Understanding Latent Tuberculosis: A Moving Target

Philana Ling Lin and JoAnne L. Flynn

*J Immunol* 2010; 185:15-22; doi: 10.4049/jimmunol.0903856
http://www.jimmunol.org/content/185/1/15

---

**References**

This article cites 120 articles, 32 of which you can access for free at:
http://www.jimmunol.org/content/185/1/15.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Understanding Latent Tuberculosis: A Moving Target

Philana Ling Lin* and JoAnne L. Flynn†

Tuberculosis (TB) remains a threat to the health of people worldwide. Infection with Mycobacterium tuberculosis can result in active TB or, more commonly, latent infection. Latently infected persons, of which there are estimated to be ~2 billion in the world, represent an enormous reservoir of potential reactivation TB, which can spread to other people. The immunology of TB is complex and multifaceted. Identifying the immune mechanisms that lead to control of initial infection and prevent reactivation of latent infection is crucial to combating this disease. *The Journal of Immunology, 2010, 185: 15–22.

In 1991, the World Health Organization recognized the resurgence of tuberculosis (TB) as a global health problem. Despite the initiation of directly observed therapy strategies, TB accounts for 1.8 million deaths in 2008, second only to HIV as an infectious cause of death. New reported cases of TB increased from 8.0 million in 1997 to 9.4 million in 2008 (1). Poverty, the advent of multidrug-resistant cases, imperfect diagnostic assays, poor access to health care, limited vaccine efficacy, and availability of new drugs remain obstacles. The HIV epidemic, especially in poverty-affected countries, is among the most important contributors to the resurgence of TB.

The epidemiology of TB is a key component to understanding the complexity of this global health burden. Mycobacterium tuberculosis, the etiologic agent of TB, is an acid-fast bacillus. Person-to-person transmission of M. tuberculosis occurs by aerosolized droplets generated by a person with active disease, which are inhaled into the large and small airways, where infection can be established. An estimated 30% of exposed individuals will have evidence of infection by tuberculin skin test (TST) (2). Among those infected, only 5–10% will develop clinical manifestations of active TB disease within 2 y postexposure (known as primary TB). The majority of infected individuals develop latent infection defined as having evidence of M. tuberculosis infection by immunologic tests (TST or IFN-γ release assay [IGRA]) without clinical signs or symptoms of disease and a normal chest radiograph. This represents a state of equilibrium in which the host is able to control the infection but not completely eradicate the bacteria. Patients with latent infection are the largest reservoir for potential transmission. Although most patients with latent infection will not die of TB, the greatest danger is in reactivation (active TB after remote infection) cases and the subsequent silent spread to close contacts. The majority of active TB disease in low-prevalence regions, such as the United States, is attributed to reactivation TB (47–87%) (3–6). In contrast, recent infection accounts for the majority of active TB cases in highly endemic areas (7). The risk of reactivation TB is estimated as 10% per lifetime. Impaired immunity, as in the case of HIV infection, increases the risk to 10% per year (8). Active TB, either from recent infection or reactivation, results in contagious spread of the pathogen. For every year that a single person with active TB is untreated, he or she will infect an average of 10–15 people (1). HIV-negative people are estimated to be sputum smear positive (i.e., with acid fast bacilli observed in sputum) 1–3 y prior to diagnosis in resource-poor settings (9, 10). By these estimates, a single person with active TB could infect as many as 45 other individuals. It is no wonder that this disease remains a global health threat for which the incidence has not been reduced despite tremendous efforts.

A growing amount of clinical and epidemiologic data has recently challenged the notion that M. tuberculosis infection exists only as a bimodal distinction of latent infection and active TB (11). In fact, a variable spectrum of latent infection likely exists just as active TB has varying degrees of severity. In this review, we discuss the immunologic establishment and maintenance of latent infection, limitations of the data and how they relate specifically to human infection, risk factors of reactivation, and potential mechanisms, and the changing paradigm of latent infection.

