their lack of capsular containment and dependence on inflammatory stimuli for their development (47). IL-17 and IL-23 produced by either T helper 17 (Th17) cells or group 3 innate lymphoid cells (ILC3s) promote the formation of TLS (48–50). Furthermore, inflammation induces stromal cells to transform into fDCs and support HEV formation, also triggering them to produce chemokines (e.g., CXCL12, CXCL13, CCL19, CCL21) that recruit and organize T, B, and myeloid cells into TB granuloma-associated TLS (51). Once TLS are established, Tfh cells are required for their persistence. Interestingly, TLS are larger and more organized in NHP with relatively controlled Mtb infection in comparison to NHP with poorly controlled infection (52). Thus, TLS seem to be host protective in TB, similar to other chronic infections (53, 54).

The mechanisms by which TLS promote Mtb control are still unclear. CXCR5-deficient mice lack TLS and have increased bacterial burdens, both of which are reversed by infusion of CXCR5-expressing CD4 T cells (52). Furthermore, T cell-intrinsic CXCR5 expression is required to maintain effector CD4 T cells within the lung (55). Thus, while CXCR5⁺ Tfh cells are required to maintain TLS, cellular interactions within these structures may, conversely, be required for T cell persistence. Other possible mechanisms include promoting protective antibody responses (56) and functioning as sites of efficient immune cell entry into the lung via HEVs (57). Because Th17 induction and TLS formation can be promoted by adjuvants, some vaccines may promote TLS formation more than others. Future studies investigating TLS function in TB could be aided by the finding that TLS location and structure within mouse TB granulomas become more similar to those of humans upon altering either the infectious strain (HN878) (24) or the dose (1–3 CFU) (25).

IDO

Tryptophan (Trp) is an essential amino acid for humans. Because Trp is also essential for pathogens, hosts often use Trp starvation to combat infectious agents, such as *Chlamydia trachomatis* and *Toxoplasma gondii* (58, 59). Host Trp depletion is often achieved by IFN- γ -dependent indoleamine 2,3-dioxygenase (IDO) expression, the rate-limiting enzyme in Trp catabolism. Indeed, IDO is strongly upregulated during TB and can be detected in the pleural fluid, sputum, and serum of patients with active TB (60, 61) as well as in the granuloma itself (62). However, Mtb has evolved the capacity to synthesize its own Trp, rendering host-mediated Trp starvation ineffective unless the Mtb TrpE operon is compromised (63). Although IDO activation does not appear to directly kill Mtb under normal settings, these findings open avenues for host-directed therapies that interfere with bacterial Trp generation. In a proof of concept, mice treated with 6-FABA (2-amino-6-fluorobenzoic acid), an inhibitor of Trp synthesis, have reduced bacterial loads in the lung and spleen (63). Such a therapy is particularly appealing because it presents limited off-target toxicity to the host, as humans do not express Trp synthesis machinery.

IDO also plays an immunosuppressive role, which is at least partially due to its effect on T cells, as the kynurenine metabolites produced by Trp metabolism suppress T cell proliferation and promote regulatory T cells. In line with this finding, pleural fluid from TB patients can suppress cytokine production by human peripheral blood mononuclear cells, which is overcome by inhibiting IDO, IL-10, or TGF- β (64). In NHP, IDO is strongly expressed in the phagocytic-rich,

lymphocyte-devoid border adjacent to the necrotic granuloma core, leading to the hypothesis that IDO excludes activated lymphocytes from the caseous core of the granuloma (65). This hypothesis was confirmed in NHP by inhibiting IDO *in vivo*, wherein D-1MT (1-methyl-D-tryptophan) treatment improved T cell cytokine production, proliferation, and colocalization with bacteria within the granuloma, resulting in significantly reduced bacterial loads in the bronchoalveolar lavage, lung, and even individual granulomas. These findings suggest that, in addition to rescuing impaired T cell function at the granuloma, IDO blockade may reorganize the granuloma itself, facilitating critical interactions between infected macrophages at the core of the granuloma and the microbicidal T cells normally excluded from this zone (39).

Although IDO activation was observed in the murine TB model, IDO deficiency had no effect on overall infection outcome (66). It remains unclear how well the conventional murine model of TB infection recapitulates the role of Trp metabolism and/or IDO, perhaps because the canonical caseous granuloma is not characteristic of murine TB. Future studies should investigate whether modified murine models, namely the ULD model (25), C3HeB/FeJ mice (67), or other outbred strains of mice (68), may help better reproduce the findings observed in NHP and humans in order to further dissect the mechanisms by which this pathway restricts immunity against Mtb.

FACTORS THAT PROMOTE IMMUNITY AT THE GRANULOMA

The Th1 immune response, which centers on IFN- γ production, has long been associated with protection against TB infection, but failed vaccine trials have shown that mounting a peripheral Th1 response is not sufficient to protect against disease. While some of this lack of protection is likely due to the improper localization of immune cells to the granuloma, as described in the preceding section, other immune cell axes are also involved in protection. Here, we discuss cytokines and immune cell types that contribute to granuloma organization or protection within these structures. Our discussion is not comprehensive. IL-1, for example, is critical for protection, but because little is known about how its activity relates to granulomas, we do not discuss it here. Similarly, T cell-intrinsic expression of CD153, a cell surface receptor and member of the TNF superfamily, is also important for pulmonary immunity against Mtb and is expressed by granuloma-resident T cells in NHP (69). However, because its specific role in immunity is not yet understood, we do not discuss it further.

IFN- γ

IFN- γ is essential for immune containment of tuberculosis, as humans and mice with deficiencies in the IFN- γ /IFN γ axis rapidly succumb to mycobacterial infections (70-72). Nevertheless, clinical vaccines that generate robust peripheral IFN- γ responses have variable efficacy (73-75), increased IFN- γ production from PD-1 blockade/deficiency can exacerbate disease (76, 77), and it has been reported that IFN- γ accounts for only 30% of CD4 T cell-mediated immunity in the lung (145). However, these studies did not measure IFN- γ production specifically within the granuloma, and IFN- γ levels in the blood or lung homogenate may not accurately reflect the site of infection. Supporting this idea, serial intravascular staining in NHP has shown that most IFN- γ -producing cells within the granuloma have been in the lung parenchyma for >24 h (44), suggesting that cytokine-producing cells are not recent immigrants from the blood. Interestingly, recent studies have shown that there is little IFN- γ production within pulmonary granulomas (26, 79) and that Mtb-specific Th1 effector cells rapidly lose the capacity to produce IFN- γ in the granuloma (26). Furthermore, an increased proportion of IFN- γ -producing cells within the granuloma, but not distal lung regions, was associated with protection in mice whose T cells lack TGF- β (26). Together, these data suggest that granuloma-level IFN- γ responses are not

accurately represented by sampling of blood or lung homogenates. Although increased systemic IFN- γ responses appear not to be protective and may even be harmful, targeted increases of IFN- γ at the site of infection may still have the potential to be protective, and such localized changes can only be elucidated by measuring responses within the granuloma.

IL-17

IL-17 has a well-described role in the control of extracellular bacteria and is implicated in the recruitment and activation of neutrophils as well as in the formation of TLS (80). Although IL-17 deficiency has no impact on bacterial burdens in unimmunized C57BL/6 mice infected with common experimental Mtb strains (81), it results in delayed Th1 cell recruitment to the lung and increased bacterial burdens in mice immunized with a Th17-inducing vaccine (82). Recently, unimmunized C57BL/6 mice infected with the Beijing strain HN878 were also shown to be dependent on IL-17 for optimal control of pulmonary Mtb infection, and IL-17-deficient mice had fewer lymphoid follicles and iNOS⁺ macrophages within the granuloma, a phenotype reversed by IL-17 overexpression (83). In humans, IL-17A/F transcription is upregulated in resected pulmonary tuberculosis granulomas (84). It remains unknown which cell types are the source of IL-17 during TB, although Th17 cells, ILC3s, and $\gamma\delta$ T cells are all candidates (83–86). ILC3s accumulate in the lung during the early stages of murine Mtb infection (86); consistent with this finding, ILC3s are elevated in lung tissues of patients with pulmonary TB yet depleted in the blood, underscoring the importance of evaluating responses at the site of infection. The absence of ILC3s resulted in a modest increase in lung bacterial burden as early as day 14 postinfection, which was associated with decreased IL-17 production and reduced B cell follicle generation (86). Together, these data suggest that ILC3s are recruited from the blood to the lung soon after Mtb infection, where they can shape early granuloma formation by influencing the cell types present as well as by stimulating TLS formation.

Th1/Th17 Cells

Our understanding of the role of Th1/Th17 cells is in a relatively early stage compared with the more established T helper lineages (Th1, Th2, and Th17). Th1/Th17 express both T-bet and ROR γ t, produce both IFN- γ and IL-17 (87, 88), and are induced by human Mtb infection (89–91). Recent evidence has shown an association of Th1/Th17 cells with protection against Mtb. In a cohort of South African adults, an increased population of BCG-specific IFN- γ ⁺IL-17⁺ CD4 T cells was negatively correlated with a transcriptional score predicting risk of progression to active TB in IGRA⁺ individuals following BCG immunization (92). A higher proportion of CXCR3⁺CCR6⁺ CD4⁺ T cells (purportedly Th1/Th17) was observed in asymptomatic contacts of active TB patients in comparison to contacts who developed active TB (93). In a recent NHP study, the numbers of both Th1/Th17 and Tc1/Tc17 cells (defined as CXCR3⁺CCR6⁺) were strongly anticorrelated with bacterial burden within individual granulomas (94), suggesting that successful granulomas were enriched for these cells. Importantly, these are only correlative data, and further investigation is warranted into the mechanistic role of Th1/Th17 cells in protective immunity to TB.

TNF

TNF is a pleiotropic Th1-related inflammatory cytokine that is involved in cellular chemotaxis, apoptosis, activation, and metabolic state. TNF is important for maintaining granuloma architecture and is essential for immunity to Mtb infection, as animals lacking TNF and humans undergoing TNF blockade exhibit exacerbated TB disease characterized by breakdown of the granuloma structure (95–97). However, an exaggerated TNF response is also associated

with exacerbated pathology and higher bacterial burdens (98, 99), which occur downstream of programmed macrophage necrosis triggered by mitochondrial, lysosomal, and endoplasmic reticulum cross talk (100–102). Additionally, as with IFN- γ , few T cells within the granuloma produce TNF (44, 79), and increased peripheral TNF production is associated with worse outcomes in the setting of PD-1 blockade (76, 103). Together, these findings suggest that the amount and location of the TNF response are crucial for optimal TB outcomes.

GM-CSF

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is widely appreciated for its role in driving myelopoiesis, but it also plays a role in controlling infections, including Mtb. GM-CSF is expressed in the blood (104) and granulomas (105) of human TB patients and is more pronounced in noncaseating, paucibacillary granulomas (105), suggesting that levels of this cytokine correlate with protective granulomas. In animal models, GM-CSF-deficient mice are highly susceptible to infection and generate severely necrotic lesions and lung-specific bacterial outgrowth within 35 days of infection (106, 107). GM-CSF expression has been attributed to invariant natural killer T cells (108) early postinfection and later by conventional T cells, although it is essential for T cell-mediated protection only if nonhematopoietic sources of GM-CSF are compromised (104). While GM-CSF appears to be protective in the context of TB, and even directs human macrophages to be mycobactericidal in vitro (109, 110), its overexpression also ultimately results in susceptibility and a marked absence of the hallmark granuloma, instead demonstrating diffuse interstitial inflammation that fails to restrict bacterial growth (106, 107). Thus, as with IFN- γ and TNF, an intricate balance of GM-CSF levels is required to promote immunity against TB.

