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Introduction

Mycobacterium tuberculosis is a remarkably successful human pathogen, infecting nearly 1/3 of the world's population, and causing an estimated 9 million new cases and 1.4 million deaths each year (1). Our understanding of the immunologic factors contributing to this success is incomplete. Most infected individuals acquire partially protective pathogen-specific immune responses that permit them to remain disease-free for a lifetime. However, in some individuals, disease due to reactivation will occur, often without apparent predisposing defects in immune function. Indeed, it is precisely in these cases with unimpaired cellular immune responses that lung destruction can be greatest, TB transmission most problematic, and relapse-free cure most difficult to achieve. This chapter will examine aspects of the human immune response to *M. tuberculosis* as they relate to TB pathogenesis, protection, and treatment.

Early responses to mycobacterial infection

Cellular immune responses to a wide range of intracellular pathogens including *M. tuberculosis* progress in two stages. Initial innate responses involve the direct production of cytokines, including tumor necrosis factor (TNF), interleukin 12 (IL-12) and others, by infected macrophages activated via toll-like and other pattern receptors (2-4). These signals are then amplified by adaptive responses of pathogen-specific T lymphocytes recruited to the site of infection. Interferon (IFN) γ produced by these cells serves both to augment the production of inflammatory mediators, and to activate intracellular antibacterial mechanisms by activation of Janus kinases (JAK) and subsequent phosphorylation of signal transducer and activator of transcription (STAT, particularly STAT-1) (5,6). The absolute requirement for IFNγ signaling in this process is evident in persons with hereditary lack of the IFN receptor (7,8) or with acquired high titer neutralizing IFNγ antibodies (9). Both defects cause disseminated infections due to mycobacteria (*Mtb* and NTM), Cryptococcus, Histoplasma, Salmonella, Burkholderia, and Penicillium. This spectrum of disease overlaps with that due HIV-1, in which it is due mainly to depletion of antigen-specific IFNγ-producing CD4 T lymphocytes (10).

Early T cell responses to *M. tuberculosis* infection in the human lung have been studied by Silver *et al* following segmental bronchoscopic challenge with PPD (11-13). In this model, *Mtb*-infected healthy individuals show enhanced production of IFNγ by lung-resident memory T cells. These cells normally

comprise approximately 10% of the mononuclear cells obtained by bronchoalveolar lavage, but quickly double in number in response to antigenic challenge. Responding cells also produce the IFNγ-inducible CXCR3 ligands interferon induced protein 10 (IP10, also known as CXCL10) and monokine induced by interferon γ (MIG). These chemokines facilitate the further recruitment of CD4+ T cells to the site of antigen challenge. CC chemokines do not appear to be involved in this process.

Several *in vitro* models have been used to further examine the antimycobacterial activity of these early cell interactions (14-16). The models have consisted of either whole blood or mononuclear cells to which *M. tuberculosis* or *M. bovis* BCG is added. After a period of culture, the extent of mycobacterial growth or death is measured by one of several methods (CFU counting, reporter phages, tritiated uracil incorporation, or time to detection in automated liquid culture). Findings in these models have been consistent with clinical observations regarding mycobacterial immunity. Superior restriction of intracellular mycobacterial growth has generally been observed in cultures of *Mtb*-infected or BCG-vaccinated healthy persons (14,17). Impaired responses are found in cultures in HIV-infected persons; these improve with anti-retroviral therapy (18). IFNγ and TNF are required in these models as they are *in vivo*, as the addition of neutralizing antibodies interferes with inhibition of growth. However, addition of exogenous cytokine has no clear effect on growth inhibition, which instead appears to require direct cell-to-cell contact without involvement of a recognized pair of ligands. Furthermore, the extent of growth inhibition in these models appears unrelated to either the number of IFNγ-producing cells or the amount of IFNγ produced (18,19). These observations are nonetheless consistent with longitudinal studies of close contacts of TB cases, in which the diagnosis of active tuberculosis is often preceded by striking increases in the proportions of *Mtb*-reactive IFNγ-producing CD4 T cells (20-22). This has dampened enthusiasm for IFNγ as an immune correlate of protection from TB, and focused attention instead on the potential role of growth inhibition assays as an alternative means for early evaluation of new TB vaccines (23) *NB: citation is a placeholder*.