Establishing latent infection

From the beginning. Infection first occurs when the bacilli enter the alveoli and are phagocytized by alveolar macrophage and resident dendritic cells. Dendritic cells carrying bacilli and Ags travel from the distal airways to the draining mediastinal lymph nodes, where they initiate T cell responses. Lymphocytes and macrophages then migrate to the primary site of infection to form a granuloma. In most animal models of TB, bacterial growth increases logarithmically and then reaches a plateau.

*Department of Pediatrics, Children’s Hospital of Pittsburgh of the University of Pittsburgh Medical Center, Pittsburgh, PA 15224; and †Department of Microbiology and Molecular Genetics, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

Received for publication April 9, 2010. Accepted for publication April 28, 2010.

This work was supported by National Institutes of Health Grants HL075845, HL092883, AI50732, AI37859, and HL71241 (to J.L.F.), AI063101 (to P.L.L.), the Otis Childs Foundation (to P.L.L.), and the Bill and Melinda Gates Foundation (to J.L.F. and P.L.L.).

Address correspondence and reprint requests to Dr. JoAnne L. Flynn, Department of Microbiology and Molecular Genetics, University of Pittsburgh School of Medicine, W1144 Biomedical Science Tower, Pittsburgh, PA 15261. E-mail address: joanne@pitt.edu

Abbreviations used in this paper: IGRA, IFN-γ release assay; NOS2, NO synthase 2; TB, tuberculosis; TST, tuberculin skin test.

Copyright © 2010 by The American Association of Immunologists, Inc. 0022-1767/10/$16.00
coincident with T cell response initiation, when granulomas are observed histologically (12). The granuloma, the hallmark of TB, is a foci of clonal expansion of inflammatory cells that have specific architectural structures in humans. Granulomas are thought to represent an immunologic and physical barrier to contain infection and prevent dissemination. Maintenance of the granulomas is a dynamic process of continual immunologic control of bacterial replication.

The innate immune response. In the United States, close-contact investigations revealed that an estimated 20–30% of close contacts had latent infection, and another 1% had active TB (2, 13). This indicates that 70–80% of exposed individuals do not become infected. Although difficult to prove, these data suggest that some humans are resistant to infection and that the innate immune response likely plays an important role in clinical outcome.

Host resistance against mycobacterial infection begins with the innate immune response involving interaction of the bacillus with macrophages and dendritic cells. TLRs have been recognized as important pattern recognition receptors for M. tuberculosis infection. Increased susceptibility to M. tuberculosis in MyD88-deficient mice first indicated that TLRs were important in the initial host response (14–16), although the exact TLRs involved are controversial. However, MyD88-dependent IL-1R expression is also critical for resistance to M. tuberculosis (17). In vitro and in vivo studies in murine models have implicated TLR2, TLR4, and TLR9 as important in the response to M. tuberculosis (18–24), although other studies have not supported these findings (25, 26). Genetic polymorphisms of TLR genes have been associated with increased risk of M. tuberculosis infection or disease (27–29).

Human in vitro studies have shown that TLR activation induces vitamin D-dependent production of antimicrobial peptides that have mycobactericidal activity (30, 31).

Transition to control of infection. Little is known about the transition between controlling acute infection and establishment of latent infection, in large part due to lack of appropriate animal models. In nonhuman primates, clinical outcome is both dose dependent (32) and host dependent (33). Cynomolgous macaques infected with a low dose of M. tuberculosis are the only animal model in which both active disease and latent infection have been described and characterized (34); gross signs of disease are observed as early as 4 wk postinfection (35). By 6 wk postinfection, greater production of mycobacteria-specific IFN-γ in PBMCs was observed among monkeys who later developed active disease compared with latent monkeys (34). Human contact studies, in which humans exposed to a case of active TB are followed, have also found that high responses to ESAT6 (an M. tuberculosis protein) correlated to active disease outcome (2-γ follow-up) compared with those without disease (36). In our experience, increases in IFN-γ often reflect increased bacterial burden, suggesting that control of bacterial replication early post-infection plays an important role in establishing latent infection. The amount of gross disease seen at 8 wk postinfection could not predict which monkeys would later develop latent or active disease. Yet the overall bacterial burden at 8 wk is significantly more than latently infected monkeys (P.L. Lin and J.L. Flynn, unpublished observations), suggesting that the host response of latent monkeys reduces the initial bacterial burden over time.