FACTORS THAT RESTRICT IMMUNITY AT THE GRANULOMA

The immense success of Mtb as a pathogen is in part due to the presence of inhibitory molecules and immunosuppressive factors within the granuloma that prevent efficient immune responses and subsequent bacterial eradication. The mechanisms of suppression range from host-derived soluble mediators to direct manipulation of host responses by the bacteria and even environmental stresses. Here, we highlight several responses that prevent effective bacterial control within pulmonary TB granulomas. While the cytokines IL-10 and type I interferon also have important roles in restricting immunity against Mtb, we do not discuss them here because little is known about how they function spatially within the granuloma, although excessive type I interferon production has been associated with the formation of large caseating granulomas in highly susceptible mice (23, 111).

TGF- β

TGF- β is an immunosuppressive cytokine that has pleiotropic effects on cellular proliferation, survival, differentiation, and function (112). The Mtb cell wall component lipoarabinomannan induces TGF- β by macrophages in vitro (113), and TGF- β levels within the lung rise in concert with bacterial burdens during murine infection (114). TGF- β is detected in the bronchoalveolar lavage of patients and NHP with active pulmonary TB, which subsequently decreases with treatment in NHP (115, 116). Furthermore, TGF- β has been predicted to restrict Mtb control within granulomas in an *in silico* TB granuloma model, chiefly by suppressing effector T cell function (117).

Recent mechanistic research in mice supports a key immunosuppressive role for TGF- β within the granuloma. Mice with a selective deficiency of TGF- β receptor on mature T cells have elevated pulmonary bacterial burdens (26). Using ULD Mtb infection, this phenotype was associated with increased CD4 T cell-produced IFN- γ specifically within the granuloma,

where there is robust TGF- β signaling, and not in the distal lung, where TGF- β signaling is virtually absent (26). Furthermore, TGF- β inhibited the proliferation, survival, and terminal differentiation of T effector cells (26). Further research is needed to characterize the effects of TGF- β on macrophages and nonimmune cells. However, the highly localized suppressive effects of TGF- β raise the possibility that TGF- β blockade may enhance host-protective immunity to *Mtb* without provoking a potentially harmful inflammatory response, as has been observed with checkpoint inhibitors (76, 118–123).

Preliminary studies have shown promise for the therapeutic effects of TGF- β inhibition during TB. Treatment of BALB/c mice with the TGF- β inhibitor D4476 for 14 days, initiated 10 days following intravenous *Mtb* challenge, resulted in complete absence of pulmonary granuloma formation and decreased lung bacterial burdens (124). These findings are promising, though mice are less susceptible to intravenous than aerosolized *Mtb* challenge (125), and it would not be feasible to initiate treatment at such an early state of infection in humans. In another study, inhaled small interfering RNA targeting TGF- β was initiated 60 days postinfection, after mice had formed mature granulomas, every 5 days for three doses. Despite this short treatment course and only a 25% decrease in pulmonary TGF- β levels, there was a statistically significant though modest (0.17 log) reduction in pulmonary bacterial burdens (126). Recent advances in defining the role of TGF- β in *Mtb* infection, as well as the demonstrated safety of TGF- β inhibition in clinical trials (127–129), support TGF- β blockade as an attractive candidate for host-directed therapy.

Inhibitory Receptors

There is mounting evidence that T cells in the lung parenchyma undergo functional exhaustion during TB due to chronic antigen exposure. For example, parenchymal T cells mount poorer IFN- γ responses to 6-kDa early secreted antigenic target (ESAT-6) (130–133), a chronically expressed *Mtb* antigen, compared with T cells that recognize Ag85B, a transiently expressed *Mtb* antigen (134). Furthermore, exhaustion-associated inhibitory receptors, such as T cell immunoglobulin and mucin domain-containing protein 3 (TIM3), lymphocyte-activating 3, cytotoxic T lymphocyte-associated protein 4 (CTLA-4), and PD-1 (135), are enriched on lung T cells relative to blood T cells during TB (27, 131, 133, 136, 137). In the mouse, T cell exhaustion has functional consequences, as TIM3 knockouts survive longer than wild-type mice, with decreased pulmonary and splenic bacterial loads, and intravenous ESAT-6 infusions fail to reduce the lung bacterial load compared with Ag85B (131, 136). Inhibitory markers and exhaustion may also affect vaccination, as increased CTLA-4 expression in mice coincided with loss of BCG-mediated protection (138), although CTLA-4 blockade alone does not improve disease outcome upon mycobacterial challenge despite enhancing peripheral immune responses (139).

Immune checkpoint blockade has become an exciting arena in the fight against malignancy. In TB, exhaustion-associated inhibitory receptors (140) are coexpressed on lung T cells (131–133, 136), antigen-specific CD4 T cells express higher levels of PD-1 during active TB than in asymptomatic infection (141), and the granuloma core is enriched for PD-L1 (62, 142). Therefore, one might predict that inhibitory receptor blockade would improve TB outcomes. Surprisingly, deficiencies in the PD-1/PD-L1 axis actually exacerbate disease. PD-1-deficient mice are highly susceptible to *Mtb* and display diffuse necrotic lesions in the lung (77, 143, 144). Their extreme susceptibility is the consequence of a hyper-Th1 response, as depletion of either CD4 T cells or IFN- γ can reverse this susceptibility (143, 145). In vitro models have suggested that overproduction of TNF- α may also drive anti-PD-1 susceptibility, emphasizing the potentially detrimental role of hyperinflammation during TB (146). Similar observations have been made in NHP, where PD-1 blockade led to worse TB disease despite enhancing the

numbers of functional Mtb-specific T cells (147). Corroborating data from animal models, there is now overwhelming evidence that some human patients undergoing anti-PD-1/PD-L1 therapy for cancer experience TB reactivation (76) and an associated hyper-Th1 immune response (76).

In addition to its expression on exhausted T cells, PD-1 is highly expressed on Tfh cells and can be an indicator of antigen sensing (148). Thus, not all cells that express PD-1 are nonfunctional, and truly exhausted T cells may require expression of multiple inhibitory receptors (136). This hypothesis is supported by data showing that Mtb-specific PD-1⁺ CD4 T cells are more proliferative, can mount recall responses, and are long lived (131, 132, 146, 149). In addition, PD-1⁺ CD4 T cells provide significantly enhanced protection upon adoptive transfer into recipient mice compared with T cells expressing KLRG1 (killer cell lectin-like receptor G1), a marker of terminal differentiation (131, 133, 145). Consistent with the idea that PD-1 may also mark proximity to antigen, few T cells in the NHP model coexpress other inhibitory receptors within granulomas, suggesting that exhaustion is not the sole factor driving ineffective T cell responses (79). Instead, the granuloma structure may prevent exhaustion by limiting the interactions of T cells with Mtb antigen (79). While the degree of exhaustion could depend on the animal model used and the antigen being measured, PD-1 may limit T cell terminal differentiation by modulating the threshold for antigen stimulation, thus enabling cells to produce cytokines in the face of antigen without causing immune-mediated pathology (150). If that is the case, blocking inhibitory receptors could increase cytokine production by already functional cells, including cells that are not colocalized with Mtb-infected cells, leading to immune-mediated damage. In addition, T cells may be suppressed by other pathways that are not reversed by PD-1 blockade. Further studies are needed to determine whether anti-PD-1 combined with other approaches could be helpful. Indeed, one study has shown that combinatorial treatment of mice with anti-PD-1 and standard antimicrobial therapy led to enhanced IFN- γ responses, decreased Mtb burdens, and improved lung pathology (151). Perhaps new combinatorial regimens targeting other immunosuppressive pathways that either inhibit the ability of T cells to localize to the granuloma, such as IDO signaling (39), or specifically suppress T cell function near the granuloma, such as TGF- β signaling (26), along with anti-PD-1 and/or antibiotic treatment, could also be helpful.

Limitations in Antigen Presentation

A large body of literature, reviewed elsewhere (152, 153), suggests that Mtb can modulate the antigen presentation pathway to subvert T cell activation via numerous mechanisms, including inhibition of phagolysosomal fusion/acidification, autophagy, and surface MHC-II expression. How these molecular mechanisms operate *in vivo*, however, is less well studied. Given the importance of direct interactions between MHC-II molecules and T cells for bacterial control (154), impaired antigen presentation could have strong implications for infection outcomes. This idea is supported by findings that the same Ag85B antigen is more efficiently presented by BCG-infected cells than by virulent Mtb-infected cells (155). Several groups have provided data that antigen presentation is also limited within the granuloma. For example, Egen et al. (156) showed that Mtb-specific T cells within hepatic granulomas failed to demonstrate significant antigen-induced migration arrest or cytokine production relative to T cells with an irrelevant antigen specificity (ovalbumin), despite responding robustly to exogenous antigen, suggesting that antigen is limiting within the granuloma.

One mechanism that may contribute to this impaired antigen sensing is the active export of antigen from infected cells in a Kinesin-2-dependent manner. As a result, antigen is diverted away from the MHC pathway and instead presented by uninfected cells that take up the antigen within the lung (157). Consistent with this idea, recent data in mice showed that T cell sensing of antigen,

as measured by phosphorylation of the ribosomal protein S6 (downstream of T cell receptor signaling), predominantly occurs distal to Mtb-infected cells early postinfection, suggesting that antigen can become segregated from infected cells (158). CD4 T cells ultimately phosphorylate S6 within the granuloma, an indication that they have sensed antigen, but the proportion of T cells that has sensed antigen and produced IFN- γ remains unchanged relative to distally located T cells, despite the disproportionate level of antigen within the granuloma (26). It would be interesting to measure antigen sensing among T cells within human and NHP granulomas to determine whether T cell receptor engagement is also restricted to the periphery of the granuloma in other host settings. Although enhanced cross-presentation following antigen export could be a mechanism to overcome suppressed antigen presentation, this process ultimately favors the bacteria, as reversal of antigen export via deletion of Kinesin-2 results in increased activation of Mtb-specific CD4 T cells and improved bacterial control (157). Together, these findings suggest that antigen presentation may be ineffective within heavily infected regions of the lung.