Granuloma formation and mycobacterial persistence

As a group, these *in vitro* models have consistently revealed that the early macrophage-T cell interactions result in inhibition of mycobacterial growth, but they also have revealed the apparent lack of expression of true bactericidal activity. The persistence of viable *M. tuberculosis* despite the early cellular immune response is likely the key factor driving the formation of the dense, fibrous,

multilayered structures that are characteristic of human TB granulomas (figure 1). Mature TB granulomas contain a core of acidic caseous material lacking oxygen and nutrients, incapable of supporting mycobacterial replication (24). Mycobacterial survival under these harsh circumstances is facilitated by a dormancy response that limits bacterial cell wall synthesis, cell division, and dependence on aerobic respiration (25,26). The extent to which the granulomatous host response is ultimately successful in eradicating latent *Mtb* infection is not known. One study of 22 adults with LTBI re-examined 19 years after an initial positive tuberculin skin test (TST) found that most had maintained reactivity despite a lack of recognized ongoing exogenous re-exposure (27). Indeed, only 1 of the 22 subjects showed evidence of boosted responses on repeated testing, a phenomenon thought to reflect re-expansion of otherwise waning T cell recall responses due to exogenous antigen. The remarkable longevity of these TST responses supports the concept of continued endogenous antigenic re-exposure due to mycobacterial persistence in persons with LTBI.

However, to be of clinical consequence, this persistence must be accompanied by the capacity to reactivate. The most relevant data in this regard come from experience with anti-TNF monoclonal antibodies (mAbs) garnered prior to widespread implementation of LTBI testing and treatment. It is now recognized that the TNF mAbs constitute a strong risk for reactivation of LTBI, which, if untreated, produces LTBI reactivation rates many times that in the general population (28). Most of that risk is concentrated in the first few months of anti-TNF treatment (29), suggesting a model in which the subsequent decline of TB cases is due to depletion of “reactivatable” LTBI. A formal analysis of these data using hidden Markov modeling indicated a strikingly low proportion of susceptible individuals (0.015%) and a very high monthly rate of reactivation (20%) (30). The proportion of susceptible individuals could increase, of course, should immunosuppressives with greater potential for LTBI reactivation be developed in the future. In any case, these findings support the concept that over time, the human immune response can indeed eradicate many instances of latent *Mtb* infection, but that the process is at best a slow one.

Reducing the risk of reactivation

The greatest body of experience in the detection of latent TB infection prior to treatment with biologic (anti-cytokine) therapies exists for the TST, which unfortunately neither distinguishes persistent *M. tuberculosis* infection from immunologic memory of resolved prior infection, nor from sensitization due

to non-tuberculous mycobacteria or BCG. However, studies in TB-endemic regions indicate that in the absence of exposure to an index TB case within a household, TST responses in BCG-vaccinated decline in a majority of infants to <5mm within the first year of life (31). Large reactions in adults from TB endemic regions are unlikely to be due to childhood BCG vaccination, but instead are more likely to reflect true *M. tuberculosis* infection (32,33).