Adaptive immune responses in establishing and maintaining latency.

T cells. The cell-mediated immune system is critical to overcoming acute M. tuberculosis infection. Cells found within the granuloma include CD4 and CD8 T cells, B cells, macrophages, neutrophils, fibroblasts, and multinucleated giant cells. Murine models have shown that CD4 T cells are required for control of acute and chronic infection (reviewed in Ref. 37). CD4 T cells are major producers of IFN-γ, contribute to TNF production, and are important for optimal CD8 T cell function. This has been confirmed in patients with HIV in whom the risk of TB increases with decreasing CD4 T cell counts (38), and in nonhuman primates with SIV, in which the initial reduction in CD4 T cell levels was associated with time to reactivation of latent infection (39). Furthermore, the prevalence of extrapulmonary TB (an indicator of severe disease) was inversely proportional to CD4 T cell count (40).

Although initially controversial, CD8 T cells play an important role in the immune response to FB. In some murine acute infection studies, mice lacking functional MHC class I had higher bacterial burden compared with wild-type controls (reviewed in Ref. 41). In mice with minimal bacterial loads due to antibiotic treatment, deletion of CD8 T cells resulted in exacerbation of infection (42). CD8 T cells can also produce IFN-γ (though less than CD4 T cells, at least in mice) (43) and TNF, but are best known for their cytotoxic capacity against infected cells. CD8 T cells can secrete perforin that allows pore formation into the cellular membrane of an infected cells and delivery of granule-associated proteins, such as granzymes, resulting in apoptosis. Perforin from CD8 T cells has been shown to play an important protective role during acute infection in mice (44). Human CD8 T cells also produce granulysin, which has direct antimycobacterial activity (45), are cytolytic, and produce IFN-γ (46), but their role in human TB remains unclear. In rhesus macaques, CD8 depletion resulted in impaired Bacillus Calmette-Guérin–induced immune response during acute M. tuberculosis infection (47), suggesting that CD8 T cells play an important role in the protective response.

Macrophage activation. Macrophage activation is the key to mycobacterial killing. IFN-γ (primarily from T cells) seems to be an essential cytokine for macrophage activation. In mice, the generation of reactive nitrogen intermediates by NO synthase 2 (NOS2) is important during early and chronic infection (48–50), and macrophage activation is synonymous with the production of NOS2 in this model. Although NOS2 expression and function has been observed in human samples (51, 52), its role in human TB warrants further investigation. In humans, alterations in the NOS2A gene have been associated with increased susceptibility to TB (28).