Hypoxia

Hypoxia is a feature of the caseum of necrotic granulomas that likely results from poor vascularization (16). Mtb has adapted to life in the lipid-rich granuloma core (159, 160), where it persists in foamy macrophages (161) and is recalcitrant to drug-mediated killing (162). This bacterial persistence may relate to the highly immunosuppressive nature of the granuloma core, which is enriched for Foxp3, IL-10 (163), and PD-L1 (62, 142) expression relative to the granuloma rim. However, despite enabling bacterial persistence, the low-oxygen environment of the granuloma actually limits intracellular mycobacterial growth (164, 165). In vitro models of hypoxia have shown that limiting oxygen can drive bacteria into a nonreplicative state and prevent bacterial spread within the host (162, 166). A 1965 study found that open cavities with access to the airways had significantly higher bacterial burdens than necrotic lesions, indicating the favorable effect of aeration on replication (167). The restrictive nature of the hypoxic granuloma is multifactorial: Antimicrobial effectors are enriched in the hypoxic caseum of granulomas (168), hypoxia induces bacteriostatic PD-1 expression (146) and T cell granulysin (169), and macrophages and T cells grown in hypoxic conditions shift toward glycolytic metabolism, likely dependent on the transcription factor hypoxia-inducible factor (HIF)-1 α (170–172). Importantly, glycolytic macrophages control Mtb better than macrophages undergoing oxidative phosphorylation (173). Ablation of myeloid HIF-1 α in a *Mycobacterium avium* mouse model of necrotizing granulomas (174) resulted in enhanced granuloma necrosis and an inability to clear infection, consistent with the idea that macrophages become more microbicidal in response to hypoxia. Notably, HIF-1 α signaling controls much of the IFN- γ -dependent immune response during Mtb and, thus, is a driver of more than just hypoxic responses in the macrophage (175).

Deficient vascularization, and the subsequent deprivation of nutrients and oxygen, is one cause of hypoxia that leads to necrotic granulomas (16). However, early-stage granulomas appear to be well vascularized (174) with dysfunctional vessels (176), and this early vascularization may be a strategy used by Mtb to disseminate. Zebrafish studies have shown that induction of vascular endothelial growth factor (VEGF) and angiopoietin-2 by *Mycobacterium marinum* leads to increased bacterial dissemination and burden, suggesting that bacteria manipulate neovascularization and vascular permeability to enhance pathogenicity (176, 177). Consistent with this idea, human macrophages infected with Mtb upregulate angiogenic factors, including VEGF, and treatment of Mtb-infected mice with anti-VEGF reduces bacterial dissemination to the spleen (178). Granuloma types may differ in their level of vascularization and therefore their degree of hypoxia. A study of human subjects with active cavitary or nonprogressive tuberculoma disease reported higher levels of CD31 $^{+}$ endothelial cells and macrophages in the lung tissue of tuberculoma patients (179).

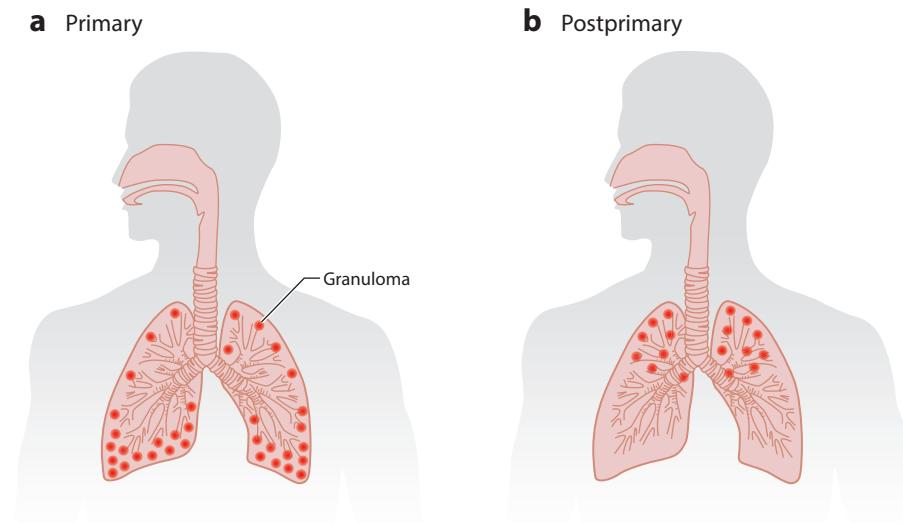


Figure 2

Primary and postprimary tuberculosis (TB) present in different lung locations. Anatomical records from the pre-antibiotic era clearly demonstrate that the pulmonary presentations of (a) primary and (b) postprimary tuberculosis are distinct. This led some to believe that these pathologies represented separate diseases, although it is now clear that they are indeed caused by the same microorganism. While primary TB tends to present among children as lesions within the well-ventilated lung bases, postprimary TB, which is more prevalent among adults living in endemic regions who have likely been repeatedly exposed to the bacteria over their lifetime, presents as lesions within the poorly ventilated lung apices. These distinct presentations indicate that prior *Mycobacterium tuberculosis* infection likely provides protective immunity within the lung bases, where *M. tuberculosis* often implants upon inhalation. Unfortunately, the protective immunity conferred by prior exposure is ineffective in the apical regions of the lung that are highly vulnerable to infection. Figure inspired by References 182 and 186.

While the tuberculomas contained live, culturable bacteria, these bacteria were contained and the patients had negative sputum cultures, suggesting that access to oxygen and nutrients via blood vessels can also promote immunity and help limit bacterial spread (179). Overall, oxygen levels can simultaneously restrict bacterial growth, promote antibacterial effector function by macrophages, and provide a lipid-rich haven in which the bacteria have learned to adapt over thousands of years of evolution, underscoring the complicated nature of this host-pathogen interaction.

POSTPRIMARY TUBERCULOSIS: A DISTINCT PATHOGENESIS

Most TB occurs in adults living in endemic regions who were first exposed to *Mtb* many years prior and are repeatedly reexposed throughout their lives. In these individuals, TB pathogenesis and the corresponding immune cell organization at pulmonary sites of infection are very different than in those with primary TB. Whereas primary TB granulomas localize to the well-ventilated lung bases, postprimary TB lesions occur in the poorly ventilated lung apices (Figure 2). While stereotypic granuloma development is central to the pathogenesis of primary TB, postprimary TB seems to be driven by early lesions of alveolitis and bronchogenic pneumonia (Figure 3). In TB-endemic regions, primary TB usually occurs in children, whereas adults exhibit postprimary TB. However, children with prior *Mtb* exposure can develop “adult-like” pathologies of postprimary TB, and conversely, adults without prior *Mtb* exposure can present with primary TB pathology,

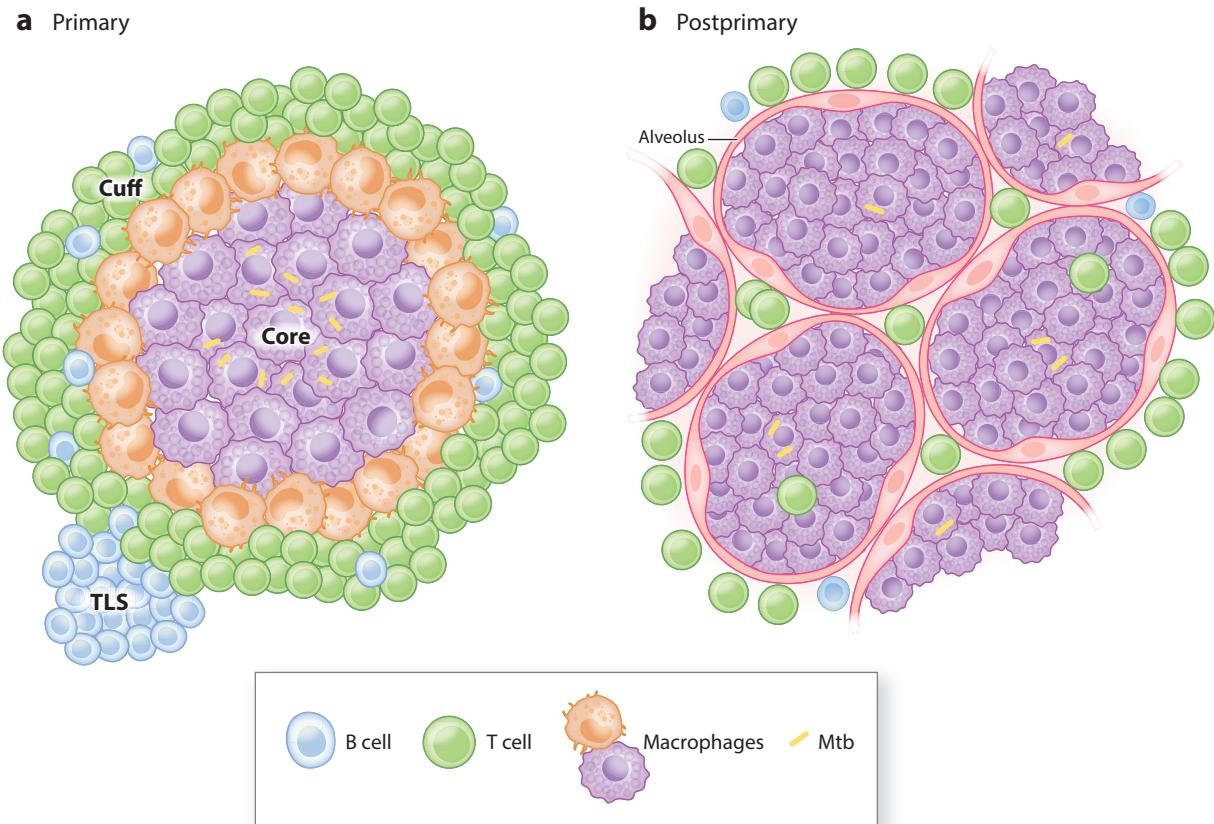


Figure 3

Lesions associated with primary and postprimary tuberculosis (TB) have distinct pathological features. In addition to occurring in distinct locations, (a) primary and (b) postprimary pulmonary TB lesions possess vastly different pathologic features. The primary granuloma is the classic tubercle, with a core of infected foamy macrophages, surrounded by a layer of epithelioid macrophages, all encompassed by a T cell-rich lymphocytic cuff containing tertiary lymphoid structures (TLS). In contrast, postprimary TB lesions are typified by an alveolitis (or consolidative) response, with clusters of infected macrophages contained within alveolar sacs, and infiltrating lymphocytes within the surrounding alveolar walls. Caseating and cavitary lesions can form in postprimary TB, though this is generally a later-stage finding when consolidation spreads to take up large regions of the lung, followed by central necrosis. These insights suggest that distinct processes may govern the biology of these different clinical entities. Figure inspired by References 183 and 184.

showing the importance of acquired immunity in driving the different outcomes (4). An unfortunate example of the latter occurred when Senegalese troops without prior *Mtb* exposure were brought to Europe to fight in World War I and were decimated by a form of TB similar to primary TB in children (180). In the nineteenth century, before it became clear that preexisting immunity drives these distinct outcomes, a great debate raged about whether these pathologies represented different manifestations of the same disease or completely different diseases (6). In brief, René Laennec (2) favored the idea of a single disease with two distinct pathologies, tubercular infiltration (granuloma) and tubercular granulation (pneumonia). Rudolf Virchow (3) countered that the two pathologies were so different microscopically that they must represent two distinct diseases. He believed that the tubercle of primary TB was a type of tumor, whereas the infiltration of postprimary TB was an inflammatory process. Only after Robert Koch (181) discovered the

Table 1 Features of primary and postprimary TB granulomas

Primary	Postprimary
Subpleural, lung bases	Lung apices
Organized, with or without central necrosis	Alveolitis
Airway deposition	Suspected to be either airway or hematogenous deposition
No evidence of previous mycobacterial exposure	Preexisting immunity to mycobacteria
Children from TB-endemic regions, adults from non-TB-endemic regions	Adolescents and adults from TB-endemic regions
Well described in animal models	Paucity of animal models

Abbreviation: TB, tuberculosis.

tuberculous bacilli, which were subsequently isolated from patients with each type of pathology, was the debate finally settled. Here, we discuss how the unique anatomic locations and pathologies of postprimary and primary TB suggest important differences in pathogenesis (Table 1).