Among all current treatments for chronic inflammatory conditions such as RA, anti-TNF mAbs pose the greatest TB reactivation risks, which are 2 to 14-fold those of soluble TNF receptor, and are accompanied by greater risk of extrapulmonary and/or disseminated disease (29,34,35). The reduced ability of soluble TNF receptor to reactivate LTBI is consistent with its lack of efficacy in chronic granulomatous inflammatory conditions (36-38). Corticosteroids pose a dose-dependent TB risk, with those of daily doses ≥15mg comparable to TNF mAbs (adjusted OR=7.7, 95% CI 2.8 to 21.4) (35,39). The TB risks posed by other, newer therapies are less well understood. A recent post-marketing survey of tocilizumab (anti-IL-6 antibody) in Japan reported 4 TB cases among 3881 treated patients, a rate of 220 cases per 100,000 patient-years (40). Like TNF mAbs, TB cases appeared early during treatment, consistent with reactivation. In contrast, a prospective study of abatacept (which blocks the interaction of monocyte CD80/86 with CTLA-4 on lymphocytes, preventing activation) found no TB cases, *vs*. 2 in infliximab-treated controls (41). Unlike anti-TNF mAbs, abatacept has no effect on bacillary number or animal survival in a murine model of chronic *Mtb* infection (42). Tofacitinib, a Janus kinase (JAK) inhibitor, has been reported to cause dose-dependent increases in bacillary numbers in a chronic murine *Mtb* infection model (43). However, an analysis of TB incidence in global tofacitinib phase 2, 3 and extension trials in RA found that TB case rates in treated patients were reduced in relation to those in the general population, and that incident cases occurred late during treatment, consistent with progression of new infection rather than reactivation (44). These findings may indicate that the TB risk of tofacitinib is low and/or that cases have been preventive by an effective LTBI screening and treatment program.

TST responses are reduced in rheumatoid arthritis (RA), both due to underlying disease activity and to a lesser extent to its treatment (45-47). TST sensitivity may be increased by reducing the threshold for positivity (5mm) and by repeated testing (boosting), 7-10 days after a negative test. Both strategies increase test sensitivity at the cost of reduced specificity (48,49). Sensitivity can be further improved by chest radiography to identify regional scarring and hilar lymphadenopathy. LTBI screening strategies

should in practice attempt to balance the risks of over-diagnosis (unnecessary treatment and its associated toxicities) with those of under-diagnosis (disseminated, rapidly progressive TB). In this context RA patients treated with anti-TNF mAbs represent a “worst case” scenario in which it may be appropriate to accept reduced specificity in return for enhanced sensitivity.

The most comprehensive data regarding management of infection risks of biologic therapies come from European country-wide case registries. In Spain, the implementation of boosted TST with a 5mm threshold coupled with 9 months of isoniazid treatment reduced TB incidence due to the TNF mAb infliximab by 74%, with no TB cases occurring in INH-treated patients (50). INH was well tolerated: elevated transaminases occurred in only 7/324 patients, with no hospitalizations, liver failures, or deaths. Treatment of these 324 individuals appeared to prevent approximately 7 TB cases. The corresponding French experience using a single (unboosted) 5mm TST also found no TB cases in INH-treated patients (51). However, in contrast, 30 of 45 TB cases in persons with known TST results occurred in those with reactions <5mm. The inadequate sensitivity of a single TST, even with a 5mm threshold, was subsequently confirmed in follow-up data from Spain, in which TB cases arose only from patients in whom the second TST was omitted or ignored (52).

These experiences argue strongly against the sole use of single TST screening prior starting anti-TNF treatment, even with a 5mm threshold. They also provide interesting insights into INH treatment of LTBI. Large preventive therapy studies in the 1970s and 80s established efficacy rates of 65% and 75% for isoniazid treatment durations of 24 and 52 weeks, respectively; in contrast, a 12 week regimen was only 21% effective (53,54). The Spanish experience, in which 9 months of treatment with INH was started 1 month prior to starting anti-TNF treatment, indicates that concomitant TNF blockade does not interfere with treatment of latent TB infection. It also indicates that TB prevention does not require the eradication of latent infection prior to starting TNF blockade, but merely that preventive treatment is in place. Current recommendations for 1 month of INH prior to starting anti-TNF therapy are therefore useful mainly to ensure adequate INH tolerability.