Cytokines. Cytokines play a critical role during primary and latent infection. IL-12 is important in the Th1 response, and mice deficient in IL-12 had poor survival and increased bacterial burden compared with controls (53). In humans, genetic defects in the IL-12/IL-23/IFN-γ axis are associated with severe disseminated mycobacterial disease (54). Murine studies have shown that IFN-γ produced by T cells is critical for early protection and essential for inducing NOS2 (reviewed in Refs. 37, 55). Humans also produce IFN-γ in response to mycobacterial Ags, which is the basis for the diagnostic IGRA. In humans, IFN-γ also induces autophagy (56) as a mechanism of reducing mycobacterial burden. Genetic defects in IFN-γR
active TB cases have been associated with greater production of IFN-γ or ELISPOTs) has been used to detect differences between severity of disease, and presence of coinfections or other comorbidities. In TB, the time and frequency of exposure, strain, inoculum, and other factors can affect the outcome of infection. Human studies, arguably the most relevant, can be challenging. The recent emphasis in multifunctional T cells (producing multiple cytokines) has led to interesting findings in memory T cell responses. Using tetramers, Caccamo et al. (88) observed that latently infected patients had a much higher proportion of Ag-specific CD8 T cells producing both IFN-γ and IL-2 compared with active TB cases. Latent infection was also associated with CD8 T cells with a terminally differentiated phenotype compared with active TB cases with predominantly central and effector memory T cell phenotypes (88). These studies were consistent with murine studies suggesting that vaccine-induced protection occurred via multifunctional T cells (89). However, Sutherland et al. (90) found higher frequencies of M. tuberculosis-specific CD4 T cells producing two or more of the cytokines TNF, IL-2, and IFN-γ among TB cases compared with contacts. Children with TB disease had a higher frequency of specific effector memory CD4 T cells expressing IFN-γ, TNF, and/or IL-2 in response to M. tuberculosis compared with latent infection (91). These last two studies contradict the notion that multifunctional T cells are protective, but may instead increase as bacterial load increases. Recently, IL-17–producing cells have been shown to mediate vaccine-induced protection.
in mice (92). Other murine studies have suggested that IL-17 is produced by the γδ T cells in the lungs early in infection, suggesting that this cytokine could have different roles at different stages of infection (93). In humans, an increased frequency of IL-17+ T cells was associated with latent infection (90). In another human study, more severe disease was associated with reduced IL-17+ T cells (94). Thus, the relative contribution of multifunctional T cells as well as Th17 cells still remains to be determined in human *M. tuberculosis* infection and even in animal models.

In short, limited data prevents us from accurately interpreting which immunologic responses are protective or contribute to the establishment of latency in humans. Presumably proinflammatory Th1 cytokines, such as IFN-γ and TNF, would promote bacterial killing necessary to establish latent infection, yet these are seen more in active disease patients, probably due to increased antigenic stimulation from higher bacterial loads. Likewise, multifunctional cytokine-producing T cells, shown to be protective in other infectious models, have not been clearly established as a surrogate marker for protection, yet human vaccine studies are actively using this as a primary endpoint. Anti-inflammatory factors are presumably necessary to limit pathology and inflammation during initial infection, active disease, or even during latency. The anti-inflammatory factors may include regulatory T cells, IL-10, TGF-β, alternatively activated macrophages, and even Th2 T cell responses. The reports of immune reconstitution disease (immune reconstitution inflammatory syndrome, IRIS) in HIV+ patients following initiation of antiretroviral therapy leading to unmasking of subclinical *M. tuberculosis* infection or even reactivation of latent infection (95, 96) suggests that increased inflammation during the subclinical or latent phase is not beneficial to control of infection. The available data support that a complex balance between inflammation and anti-inflammatory factors is crucial to optimal control of all phases of *M. tuberculosis* infection (Fig. 1). This balance likely varies during the course of infection and disease, from individual to individual, and even within individual granulomas in an infected lung. The complexity of the host immune response interactions makes it difficult to assign a positive or negative role to each response, as the real answer likely lies in the context of the response, and more specifically, in the context of each individual granuloma, in which the balance of pro- and anti-inflammatory responses is reached and maintained and where the host-pathogen standoff takes place.

The bacillus. There has been a major increase in our understanding of the virulence factors associated with *M. tuberculosis*, and many of these virulence factors appear to modulate host responses. This is an important area of study and will be enlightening with respect to host-pathogen interactions and outcome of infection. For the purposes of this review, we focus on the broader idea that the infecting strain can play an important role in the outcome of infection or severity of disease. For many years, it was believed that *M. tuberculosis* was relatively stable in its genomic composition. However, recent evidence demonstrates that the genome of *M. tuberculosis* has much more plasticity than previously appreciated and that there are major differences among strains and isolates that may contribute to virulence and outcome of infection. There are also data that certain clades of *M. tuberculosis* are associated with populations from specific geographic regions and appear to have coevolved with those populations (97). The Beijing strain has emerged in the past decade as a major cause of TB and accounts for ∼50% of strains from east Asia. There are data that Beijing strains are more drug resistant, and some, but not all, studies have demonstrated that more severe disease is associated with Beijing strain infections (reviewed in Ref. 98). In guinea pigs, some Beijing strains were found to be more virulent than non-Beijing strains, but this was not universally true (99).