Apical Location of Postprimary Tuberculosis

The observation that postprimary TB lesions localize to poorly ventilated lung apices demonstrates that prior *Mtb* exposure provides protective immunity in well-ventilated lung bases, where inhaled *Mtb* are most likely to implant. This provides an anatomical context for the observation that prior *Mtb* exposure (as measured by tuberculin skin test positivity) conferred ~79% protection against TB disease in nursing students heavily exposed to *Mtb* when compared with their *Mtb*-naïve (tuberculin skin test-negative) counterparts (185). Thus, among those with prior *Mtb* exposure, TB disease occurs only when *Mtb* is able to reach the apical regions of the lung, which are vulnerable to *Mtb* infection.

Why are the lung apices vulnerable? The explanation undoubtedly stems from the relatively low blood pressure that perfuses the lung, which is approximately one-fifth the systemic blood pressure (14). As a result, pulmonary blood pressure is gravity dependent, and the uppermost lung regions (apices in humans) are relatively starved for blood flow. Mechanistic evidence that the vulnerability is gravity dependent comes from Medlar & Sasano (186), who observed that TB in rabbits and other four-legged animals occurs primarily in the dorsal regions of the lungs, which are uppermost when the animals are on their feet. However, when rabbits are held in an erect position by a harness, TB lesions shift to the apices, similar to humans. In bats, which sleep hanging upside down, TB lesions develop at the bases of their lungs near the diaphragm (187, 188).

Why does low pulmonary blood pressure lead to TB vulnerability? The most favored explanation relates to the high oxygen concentrations in the lung apices. Although ventilation is lower in the apices relative to the bases, perfusion is even further decreased. This results in an increased ratio of ventilation to perfusion in the apices and increased oxygen concentration, thereby providing an environment supportive of *Mtb* replication. In addition, because of decreased perfusion, immune cell recruitment to these regions is limited. Furthermore, delivery of soluble factors, such as antibodies and complement components, is restricted due to low oncotic pressures. Finally, the lung apices have limited lymphatic flow (189), thus minimizing lymphatic drainage to the lymph node, clearance of *Mtb* antigen/debris, and T cell priming. The relative importance of each of these factors for the vulnerability of the lung apices to *Mtb* infection is unknown.

How does *Mtb* reach the lung apices? This is another long-standing debate in the TB field, with some investigators favoring exogenous reinfection of the lung apices via exposure to newly inhaled *Mtb* (190) and others arguing for endogenous reactivation through hematogenous spread, often from a years-prior primary infection (191). Excellent reviews detailing the history of both

perspectives exist, so we do not repeat them here (191). There is evidence that both pathways are possible (192–194), and exogenous reinfection may be more common in endemic countries; conversely, endogenous reactivation may be more common in areas with low ongoing transmission (13). In recent years, however, studies using molecular fingerprinting of *Mtb* strains have shown that reinfection is much more common than previously believed, even in low-endemic countries (195, 196). Thus, while remote reactivation of hematogenously spread strains can occur, the greatest global burden of postprimary TB is due to reinfection, especially in high-endemic regions (195). The observation that individuals with positive TB immunoreactivity tests are less likely to develop initial lesions in the bases of the lung, where inhaled *Mtb* is most likely to implant, suggests that postprimary TB arises in the apices, where *Mtb* is less likely to implant but more vulnerable to establishment of infection.

Pathology of Postprimary *M. tuberculosis* Lesions

Decades of animal research have presented the granuloma as the nexus of the host response to *Mtb* infection, a belief that typically draws no distinction between primary and postprimary TB. Hunter (6, 7) and Hunter & Actor (8) have challenged this idea by combining a thorough review of literature from postmortems in the pre-antibiotic era with their own analysis of human TB pathology. They posit that historically there was widespread recognition of two forms of human TB, one in which the tubercle (stereotypic granuloma) was central to pathogenesis (primary TB) and another in which pathogenesis was driven by alveolitis and pneumonia (postprimary TB). Because human postmortems are now rare, our understanding of TB pathogenesis is driven by animal models that largely model granuloma-driven primary TB. As a result, recognition of the role of pneumonia in driving postprimary TB has largely been lost. We have reviewed Hunter's research together with many of the primary studies upon which they were based (4, 5) and find the arguments to be quite convincing.

The consensus view of TB pathogenesis from decades of postmortem analyses was that the earliest pulmonary lesions of postprimary TB represent areas of caseous pneumonia rather than granulomas (4, 6). As summarized by Hunter (6), the process starts in the alveoli and small bronchi, which fill with inflammatory cells. As the pneumonia spreads, it eventually undergoes necrosis and caseation, producing cavities. Bronchial spread can subsequently occur, an underappreciated form of local dissemination (197). This alveolitis/pneumonia with bronchial obstruction and spread can readily be visualized by computed tomography scans; the “tree-in-bud” sign is recognized as a characteristic radiologic sign of early-stage postprimary TB (198). Stereotypic granuloma formation can eventually occur in postprimary TB, but according to Hunter (6), it is a late event triggered in response to the caseating pneumonia. Medlar (199) examined numerous postprimary TB granulomas with various stages of caseation and found no evidence that they resulted from an early noncaseating granuloma that subsequently underwent central necrosis. Thus, the prevailing opinion from the pre-antibiotic era was that granulomas in postprimary TB formed in response to cavitary lesions and did not precede them.

IMPLICATIONS FOR UNDERSTANDING IMMUNITY AT PULMONARY SITES OF *M. TUBERCULOSIS* INFECTION

In contrast to the recent advances in our understanding of primary TB granuloma biology, most of our understanding of the pathogenesis of postprimary TB was obtained decades ago, and little is known about the local immune responses that operate in the most prevalent form of global TB. What are the molecular and cellular immune mediators that drive the pathogenesis of postprimary

TB alveolitis and pneumonia? What factors govern immune cell trafficking and organization? Do TLS develop in conjunction with postprimary lesions? Do T cells and Mtb-infected macrophages colocalize, or are they segregated as they are in primary granulomas? Is protective cytokine production restricted at postprimary sites of infection as in primary TB? What is the role of TGF- β , inhibitory receptors, and other immunosuppressive mediators? Answers to these and similar questions will help guide the development of new approaches to prevent and treat TB in endemic regions, where most adults have a history of prior Mtb infection. Modern tools of immunology need to be utilized to study immune cell organization and function in human lung tissues representing the spectrum of TB pathology. Exciting new approaches in single-cell biology, multiparameter imaging, and computational analysis will greatly aid these efforts (25, 26, 62, 200) and would be further advanced by access to postmortem lung tissue from individuals with early stages of Mtb infection, including asymptomatic and subclinical disease. New animal models of postprimary TB need to be developed so that mechanistic studies can be performed in parallel with these human investigations. For example, in mice with a contained lymph node Mtb infection (201), could pulmonary lesions that result from subsequent Mtb challenge resemble those in human postprimary Mtb infection? Could hematogenously seeded lung lesions in ULD Mtb-infected mice (25) differ from those seeded by aerosolization and contain features of human postprimary TB? Could pre-existing immunity due to vaccination also induce changes at Mtb infection sites, similar to prior Mtb exposure? There are data to support this idea, as aerosol Mtb infection of BCG-immunized guinea pigs leads to smaller-sized lesions with a complete absence of central necrosis, reminiscent of secondary granulomas seeded by hematogenous spread (202, 203). Whether these lesions resemble those in postprimary TB will require further studies. However, studying how different vaccines alter immune cell interactions at Mtb infection sites may provide insight into the biology of primary TB granulomas and help parse out different components of immunity that govern the biology of postprimary TB lesions. A fresh analysis of pulmonary Mtb infection sites in humans and new animal models using modern tools is needed to determine how prior or ongoing Mtb exposure shapes the architecture of the host response to Mtb and to reveal new insights into TB pathogenesis.

CONCLUDING REMARKS

The last decade has witnessed great advances in our understanding of the primary TB granuloma. Using modern immunologic tools in animal TB models, we have gained insights into the factors that both mediate and restrict immunity within these structures. However, our understanding of immune cell interactions and functionality at sites of infection in postprimary TB lags behind; most was generated almost a century ago, in the pre-antibiotic era, when human postmortem studies were common. Animal models for postprimary TB have not been adequately developed; thus, modern tools have not been used to investigate its pathogenesis. Recent insights into primary TB granuloma biology suggest a plethora of new strategies to prevent and treat TB. However, we should not assume that targetable pathways discovered by studying granulomas in primary TB will necessarily translate to the setting of postprimary TB. The pathogenesis of postprimary TB is quite distinct, and the pulmonary lesions differ from those of primary TB in their location and cellular organization. Nevertheless, the progress made in understanding primary TB granulomas has provided a wealth of hypotheses that need to be tested. Doing so will require an integrated approach, using modern tools to study the spectrum of postprimary TB in the human lung, along with mechanistic research in newly developed animal models. To move forward in TB, we need to look to the past and build on the strong foundation that was set by the great TB investigators that preceded us. Only then can we maximize the utility of the tools at our disposal.