The specificity of testing for latent TB infection can be improved by the use of the antigens ESAT-6, CFP-10, and TB7.7, which are encoded by genes absent from all BCG strains (55). T cell responses to these antigens are detected by *in vitro* assays by the release of IFNγ, either by ELISA (Quantiferon TB Gold In-tube [QFT-GIT], Cellestis) or by ELISPOT (T spot-TB, Oxford Immunotech). Some studies have reported

reduced sensitivity of testing with ESAT-6 and CFP-10 compared to TST although others have not (33,56). In the largest reported global study of the use of TST and QFT-GIT prior to anti-TNF mAb treatment (golimumab) involving 2282 patients, single (unboosted) TST and QFT-GIT were concordant in only a minority of those with either test positive (κ =.22) (57). Five TB cases, diagnosed from 3 to 11 months after starting anti-TNF treatment (median, 7 months), occurred among 2210 patients treated for 1 year (58). Of these, 2 had positive unboosted TST results at screening (range 0 to 15mm, median 3mm); all had negative QFT-GIT results. Further studies are warranted to determine if a lower threshold for QFT-GIT positivity (*e.g*., 0.1 rather than the current 0.35) might be more appropriate for testing in this high-risk patient population.

Enhancing the effects of concurrent TB chemotherapy

The goals of adjuvant immunotherapy in TB include enhanced bacillary clearance and reduced lung damage. The largest published randomized controlled trial of adjunctive IFNγ in TB compared the effects of standard therapy plus IFNγ 200 μg given thrice weekly for 4 months by aerosol or by subcutaneous injection *vs*. standard therapy alone in a total of 89 patients with drug-sensitive pulmonary TB (59). There was no effect of either adjunctive treatment on sputum culture conversion or high resolution chest computed tomography. A study conducted by Intermune compared aerosolized IFNγ 500 μg thrice weekly *vs*. placebo for 6 months in a total of 80 patients with MDR-TB, all of whom also received standardized therapy with second-line drugs (60). The study was halted prematurely by its external monitoring board due to a trend toward increased mortality in the experimental arm (10 deaths, *vs*. 5 in controls, P=0.14), with no effect on sputum culture or chest radiography. The study findings have never been published. Several other studies with fewer than 10 subjects per arm in which adjunctive IFNγ or IFNα was given by aerosol or subcutaneous injection have reported inconsistent results (61-65). These clinical findings mirror the lack of consistent effect of added IFNγ on intracellular *M. tuberculosis* using *in vitro* models. One additional study examined the effects of interleukin 2 (IL-2), a cytokine that promotes T cell proliferation and differentiation. Adjunctive IL-2 (225,000 IU) given subcutaneously twice daily for 1 month *vs*. placebo in 110 patients with drug sensitive TB tended to delay rather than enhance sputum culture conversion (66).

These findings stand in contrast to those of a study of high dose methylprednisolone, a corticosteroid with broadly immunosuppressive properties. This trial examined the effects of adjunctive

methylprednisolone 2.75 mg/kg/d for one month *vs*. placebo in 189 subjects with drug-sensitive TB (67). The methylprednisolone dose, which was selected as that required to reduce TNF production at least by half, was tapered to zero during the subsequent month, with subjects receiving on average a cumulative dose of 6500 mg, substantially greater than in any prior TB trials. Fifty percent of prednisolone-treated subjects converted to sputum culture negative after 1 month *vs*. 10% in the placebo arm (*P*=0.001). The magnitude of this effect is greater than has been reported in any other studies of adjunctive TB immunotherapy. No serious opportunistic infections occurred. However, other early serious adverse events, consisting of expected gluco- and mineralocorticoid toxicities (hypertension, edema, hyperglycemia, and one death due to hypertensive crisis) occurred significantly more often in the prednisolone arm. Two other prospective randomized trials of adjunctive corticosteroids given at lower doses have observed similar, albeit smaller, salutary effects on sputum culture, chest radiography and symptoms (68,69), as has one small trial of adjunctive etanercept (soluble TNF receptor) 25 mg twice weekly for month (70). These findings support the concept that adjunctive treatments to dampen the inflammatory response during early TB treatment may improve outcomes, possibly by facilitating drug entry into lung lesions and by enhancing the susceptibility of sequestered mycobacteria to the bactericidal activity of these drugs by restoring aerobic patterns of mycobacterial respiration, metabolism, biosynthesis and growth (71).