A small study in the Gambia indicated that contacts infected with *Mycobacterium africanum*, a member of the *M. tuberculosis* complex, were less likely to progress to active TB (i.e., more likely to develop latency) than those infected with *M. tuberculosis* strains, including the Beijing strain (100). In one study, strains of a Euro-American lineage were more likely to cause pulmonary disease rather than meningeal TB compared with other strains (101). In this same study, Beijing strains were associated with individuals with polymorphisms in the TLR2 gene, which was previously shown to be involved in susceptibility to TB (102). Thus, the infecting strain has a role in influencing infection outcome, with the final outcome likely dependent on contributions from both host and bacillus.

**Latent infection: the spectrum.** The classic teaching of TB is that it exists strictly as active disease or latent infection without overlap. There is now growing evidence that, like active TB (which can manifest on a continuum of severity), there is likely a spectrum of latent infection. A simple review of IGRA results shows that although the overall mean of IFN-γ production is greater in active disease than among latent patients, there is a tremendous degree of variability within the latent as well as active groups, impairing the predictive value of the assay itself (72). Barry et al. (11) recently published a review suggesting that the paradigm of latent infection is changing.

The diagnosis of latent infection is made on the basis of a positive TST or blood test (IGRA) without symptoms and signs (x-ray) of disease, with no further workup required,
which limits the ability to detect subclinical disease. In a study of 601 cases of culture-positive TB cases, 9% had normal chest x-rays, and many were asymptomatic. Five percent of these subclinical cases were non-HIV infected, and 22% were HIV infected, a known risk factor for atypical chest x-ray findings (40, 103). These data suggest that subclinical disease occurs, but at a relatively low prevalence. However, subclinical disease (sputum positive despite normal chest x-ray and lack of signs or symptoms of disease with positive TST or blood test) is dramatically higher in HIV+ patients who live in a high TB endemic area (10, 104, 105). This has resulted in sputum as a standard screening practice for TB in areas with high endemic rates of both HIV and TB (38). A thorough and systematic investigation of subclinical rates of TB among immune competent individuals has not been performed. Recent advances in medical imaging have anecdotally noted metabolically active lesions in humans with latent infection, suggesting that latency can be a dynamic process (106, 107). In the nonhuman primate model, ~5% of infected monkeys develop subclinical infection (34), and preliminary data suggests that they may be more susceptible to reactivation disease (P.L. Lin and J.L. Flynn, unpublished observation).

It stands to reason that where a person lies on the spectrum of latent infection predicts their relative risk of reactivation (Fig. 1). For instance, some humans with unrecognized subclinical TB would otherwise be called latently infected and likely have a high rate of reactivation, whereas patients without subclinical disease are likely to have a lower risk. Conversely, some humans are unlikely to ever reactivate despite immune suppression; this may be due to actual clearance of the infection or to bacilli existing in a truly dormant state, and distinguishing these states is impossible in humans given current technologies. Biomarkers that can discriminate the position on the spectrum of latency are urgently needed to identify those infected individuals for whom immune interventions (such as anti-TNF therapy) may be most risky and those for whom antibiotic therapy is most beneficial.

Risk factors for reactivation

The current treatment for latent infection consists of 9 mo of isoniazid (108), a drug for which the efficacy is dependent on mycolic acid synthesis during active replication. It has been shown that M. tuberculosis can persist for decades within a human host (109–111), and therefore it is logical to think of latent infection as a dynamic process of bacterial persistence and immunologic control. Based on epidemiologic studies, known risk factors for reactivation of latent infection include: HIV, malnutrition, tobacco smoke, indoor air pollution, alcoholism, silicosis, insulin dependent diabetes, renal failure, malignancy, and immune suppressive treatment, such as glucocorticoids (112–114).