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LITERATURE CITED

1. Opie EL. 1927. Tubercle bacilli in latent tuberculous lesions and in the lung tissue without tuberculous lesions. *Arch. Pathol. Lab. Med.* 4:1–21
2. Laennec RTH. 1979 (1821). *A Treatise on the Diseases of the Chest: In Which They Are Described According to Their Anatomical Characters, and Their Diagnosis Established on a New Principle by Means of Acoustick Instruments*, transl. J Forbes. Omaha, NE: Gryphon Class. Med. Libr. (From French)
3. Virchow R. 1863. *Cellular Pathology as Based Upon Physiological and Pathological Histology*. Philadelphia: Lippincott
4. Rich AR. 1944. *The Pathogenesis of Tuberculosis*. Springfield, IL: Thomas
5. Osler W. 1920. *The Principles and Practice of Medicine*. New York: Appleton
6. Hunter RL. 2016. Tuberculosis as a three-act play: a new paradigm for the pathogenesis of pulmonary tuberculosis. *Tuberculosis* 97:8–17
7. Hunter RL. 2011. Pathology of post primary tuberculosis of the lung: an illustrated critical review. *Tuberculosis* 91:497–509
8. Hunter RL, Actor J. 2019. The pathogenesis of post-primary tuberculosis: a game changer for vaccine development. *Tuberculosis* 116(Suppl.):S114–17
9. Ravesloot-Chávez MM, Van Dis E, Stanley SA. 2021. The innate immune response to *Mycobacterium tuberculosis* infection. *Annu. Rev. Immunol.* 39:611–37
10. Khader SA, Divangahi M, Hanekom W, Hill PC, Maeurer M, et al. 2019. Targeting innate immunity for tuberculosis vaccination. *J. Clin. Investig.* 129:3482–91
11. Netea MG, Dominguez-Andres J, Barreiro LB, Chavakis T, Divangahi M, et al. 2020. Defining trained immunity and its role in health and disease. *Nat. Rev. Immunol.* 20:375–88
12. Cohen SB, Gern BH, Delahaye JL, Adams KN, Plumlee CR, et al. 2018. Alveolar macrophages provide an early *Mycobacterium tuberculosis* niche and initiate dissemination. *Cell Host Microbe* 24:439–46.e4
13. Balasubramanian V, Wiegshaus EH, Taylor BT, Smith DW. 1994. Pathogenesis of tuberculosis: pathway to apical localization. *Tuberc. Lung Dis.* 75:168–78
14. Murray JF. 2003. Bill Dock and the location of pulmonary tuberculosis: how bed rest might have helped consumption. *Am. J. Respir. Crit. Care Med.* 168:1029–33
15. Bucsan AN, Mehra S, Khader SA, Kaushal D. 2019. The current state of animal models and genomic approaches towards identifying and validating molecular determinants of *Mycobacterium tuberculosis* infection and tuberculosis disease. *Pathog. Dis.* 77:ftz037
16. Tsai MC, Chakravarty S, Zhu G, Xu J, Tanaka K, et al. 2006. Characterization of the tuberculous granuloma in murine and human lungs: cellular composition and relative tissue oxygen tension. *Cell. Microbiol.* 8:218–32
17. Ulrichs T, Kosmiadi GA, Trusov V, Jörg S, Pradl L, et al. 2004. Human tuberculous granulomas induce peripheral lymphoid follicle-like structures to orchestrate local host defence in the lung. *J. Pathol.* 204:217–28
18. Orme IM, Ordway DJ. 2016. Mouse and guinea pig models of tuberculosis. *Microbiol. Spectr.* 4:4
19. Dannenberg AM Jr. 2006. *Pathogenesis of Human Pulmonary Tuberculosis: Insights from the Rabbit Model*. Washington, DC: ASM Press
20. Ramakrishnan L. 2013. Looking within the zebrafish to understand the tuberculous granuloma. *Adv. Exp. Med. Biol.* 783:251–66
21. Pan H, Yan B-S, Rojas M, Shebzukhov YV, Zhou H, et al. 2005. *Ipr1* gene mediates innate immunity to tuberculosis. *Nature* 434:767–72
22. Verma S, Bhatt K, Lovey A, Ribeiro-Rodrigues R, Durbin J, et al. 2019. Transmission phenotype of *Mycobacterium tuberculosis* strains is mechanistically linked to induction of distinct pulmonary pathology. *PLOS Pathog.* 15:e1007613

23. Ji DX, Witt KC, Kotov DI, Margolis SR, et al. 2021. Role of the transcriptional regulator SP140 in resistance to bacterial infections via repression of type I interferons. *eLife* 10:e67290
24. Chorenó-Parra JA, Bobba S, Rangel-Moreno J, Ahmed M, Mehra S, et al. 2020. *Mycobacterium tuberculosis* HN878 infection induces human-like B-cell follicles in mice. *J. Infect. Dis.* 221:1636–46
25. Plumlee CR, Duffy FJ, Gern BH, Delahaye JL, Cohen SB, et al. 2021. Ultra-low dose aerosol infection of mice with *Mycobacterium tuberculosis* more closely models human tuberculosis. *Cell Host Microbe* 29:68–82.e5
26. Gern BH, Adams KN, Plumlee CR, Stoltzfus CR, Shehata L, et al. 2021. TGF β restricts expansion, survival, and function of T cells within the tuberculous granuloma. *Cell Host Microbe* 29:594–606.e6
27. Kauffman KD, Sallin MA, Sakai S, Kamenyeva O, Kabat J, et al. 2018. Defective positioning in granulomas but not lung-homing limits CD4 T-cell interactions with *Mycobacterium tuberculosis*–infected macrophages in rhesus macaques. *Mucosal Immunol.* 11:462–73
28. Ramakrishnan L. 2012. Revisiting the role of the granuloma in tuberculosis. *Nat. Rev. Immunol.* 12:352–66
29. Williams GT, Williams WJ. 1983. Granulomatous inflammation—a review. *J. Clin. Pathol.* 36:723–33
30. Pagan AJ, Ramakrishnan L. 2018. The formation and function of granulomas. *Annu. Rev. Immunol.* 36:639–65
31. Williams WJ, James EM, Erasmus DA, Davies T. 1970. The fine structure of sarcoid and tuberculous granulomas. *Postgrad. Med. J.* 46:496–500
32. Gideon HP, Phuah J, Junecko BA, Mattila JT. 2019. Neutrophils express pro- and anti-inflammatory cytokines in granulomas from *Mycobacterium tuberculosis*–infected cynomolgus macaques. *Mucosal Immunol.* 12:1370–81
33. Mattila JT, Ojo OO, Kepka-Lenhart D, Marino S, Kim JH, et al. 2013. Microenvironments in tuberculous granulomas are delineated by distinct populations of macrophage subsets and expression of nitric oxide synthase and arginase isoforms. *J. Immunol.* 191:773–84
34. Bouley DM, Ghori N, Mercer KL, Falkow S, Ramakrishnan L. 2001. Dynamic nature of host-pathogen interactions in *Mycobacterium marinum* granulomas. *Infect. Immun.* 69:7820–31
35. Cronan MR, Hughes EJ, Brewer WJ, Viswanathan G, Hunt EG, et al. 2021. A non-canonical type 2 immune response coordinates tuberculous granuloma formation and epithelialization. *Cell* 184:1757–74.e14
36. Cronan MR, Beerman RW, Rosenberg AF, Saelens JW, Johnson MG, et al. 2016. Macrophage epithelial reprogramming underlies mycobacterial granuloma formation and promotes infection. *Immunity* 45:861–76
37. Jankovic D, Kullberg MC, Noben-Trauth N, Caspar P, Ward JM, et al. 1999. Schistosome-infected IL-4 receptor knockout (KO) mice, in contrast to IL-4 KO mice, fail to develop granulomatous pathology while maintaining the same lymphokine expression profile. *J. Immunol.* 163:337–42
38. Van den Bossche J, Bogaert P, van Hengel J, Guerin CJ, Berx G, et al. 2009. Alternatively activated macrophages engage in homotypic and heterotypic interactions through IL-4 and polyamine-induced E-cadherin/catenin complexes. *Blood* 114:4664–74
39. Gautam US, Foreman TW, Bucsan AN, Veatch AV, Alvarez X, et al. 2018. In vivo inhibition of tryptophan catabolism reorganizes the tuberculoma and augments immune-mediated control of *Mycobacterium tuberculosis*. *PNAS* 115:E62–71
40. Cronan MR, Matty MA, Rosenberg AF, Blanc L, Pyle CJ, et al. 2018. An explant technique for high-resolution imaging and manipulation of mycobacterial granulomas. *Nat. Methods* 15:1098–107
41. Srinivas V, Arrieta-Ortiz ML, Peterson EJR, Baliga NS. 2020. PerSort facilitates characterization and elimination of persister subpopulation in mycobacteria. *mSystems* 5(6):01127
42. Laufer JM, Legler DF. 2018. Beyond migration—chemokines in lymphocyte priming, differentiation, and modulating effector functions. *J. Leukoc. Biol.* 104:301–12
43. Hoft SG, Sallin MA, Kauffman KD, Sakai S, Ganusov VV, Barber DL. 2019. The rate of CD4 T cell entry into the lungs during *Mycobacterium tuberculosis* infection is determined by partial and opposing effects of multiple chemokine receptors. *Infect. Immun.* 87:e00841
44. Potter EL, Gideon HP, Tkachev V, Fabozzi G, Chassakiros A, et al. 2021. Measurement of leukocyte trafficking kinetics in macaques by serial intravascular staining. *Sci. Transl. Med.* 13:eabb4582

45. Shanmugasundaram U, Bucsan AN, Ganatra SR, Ibegbu C, Quezada M, et al. 2020. Pulmonary *Mycobacterium tuberculosis* control associates with CXCR3- and CCR6-expressing antigen-specific Th1 and Th17 cell recruitment. *JCI Insight* 5:e137858
46. Kahnert A, Hopken UE, Stein M, Bandermann S, Lipp M, Kaufmann SH. 2007. *Mycobacterium tuberculosis* triggers formation of lymphoid structure in murine lungs. *J. Infect. Dis.* 195:46–54
47. Yin C, Mohanta S, Maffia P, Habenicht AJ. 2017. Tertiary lymphoid organs (TLOs): powerhouses of disease immunity. *Front. Immunol.* 8:228
48. Monin L, Griffiths KL, Slight S, Lin Y, Rangel-Moreno J, Khader SA. 2015. Immune requirements for protective Th17 recall responses to *Mycobacterium tuberculosis* challenge. *Mucosal Immunol.* 8:1099–109
49. Khader SA, Guglani L, Rangel-Moreno J, Gopal R, Junecko BA, et al. 2011. IL-23 is required for long-term control of *Mycobacterium tuberculosis* and B cell follicle formation in the infected lung. *J. Immunol.* 187:5402–7
50. Gopal R, Rangel-Moreno J, Slight S, Lin Y, Nawar HF, et al. 2013. Interleukin-17-dependent CXCL13 mediates mucosal vaccine-induced immunity against tuberculosis. *Mucosal Immunol.* 6:972–84
51. Jones GW, Hill DG, Jones SA. 2016. Understanding immune cells in tertiary lymphoid organ development: It is all starting to come together. *Front. Immunol.* 7:401
52. Slight SR, Rangel-Moreno J, Gopal R, Lin Y, Fallert Junecko BA, et al. 2013. CXCR5⁺ T helper cells mediate protective immunity against tuberculosis. *J. Clin. Investig.* 123:712–26
53. Neyt K, Perros F, GeurtsvanKessel CH, Hammad H, Lambrecht BN. 2012. Tertiary lymphoid organs in infection and autoimmunity. *Trends Immunol.* 33:297–305
54. Moyron-Quiroz JE, Rangel-Moreno J, Kusser K, Hartson L, Sprague F, et al. 2004. Role of inducible bronchus associated lymphoid tissue (iBALT) in respiratory immunity. *Nat. Med.* 10:927–34
55. Day TA, Koch M, Nouailles G, Jacobsen M, Kosmiadi GA, et al. 2010. Secondary lymphoid organs are dispensable for the development of T-cell-mediated immunity during tuberculosis. *Eur. J. Immunol.* 40:1663–73
56. Lu LL, Chung AW, Rosebrock TR, Ghebremichael M, Yu WH, et al. 2016. A functional role for antibodies in tuberculosis. *Cell* 167:433–43.e14
57. Peske JD, Thompson ED, Gemta L, Baylis RA, Fu YX, Engelhard VH. 2015. Effector lymphocyte-induced lymph node-like vasculature enables naive T-cell entry into tumours and enhanced anti-tumour immunity. *Nat. Commun.* 6:7114
58. Leonhardt RM, Lee SJ, Kavathas PB, Cresswell P. 2007. Severe tryptophan starvation blocks onset of conventional persistence and reduces reactivation of *Chlamydia trachomatis*. *Infect. Immun.* 75:5105–17
59. Pfefferkorn ER, Eckel M, Rebhun S. 1986. Interferon- γ suppresses the growth of *Toxoplasma gondii* in human fibroblasts through starvation for tryptophan. *Mol. Biochem. Parasitol.* 20:215–24
60. Almeida AS, Lago PM, Boechat N, Huard RC, Lazzarini LC, et al. 2009. Tuberculosis is associated with a down-modulatory lung immune response that impairs Th1-type immunity. *J. Immunol.* 183:718–31
61. Suzuki Y, Miwa S, Akamatsu T, Suzuki M, Fujie M, et al. 2013. Indoleamine 2,3-dioxygenase in the pathogenesis of tuberculous pleurisy. *Int. J. Tuberc. Lung Dis.* 17:1501–6
62. McCaffrey EF, Donato M, Keren L, Chen Z, Delmastro A, et al. 2022. The immunoregulatory landscape of human tuberculosis granulomas. *Nat. Immunol.* 23:318–29
63. Zhang YJ, Reddy MC, Ioerger TR, Rothchild AC, Dartois V, et al. 2013. Tryptophan biosynthesis protects mycobacteria from CD4 T-cell-mediated killing. *Cell* 155:1296–308
64. Li Q, Li L, Liu Y, Fu X, Qiao D, et al. 2011. Pleural fluid from tuberculous pleurisy inhibits the functions of T cells and the differentiation of Th1 cells via immunosuppressive factors. *Cell Mol. Immunol.* 8:172–80
65. Mehra S, Alvarez X, Didier PJ, Doyle LA, Blanchard JL, et al. 2013. Granuloma correlates of protection against tuberculosis and mechanisms of immune modulation by *Mycobacterium tuberculosis*. *J. Infect. Dis.* 207:1115–27
66. Blumenthal A, Nagalingam G, Huch JH, Walker L, Guillemin GJ, et al. 2012. *M. tuberculosis* induces potent activation of IDO-1, but this is not essential for the immunological control of infection. *PLOS ONE* 7:e37314
67. Kramnik I, Dietrich WF, Demant P, Bloom BR. 2000. Genetic control of resistance to experimental infection with virulent *Mycobacterium tuberculosis*. *PNAS* 97:8560–65