HIV infection and treatment with TNF inhibitors are the most well-described risk factors for reactivation. TNF inhibitors were first introduced more than a decade ago for treatment of inflammatory diseases. An increased incidence of TB (presumed to be reactivation of latent infection) was noted among patients on TNF inhibitors (reviewed in Refs. 64, 115). Reactivation could also be induced by TNF neutralization in nonhuman primates with true latent infection; unlike the murine model, granuloma structure was completely normal, as confirmed in the human literature (62). Studies in humans treated with TNF inhibitors showed that cells in blood had decreased T cell activation, IFN-γ production and proliferation, and decreased CD8 memory T cells with reduced granulysin, though these data did not correlate to reactivation cases (116–118). However, in monkeys, we found appropriate levels of IFN-γ within mediastinal lymph nodes, suggesting that immunologic factors in the blood do not necessarily correlate with regional disease. TNF neutralization altered chemokine receptor expression, impaired cellular recruitment (i.e., T cells) to disease sites, and resulted in a disproportionate degree of extrapulmonary disease (62). More importantly, although a high rate of reactivation (~65%) was noted in latently infected monkeys, not all monkeys reactivated following short-term anti-TNF treatment (62), indicating that TNF is an important but not always a critical factor in maintaining latent infection and that the spectrum of latent infection likely plays an important role on the overall risk of reactivation.

HIV remains the most common risk of reactivation TB. The immune suppression by HIV has been an overwhelming factor in the resurgence of TB as a global health threat. The risk of reactivation among HIV patients is almost 10-fold higher than non-HIV patients (113). Prior to the HIV epidemic, 85% of cases of TB were limited to only pulmonary involvement (119). In contrast, a disproportionately high rate of disseminated, extrapulmonary disease was observed in advanced HIV-infected patients with TB (5). The greatest risk of TB was associated with CD4 T cell counts <200 cells/ml regardless of antiretroviral therapy (38, 95), suggesting that CD4 T cells are critical to maintaining latent infection. Even under optimal conditions when CD4 T cell counts improve, the risk of TB is still increased (120, 121), indicating that HIV infection induces additional immune deficiencies independent of CD4 count. Latently infected monkeys were infected with SIV, and all monkeys develop reactivation TB early or late after SIV infection (39). Early reactivation was associated with more severe T cell depletion soon after SIV infection with poor recovery of T cells thereafter, which agrees with human data. The immunologic interactions reported between HIV and M. tuberculosis from various studies include: HIV-induced loss of mycobacterial specific CD4 T cells, M. tuberculosis-induced increases in HIV load in serum and macrophages, shift from Th1 to Th2 response via alterations in IL-10, regulatory T cells, IL-12, IL-4, and TNF, loss of granuloma integrity, and alterations in apoptotic mechanisms (reviewed in Ref. 122).

In summary, the immunology of TB is complex, and understanding latent M. tuberculosis infection is even more challenging due to the difficulties in obtaining human data from the site of infection. Only recently have animal models (nonhuman primates) that adequately reflect human latent infection been described. The more sophisticated analysis of human samples, in conjunction with targeted studies in the nonhuman primate model of latent infection, should provide more answers in the near future regarding the various manifestations and consequences of latent TB. This will lead to improved therapy and perhaps even preventive strategies to reduce reactivation.

Acknowledgments

We thank the intellectual and scientific contributions of the members of the Gates Grand Challenge 11 program, especially Douglas Young, Clifton Barry,
III, and Robert Wilkinson, in developing the idea of the spectrum of latency. We also thank the members of the Flynn and Lin laboratories for helpful discussions and scientific data.

Disclosures

The authors have no financial conflicts of interest.

References