68. Svenson KL, Gatti DM, Valdar W, Welsh CE, Cheng R, et al. 2012. High-resolution genetic mapping using the mouse Diversity Outbred population. *Genetics* 190:437–47
69. Sallin MA, Kauffman KD, Riou C, Du Bruyn E, Foreman TW, et al. 2018. Host resistance to pulmonary *Mycobacterium tuberculosis* infection requires CD153 expression. *Nat. Microbiol.* 3:1198–205
70. Jouanguy E, Altare F, Lamhamdi S, Revy P, Emile J-F, et al. 1996. Interferon- γ -receptor deficiency in an infant with fatal Bacille Calmette–Guérin infection. *N. Engl. J. Med.* 335:1956–62
71. Green AM, DiFazio R, Flynn JL. 2013. IFN- γ from CD4 T cells is essential for host survival and enhances CD8 T cell function during *Mycobacterium tuberculosis* infection. *J. Immunol.* 190:270–77
72. Flynn JL, Chan J, Triebold KJ, Dalton DK, Stewart TA, Bloom BR. 1993. An essential role for interferon γ in resistance to *Mycobacterium tuberculosis* infection. *J. Exp. Med.* 178:2249–54
73. Tait DR, Hatherill M, Van Der Meeren O, Ginsberg AM, Van Brakel E, et al. 2019. Final analysis of a trial of M72/AS01E vaccine to prevent tuberculosis. *N. Engl. J. Med.* 381:2429–39
74. Nemes E, Geldenhuys H, Rozot V, Rutkowski KT, Ratangee F, et al. 2018. Prevention of *M. tuberculosis* infection with H4:IC31 vaccine or BCG revaccination. *N. Engl. J. Med.* 379:138–49
75. Tameris MD, Hatherill M, Landry BS, Scriba TJ, Snowden MA, et al. 2013. Safety and efficacy of MVA85A, a new tuberculosis vaccine, in infants previously vaccinated with BCG: a randomised, placebo-controlled phase 2b trial. *Lancet* 381:1021–28
76. Barber DL, Sakai S, Kudchadkar RR, Fling SP, Day TA, et al. 2019. Tuberculosis following PD-1 blockade for cancer immunotherapy. *Sci. Transl. Med.* 11:eaat2702
77. Lazar-Molnar E, Chen B, Sweeney KA, Wang EJ, Liu W, et al. 2010. Programmed death-1 (PD-1)-deficient mice are extraordinarily sensitive to tuberculosis. *PNAS* 107:13402–7
78. Deleted in proof
79. Wong EA, Joslyn L, Grant NL, Klein E, Lin PL, et al. 2018. Low levels of T cell exhaustion in tuberculous lung granulomas. *Infect. Immun.* 86:00426
80. Majumder S, McGeachy MJ. 2021. IL-17 in the pathogenesis of disease: good intentions gone awry. *Annu. Rev. Immunol.* 39:537–56
81. Khader SA, Pearl JE, Sakamoto K, Gilmartin L, Bell GK, et al. 2005. IL-23 compensates for the absence of IL-12p70 and is essential for the IL-17 response during tuberculosis but is dispensable for protection and antigen-specific IFN- γ responses if IL-12p70 is available. *J. Immunol.* 175:788–95
82. Khader SA, Bell GK, Pearl JE, Fountain JJ, Rangel-Moreno J, et al. 2007. IL-23 and IL-17 in the establishment of protective pulmonary CD4 $^{+}$ T cell responses after vaccination and during *Mycobacterium tuberculosis* challenge. *Nat. Immunol.* 8:369–77
83. Gopal R, Monin L, Slight S, Uche U, Blanchard E, et al. 2014. Unexpected role for IL-17 in protective immunity against hypervirulent *Mycobacterium tuberculosis* HN878 infection. *PLOS Pathog.* 10:e1004099
84. Ogongo P, Tezera LB, Ardain A, Nhamoyebonde S, Ramsuran D, et al. 2021. Tissue-resident-like CD4 $^{+}$ T cells secreting IL-17 control *Mycobacterium tuberculosis* in the human lung. *J. Clin. Investig.* 131:e142014
85. Lockhart E, Green AM, Flynn JL. 2006. IL-17 production is dominated by $\gamma\delta$ T cells rather than CD4 T cells during *Mycobacterium tuberculosis* infection. *J. Immunol.* 177:4662–69
86. Ardain A, Domingo-Gonzalez R, Das S, Kazer SW, Howard NC, et al. 2019. Group 3 innate lymphoid cells mediate early protective immunity against tuberculosis. *Nature* 570:528–32
87. Annunziato F, Cosmi L, Santarlasci V, Maggi L, Liotta F, et al. 2007. Phenotypic and functional features of human Th17 cells. *J. Exp. Med.* 204:1849–61
88. Maggi L, Santarlasci V, Capone M, Rossi MC, Querci V, et al. 2012. Distinctive features of classic and nonclassic (Th17 derived) human Th1 cells. *Eur. J. Immunol.* 42:3180–88
89. Zenaro E, Donini M, Dusi S. 2009. Induction of Th1/Th17 immune response by *Mycobacterium tuberculosis*: role of dectin-1, mannose receptor, and DC-SIGN. *J. Leukoc. Biol.* 86:1393–401
90. Nikitina IY, Panteleev AV, Kosmiadi GA, Serdyuk YV, Nenashova TA, et al. 2018. Th1, Th17, and Th1Th17 lymphocytes during tuberculosis: Th1 lymphocytes predominate and appear as low-differentiated CXCR3 $^{+}$ CCR6 $^{+}$ cells in the blood and highly differentiated CXCR3 $^{+/-}$ CCR6 $^{-}$ cells in the lungs. *J. Immunol.* 200:2090–103
91. Salgame P, Lindestam Arlehamn CS, Gerasimova A, Mele F, Henderson R, et al. 2013. Memory T cells in latent *Mycobacterium tuberculosis* infection are directed against three antigenic islands and largely contained in a CXCR3 $^{+}$ CCR6 $^{+}$ Th1 subset. *PLOS Pathog.* 9:e1003130

92. Sasse CM, Scriba TJ, Penn-Nicholson A, Shankar S, Hraha T, et al. 2017. Sequential inflammatory processes define human progression from *M. tuberculosis* infection to tuberculosis disease. *PLOS Pathog.* 13:e1006687
93. Estévez O, Anibarro L, Garet E, Martínez A, Pena A, et al. 2020. Multi-parameter flow cytometry immunophenotyping distinguishes different stages of tuberculosis infection. *J. Infect.* 81:57–71
94. Gideon HP, Hughes TK, Wadsworth MH, Tu AA, Gierahn TM, et al. 2021. Multimodal profiling of lung granulomas reveals cellular correlates of tuberculosis control. *bioRxiv* 352492. <https://doi.org/10.1101/2020.10.24.352492>
95. Keane J, Gershon S, Wise RP, Mirabile-Levens E, Kasznica J, et al. 2001. Tuberculosis associated with infliximab, a tumor necrosis factor α -neutralizing agent. *N. Engl. J. Med.* 345:1098–104
96. Flynn JL, Goldstein MM, Chan J, Triebold KJ, Pfeffer K, et al. 1995. Tumor necrosis factor- α is required in the protective immune response against mycobacterium tuberculosis in mice. *Immunity* 2:561–72
97. Clay H, Volkman HE, Ramakrishnan L. 2008. Tumor necrosis factor signaling mediates resistance to mycobacteria by inhibiting bacterial growth and macrophage death. *Immunity* 29:283–94
98. Bhattacharya B, Xiao S, Chatterjee S, Urbanowski M, Ordóñez A, et al. 2021. The integrated stress response mediates necrosis in murine *Mycobacterium tuberculosis* granulomas. *J. Clin. Investig.* 131:e130319
99. Keller C, Hoffmann R, Lang R, Brandau S, Hermann C, Ehlers S. 2006. Genetically determined susceptibility to tuberculosis in mice causally involves accelerated and enhanced recruitment of granulocytes. *Infect. Immun.* 74:4295–309
100. Roca FJ, Whitworth LJ, Redmond S, Jones AA, Ramakrishnan L. 2019. TNF induces pathogenic programmed macrophage necrosis in tuberculosis through a mitochondrial–lysosomal–endoplasmic reticulum circuit. *Cell* 178:1344–61.e11
101. Tobin DM, Roca FJ, Oh SF, McFarland R, Vickery TW, et al. 2012. Host genotype–specific therapies can optimize the inflammatory response to mycobacterial infections. *Cell* 148:434–46
102. Roca FJ, Ramakrishnan L. 2013. TNF dually mediates resistance and susceptibility to mycobacteria via mitochondrial reactive oxygen species. *Cell* 153:521–34
103. Suliman AM, Bek SA, Elkhatim MS, Husain AA, Mismar AY, et al. 2020. Tuberculosis following programmed cell death receptor 1 (PD-1) inhibitor in a patient with non-small cell lung cancer: case report and literature review. *Cancer Immunol. Immunother.* 70:935–44
104. Rothchild AC, Stowell B, Goyal G, Nunes-Alves C, Yang Q, et al. 2017. Role of granulocyte-macrophage colony-stimulating factor production by T cells during *Mycobacterium tuberculosis* infection. *mBio* 8:e01514
105. Bergeron A, Bonay M, Kambouchner M, Lecossier D, Riquet M, et al. 1997. Cytokine patterns in tuberculous and sarcoid granulomas: correlations with histopathologic features of the granulomatous response. *J. Immunol.* 159:3034–43
106. Szeliga J, Daniel DS, Yang CH, Sever-Chroneos Z, Jagannath C, Chroneos ZC. 2008. Granulocyte-macrophage colony stimulating factor-mediated innate responses in tuberculosis. *Tuberculosis* 88:7–20
107. Gonzalez-Juarrero M, Hattle JM, Izzo A, Junqueira-Kipnis AP, Shim TS, et al. 2005. Disruption of granulocyte-macrophage colony stimulating factor production in the lungs severely affects the ability of mice to control *Mycobacterium tuberculosis* infection. *J. Leukoc. Biol.* 77:914–22
108. Rothchild AC, Jayaraman P, Nunes-Alves C, Behar SM. 2014. iNKT cell production of GM-CSF controls *Mycobacterium tuberculosis*. *PLOS Pathog.* 10:e1003805
109. Bermudez LE, Young LS. 1990. Recombinant granulocyte-macrophage colony-stimulating factor activates human macrophages to inhibit growth or kill *Mycobacterium avium* complex. *J. Leukoc. Biol.* 48:67–73
110. Denis M, Ghadirian E. 1990. Granulocyte-macrophage colony-stimulating factor restricts growth of tubercle bacilli in human macrophages. *Immunol. Lett.* 24:203–6
111. Ji DX, Yamashiro LH, Chen KJ, Mukaida N, Kramnik I, Darwin KH, Vance RE. 2019. Type I interferon-driven susceptibility to *Mycobacterium tuberculosis* is mediated by IL-1Ra. *Nat. Microbiol.* 4:2128–35
112. Morikawa M, Deryck R, Miyazono K. 2016. TGF- β and the TGF- β family: context-dependent roles in cell and tissue physiology. *Cold Spring Harb. Perspect. Biol.* 8:a021873

113. Dahl KE, Shiratsuchi H, Hamilton BD, Ellner JJ, Toossi Z. 1996. Selective induction of transforming growth factor β in human monocytes by lipoarabinomannan of *Mycobacterium tuberculosis*. *Infect. Immun.* 64:399–405
114. Rook GA, Lowrie DB, Hernandez-Pando R. 2007. Immunotherapeutics for tuberculosis in experimental animals: Is there a common pathway activated by effective protocols? *J. Infect. Dis.* 196:191–98
115. DiFazio RM, Mattila JT, Klein EC, Cirrincione LR, Howard M, et al. 2016. Active transforming growth factor- β is associated with phenotypic changes in granulomas after drug treatment in pulmonary tuberculosis. *Fibrogenes. Tissue Repair* 9:6
116. Bonecini-Almeida MG, Ho JL, Boechat N, Huard RC, Chitale S, et al. 2004. Down-modulation of lung immune responses by interleukin-10 and transforming growth factor β (TGF- β) and analysis of TGF- β receptors I and II in active tuberculosis. *Infect. Immun.* 72:2628–34
117. Warsinske HC, Pierna E, Linderman JJ, Mattila JT, Kirschner DE. 2017. Deletion of TGF- β 1 increases bacterial clearance by cytotoxic T cells in a tuberculosis granuloma model. *Front. Immunol.* 8:1843
118. Anastasopoulou A, Ziogas DC, Samarkos M, Kirkwood JM, Gogas H. 2019. Reactivation of tuberculosis in cancer patients following administration of immune checkpoint inhibitors: current evidence and clinical practice recommendations. *J. Immunother. Cancer* 7:239
119. Cadranel J, Canellas A, Matton L, Darrason M, Parrot A, et al. 2019. Pulmonary complications of immune checkpoint inhibitors in patients with nonsmall cell lung cancer. *Eur. Respir. Rev.* 28:190058
120. Fujita K, Yamamoto Y, Kanai O, Okamura M, Hashimoto M, et al. 2020. Incidence of active tuberculosis in lung cancer patients receiving immune checkpoint inhibitors. *Open Forum Infect. Dis.* 7:ofaa126
121. Inthasot V, Bruyneel M, Muylle I, Ninane V. 2020. Severe pulmonary infections complicating nivolumab treatment for lung cancer: a report of two cases. *Acta Clin. Belg.* 75:308–10
122. Jensen KH, Persson G, Bondgaard AL, Pohl M. 2018. Development of pulmonary tuberculosis following treatment with anti-PD-1 for non-small cell lung cancer. *Acta Oncol.* 57:1127–28
123. van Eeden R, Rapoport BL, Smit T, Anderson R. 2019. Tuberculosis infection in a patient treated with nivolumab for non-small cell lung cancer: case report and literature review. *Front. Oncol.* 9:659
124. Jayaswal S, Kamal MA, Dua R, Gupta S, Majumdar T, et al. 2010. Identification of host-dependent survival factors for intracellular *Mycobacterium tuberculosis* through an siRNA screen. *PLOS Pathog.* 6:e1000839
125. North RJ. 1995. *Mycobacterium tuberculosis* is strikingly more virulent for mice when given via the respiratory than via the intravenous route. *J. Infect. Dis.* 172:1550–53
126. Rosas-Taraco AG, Higgins DM, Sánchez-Campillo J, Lee EJ, Orme IM, González-Juarrero M. 2011. Local pulmonary immunotherapy with siRNA targeting TGF β 1 enhances antimicrobial capacity in *Mycobacterium tuberculosis* infected mice. *Tuberculosis* 91:98–106
127. Akhurst RJ. 2017. Targeting TGF- β signaling for therapeutic gain. *Cold Spring Harb. Perspect. Biol.* 9:a022301
128. de Gramont A, Faivre S, Raymond E. 2017. Novel TGF- β inhibitors ready for prime time in onco-immunology. *Oncimmunology* 6:e1257453
129. Huynh L, Hipolito C, ten Dijke P. 2019. A perspective on the development of TGF- β inhibitors for cancer treatment. *Biomolecules* 9:743
130. Gideon HP, Phuah J, Myers AJ, Bryson BD, Rodgers MA, et al. 2015. 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
131. Moguche AO, Shafiani S, Clemons C, Larson RP, Dinh C, et al. 2015. ICOS and Bcl6-dependent pathways maintain a CD4 T cell population with memory-like properties during tuberculosis. *J. Exp. Med.* 212:715–28
132. Reiley WW, Shafiani S, Wittmer ST, Tucker-Heard G, Moon JJ, et al. 2010. Distinct functions of antigen-specific CD4 T cells during murine *Mycobacterium tuberculosis* infection. *PNAS* 107:19408–13
133. Sakai S, Kauffman KD, Schenkel JM, McBerry CC, Mayer-Barber KD, et al. 2014. Control of *Mycobacterium tuberculosis* infection by a subset of lung parenchyma-homing CD4 T cells. *J. Immunol.* 192:2965–69

134. Moguche AO, Musvossi M, Penn-Nicholson A, Plumlee CP, Mearns H, et al. 2017. Antigen availability shapes the differentiation and protective capacity of *Mycobacterium tuberculosis*-specific CD4 T cells. *Cell Host Microbe* 21:695–706.e5
135. Wherry EJ, Kurachi M. 2015. Molecular and cellular insights into T cell exhaustion. *Nat. Rev. Immunol.* 15:486–99
136. Jayaraman P, Jacques MK, Zhu C, Steblenko KM, Stowell BL, et al. 2016. TIM3 mediates T cell exhaustion during *Mycobacterium tuberculosis* infection. *PLOS Pathog.* 12:e1005490
137. Phillips BL, Mehra S, Ahsan MH, Selman M, Khader SA, Kaushal D. 2015. LAG3 expression in active *Mycobacterium tuberculosis* infections. *Am. J. Pathol.* 185:820–33
138. Nandakumar S, Kannanganat S, Posey JE, Amara RR, Sable SB. 2014. Attrition of T-cell functions and simultaneous upregulation of inhibitory markers correspond with the waning of BCG-induced protection against tuberculosis in mice. *PLOS ONE* 9:e113951
139. Kirman J, McCoy K, Hook S, Prout M, Delahunt B, et al. 1999. CTLA-4 blockade enhances the immune response induced by mycobacterial infection but does not lead to increased protection. *Infect. Immun.* 67:3786–92
140. Blackburn SD, Shin H, Haining WN, Zou T, Workman CJ, et al. 2009. Coregulation of CD8⁺ T cell exhaustion by multiple inhibitory receptors during chronic viral infection. *Nat. Immunol.* 10:29–37
141. Day CL, Abrahams DA, Bunjun R, Stone L, de Kock M, et al. 2018. PD-1 expression on *Mycobacterium tuberculosis*-specific CD4 T cells is associated with bacterial load in human tuberculosis. *Front. Immunol.* 9:1995
142. Kubo T, Hirohashi Y, Tsukahara T, Kanaseki T, Murata K, et al. 2021. Epithelioid granulomatous lesions express abundant programmed death ligand 1 (PD-L1): a discussion of adverse events in anti-PD-1 antibody-based cancer immunotherapy. *Hum. Vaccines Immunother.* 17:1940–42
143. Barber DL, Mayer-Barber KD, Feng CG, Sharpe AH, Sher A. 2011. CD4 T cells promote rather than control tuberculosis in the absence of PD-1-mediated inhibition. *J. Immunol.* 186:1598–607
144. Tousif S, Singh Y, Prasad DV, Sharma P, Van Kaer L, Das G. 2011. T cells from programmed death 1 deficient mice respond poorly to *Mycobacterium tuberculosis* infection. *PLOS ONE* 6:e19864
145. Sakai S, Kauffman KD, Sallin MA, Sharpe AH, Young HA, et al. 2016. CD4 T cell-derived IFN- γ plays a minimal role in control of pulmonary *Mycobacterium tuberculosis* infection and must be actively repressed by PD-1 to prevent lethal disease. *PLOS Pathog.* 12:e1005667
146. Tezera LB, Bielecka MK, Ogongo P, Walker NF, Ellis M, et al. 2020. Anti-PD-1 immunotherapy leads to tuberculosis reactivation via dysregulation of TNF- α . *eLife* 9:e52668
147. Kauffman KD, Sakai S, Lora NE, Namasivayam S, Baker PJ, et al. 2021. PD-1 blockade exacerbates *Mycobacterium tuberculosis* infection in rhesus macaques. *Sci. Immunol.* 6:eafb3861
148. Crotty S. 2011. Follicular helper CD4 T cells (T_{FH}). *Annu. Rev. Immunol.* 29:621–63
149. Yang Q, Zhang M, Chen Q, Chen W, Wei C, et al. 2020. Characterization of human tissue-resident memory T cells at different infection sites in patients with tuberculosis. *J. Immunol.* 204:2331–36
150. Honda T, Egen JG, Lammermann T, Kastenmuller W, Torabi-Parizi P, Germain RN. 2014. Tuning of antigen sensitivity by T cell receptor-dependent negative feedback controls T cell effector function in inflamed tissues. *Immunity* 40:235–47
151. Kamboj D, Gupta P, Basil MV, Mohan A, Guleria R, et al. 2020. Improved *Mycobacterium tuberculosis* clearance after the restoration of IFN- γ ⁺ TNF- α ⁺ CD4⁺ T cells: impact of PD-1 inhibition in active tuberculosis patients. *Eur. J. Immunol.* 50:736–47
152. Ankley L, Thomas S, Olive AJ. 2020. Fighting persistence: how chronic infections with *Mycobacterium tuberculosis* evade T cell-mediated clearance and new strategies to defeat them. *Infect. Immun.* 88:e00916
153. Goldberg MF, Saini NK, Porcelli SA. 2021. Evasion of innate and adaptive immunity by *Mycobacterium tuberculosis*. *Microbiol. Spectr.* 2. <https://doi.org/10.1128/microbiolspec.MGM2-0005-2013>
154. Srivastava S, Ernst JD. 2013. Direct recognition of infected cells by CD4 T cells is required for control of intracellular *Mycobacterium tuberculosis* in vivo. *J. Immunol.* 191:1016–20
155. Grace PS, Ernst JD. 2016. Suboptimal antigen presentation contributes to virulence of *Mycobacterium tuberculosis* in vivo. *J. Immunol.* 196:357–64

156. Egen JG, Rothfuchs AG, Feng CG, Horwitz MA, Sher A, Germain RN. 2011. Intravital imaging reveals limited antigen presentation and T cell effector function in mycobacterial granulomas. *Immunity* 34:807–19
157. Srivastava S, Grace PS, Ernst JD. 2016. Antigen export reduces antigen presentation and limits T cell control of *M. tuberculosis*. *Cell Host Microbe* 19:44–54
158. Delahaye JL, Gern BH, Cohen SB, Plumlee CR, Shafiani S, et al. 2019. Bacillus Calmette-Guérin-induced T cells shape *Mycobacterium tuberculosis* infection before reducing the bacterial burden. *J. Immunol.* 203:807–12
159. Wilburn KM, Fieweger RA, VanderVen BC. 2018. Cholesterol and fatty acids grease the wheels of *Mycobacterium tuberculosis* pathogenesis. *Pathog. Dis.* 76:fty021
160. Daniel J, Maamar H, Deb C, Sirakova TD, Kolattukudy PE. 2011. *Mycobacterium tuberculosis* uses host triacylglycerol to accumulate lipid droplets and acquires a dormancy-like phenotype in lipid-loaded macrophages. *Plos Pathog.* 7:e1002093
161. Peyron P, Vaourgeix J, Poquet Y, Levillain F, Botanch C, et al. 2008. Foamy macrophages from tuberculous patients' granulomas constitute a nutrient-rich reservoir for *M. tuberculosis* persistence. *PLOS Pathog.* 4:e1000204
162. Sarathy JP, Via LE, Weiner D, Blanc L, Boshoff H, et al. 2018. Extreme drug tolerance of *Mycobacterium tuberculosis* in caseum. *Antimicrob. Agents Chemother.* 62:e02266
163. Carow B, Hauling T, Qian X, Kramnik I, Nilsson M, Rottenberg ME. 2019. Spatial and temporal localization of immune transcripts defines hallmarks and diversity in the tuberculosis granuloma. *Nat. Commun.* 10:1823
164. Meylan PR, Richman DD, Kornbluth RS. 1992. Reduced intracellular growth of mycobacteria in human macrophages cultivated at physiologic oxygen pressure. *Am. Rev. Respir. Dis.* 145:947–53
165. Nickel D, Busch M, Mayer D, Hagemann B, Knoll V, Stenger S. 2012. Hypoxia triggers the expression of human β defensin 2 and antimicrobial activity against *Mycobacterium tuberculosis* in human macrophages. *J. Immunol.* 188:4001–7
166. Wayne LG, Hayes LG. 1996. An in vitro model for sequential study of shiftdown of *Mycobacterium tuberculosis* through two stages of nonreplicating persistence. *Infect. Immun.* 64:2062–69
167. Canetti G. 1965. Present aspects of bacterial resistance in tuberculosis. *Am. Rev. Respir. Dis.* 92:687–703
168. Marakalala MJ, Raju RM, Sharma K, Zhang YJ, Eugenin EA, et al. 2016. Inflammatory signaling in human tuberculosis granulomas is spatially organized. *Nat. Med.* 22:531–38
169. Zenk SF, Vollmer M, Schercher E, Kallert S, Kubis J, Stenger S. 2016. Hypoxia promotes *Mycobacterium tuberculosis*-specific up-regulation of granulysin in human T cells. *Med. Microbiol. Immunol.* 205:219–29
170. O'Neill LA, Pearce EJ. 2016. Immunometabolism governs dendritic cell and macrophage function. *J. Exp. Med.* 213:15–23
171. Simon LM, Axline SG, Horn BR, Robin ED. 1973. Adaptations of energy metabolism in the cultivated macrophage. *J. Exp. Med.* 138:1413–25
172. Shi L, Salamon H, Eugenin EA, Pine R, Cooper A, Gennaro ML. 2015. Infection with *Mycobacterium tuberculosis* induces the Warburg effect in mouse lungs. *Sci. Rep.* 5:18176
173. Huang L, Nazarova EV, Tan S, Liu Y, Russell DG. 2018. Growth of *Mycobacterium tuberculosis* in vivo segregates with host macrophage metabolism and ontogeny. *J. Exp. Med.* 215:1135–52
174. Cardoso MS, Silva TM, Resende M, Appelberg R, Borges M. 2015. Lack of the transcription factor hypoxia-inducible factor 1 α (HIF-1 α) in macrophages accelerates the necrosis of *Mycobacterium avium*-induced granulomas. *Infect. Immun.* 83:3534–44
175. Braverman J, Sogi KM, Benjamin D, Nomura DK, Stanley SA. 2016. HIF-1 α is an essential mediator of IFN- γ -dependent immunity to *Mycobacterium tuberculosis*. *J. Immunol.* 197:1287–97
176. Oehlers SH, Cronan MR, Beerman RW, Johnson MG, Huang J, et al. 2017. Infection-induced vascular permeability aids mycobacterial growth. *J. Infect. Dis.* 215:813–17
177. Oehlers SH, Cronan MR, Scott NR, Thomas MI, Okuda KS, et al. 2015. Interception of host angiogenic signalling limits mycobacterial growth. *Nature* 517:612–15
178. Polena H, Boudou F, Tilleul S, Dubois-Colas N, Leconte C, et al. 2016. *Mycobacterium tuberculosis* exploits the formation of new blood vessels for its dissemination. *Sci. Rep.* 6:33162

179. Ulrichs T, Kosmiadi GA, Jörg S, Pradl L, Titukhina M, et al. 2005. Differential organization of the local immune response in patients with active cavitary tuberculosis or with nonprogressive tuberculoma. *J. Infect. Dis.* 192:89–97
180. Borrel A. 1920. Pneumonie et tuberculose chez les troupes noires. *Ann. Inst. Pasteur* 34:105–48
181. Koch R. 1882. Die Aetiologie der Tuberculose. *Berl. Klin. Wochenschr.* 19(15):228–31
182. Sweany HC, Cook CE, Kegerreis R. 1931. A study of the position of primary cavities in pulmonary tuberculosis. *Am. Rev. Tuberc.* 24:558–82
183. Levine ER. 1949. Classification of reinfection pulmonary tuberculosis. In *The Fundamentals of Pulmonary Tuberculosis and Its Implications for Students, Teachers and Practicing Physicians*, ed. E Hayes, pp. 97–113. Springfield, IL: Thomas
184. Hunter RL, Jagannath C, Actor JK. 2007. Pathology of postprimary tuberculosis in humans and mice: contradiction of long-held beliefs. *Tuberculosis* 87:267–78
185. Andrews JR, Noubary F, Walensky RP, Cerdá R, Losina E, Horsburgh CR. 2012. Risk of progression to active tuberculosis following reinfection with *Mycobacterium tuberculosis*. *Clin. Infect. Dis.* 54:784–91
186. Medlar EM, Sasano KT. 1936. A study of the pathology of experimental pulmonary tuberculosis in the rabbit. *Am. Rev. Tuberc.* 34:456–76
187. Dock W. 1954. Effect of posture on alveolar gas tension in tuberculosis. *Am. Med. Assoc. Arch. Intern. Med.* 94:700–8
188. Rothlin E, Undritz E. 1952. Beitrag zur Lokalisationsregel der Tuberkulose. *Schweiz. Z. allg. Pathol. Bakteriol.* 15(6):690–700
189. Goodwin RA, Des Prez RM. 1983. Apical localization of pulmonary tuberculosis, chronic pulmonary histoplasmosis, and progressive massive fibrosis of the lung. *Chest* 83:801–5
190. Canetti G, Sutherland I, Svandova E. 1972. Endogenous reactivation and exogenous reinfection: their relative importance with regard to the development of non-primary tuberculosis. *Bull. Int. Union Tuberc.* 47:116–34
191. Stead WW. 1967. Pathogenesis of a first episode of chronic pulmonary tuberculosis in man: recrudescence of residuals of the primary infection or exogenous reinfection? *Am. Rev. Respir. Dis.* 95:729–45
192. Nardell E, McInnis B, Thomas B, Weidhaas S. 1986. Exogenous reinfection with tuberculosis in a shelter for the homeless. *N. Engl. J. Med.* 315:1570–75
193. Fine PE, Small PM. 1999. Exogenous reinfection in tuberculosis. *N. Engl. J. Med.* 341:1226–27
194. Lillebaek T, Dirksen A, Baess I, Strunge B, Thomsen VO, Andersen AB. 2002. Molecular evidence of endogenous reactivation of *Mycobacterium tuberculosis* after 33 years of latent infection. *J. Infect. Dis.* 185:401–4
195. Behr MA, Edelstein PH, Ramakrishnan L. 2018. Revisiting the timetable of tuberculosis. *BMJ* 362:k2738
196. Seidler A, Nienhaus A, Diel R. 2004. The transmission of tuberculosis in the light of new molecular biological approaches. *Occup. Environ. Med.* 61:96–102
197. Chen RY, Yu X, Smith B, Liu X, Gao J, et al. 2021. Radiological and functional evidence of the bronchial spread of tuberculosis: an observational analysis. *Lancet Microbe* 2:e518–26
198. Skoura E, Zumla A, Bomanji J. 2015. Imaging in tuberculosis. *Int. J. Infect. Dis.* 32:87–93
199. Medlar EM. 1948. The pathogenesis of minimal pulmonary tuberculosis: a study of 1,225 necropsies in cases of sudden and unexpected death. *Am. Rev. Tuberc.* 58:583–611
200. Ma F, Hughes TK, Teles RMB, Andrade PR, de Andrade Silva BJ, et al. 2021. The cellular architecture of the antimicrobial response network in human leprosy granulomas. *Nat. Immunol.* 22:839–50
201. Nemeth J, Olson GS, Rothchild AC, Jahn AN, Mai D, et al. 2020. Contained *Mycobacterium tuberculosis* infection induces concomitant and heterologous protection. *PLOS Pathog.* 16:e1008655
202. Harding GE, Smith DW. 1977. Host–parasite relationships in experimental airborne tuberculosis. VI. Influence of vaccination with Bacille Calmette–Guérin on the onset and/or extent of hematogenous dissemination of virulent *Mycobacterium tuberculosis* to the lungs. *J. Infect. Dis.* 136:439–43
203. Ly LH, Russell MI, McMurray DN. 2008. Cytokine profiles in primary and secondary pulmonary granulomas of guinea pigs with tuberculosis. *Am. J. Respir. Cell Mol. Biol.* 38:455–62

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