Reducing the risk of TB recurrence

TB patients who appear cured at end of treatment (EOT) are face 2 distinct risks for recurrent disease: relapse and reinfection. Relapses occur due to mycobacteria that persist despite treatment. The risk of relapse increases as treatment is shortened (72) and decreases with time since EOT (73). In contrast, the risk of recurrence due to reinfection increases with local TB prevalence (74) and is unaffected by time since EOT (75,76). Studies using molecular typing methods to distinguish these 2 mechanisms have revealed that immune impairment due to HIV-1 significantly increases the risk of disease due to reinfection but has little or no impact on relapse (75,76). The largest study of recurrent TB to date, conducted in South African gold miners, examined rates of initial and recurrent TB in 663 men classified according to HIV status (77). Recurrences >2 years after initial episodes were examined separately, as these are substantially more likely due to reinfection. In this heavily *Mtb*-exposed population, the risk of recurrent TB due to reinfection posed by prior TB was equal to, and additive with, that of HIV-1 infection (table 1).

The immunologic basis of this TB susceptibility is not understood, and may be heterogenous and/or multi-factorial. Nonetheless, individuals with prior TB represent an important and attractive target for novel TB vaccines, which may be expected to protect against both reactivation of persistent infection (relapse) and progression of new infection to disease (reinfection). Vaccination conceivably could serve as the last component of a 3 phase strategy of combined TB immuno/chemotherapy program (table 2), to contain persisting dormant mycobacteria and enhance protection against new infection without exacerbating immunopathology or interfering with chemotherapy.

Preventing immune reconstitution phenomena

Paradoxical immune-mediated TB exacerbation is recognized to occur in several settings. It was first described in anergic TB patients with far advanced pulmonary disease whose chest radiographs unexpectedly worsened early during treatment as their skin tests returned to positive (78). Similar cases were subsequently reported in which CNS tuberculomas appeared and progressed despite initiation of appropriate treatment (79). Miliary and early meningeal disease were often present in these subjects at diagnosis. These exacerbations were attributed to treatment-related reversal of disease-associated immunosuppression, but the underlying mechanisms were not well understood.

Since then, paradoxical worsening has been described in new settings in which the factors responsible for immune reconstitution are more apparent. Withdrawal of anti-TNF therapy is one such instance. In the absence of data from either prospective studies or retrospective case reports, most recommendations call for cessation of anti-TNF therapy in patients who develop TB. This strategy places patients at risk of paradoxical responses due to re-expression of TNF-driven inflammation (80-84). Its manifestations can include worsened fever, infiltrates, hypoxia, and lymphadenopathy, and the appearance of new lesions in previously uninvolved organs, despite microbiologic evidence for an appropriate response to treatment.

Similar paradoxical exacerbations, termed IRIS (immune reconstitution inflammatory syndrome) are also now recognized in patients with AIDS and TB beginning treatment for both infections. IRIS risk is greatest in patients with newly diagnosed TB and advanced AIDS in whom both treatments are started within the first few weeks of diagnosis. A second scenario (termed unmasking IRIS or ART-TB) has also

been described, in which the diagnosis of TB only becomes apparent after HIV therapy has been initiated. Some studies have linked immunopathogenesis of IRIS to rapid expansion of Th1 (IFNγ-producing) T cells (85,86), but others have not (87-89). Recent studies have also implicated heightened CXC chemokine responses in IRIS pathogenesis (90,91).

Management of paradoxical responses varies greatly. Many TB-IRIS cases are mild and resolve spontaneously without any specific treatment. More symptomatic cases appear to benefit from treatment with corticosteroids (92). The most problematic cases are those involving the CNS as tuberculomas or meningitis. Uncontrolled case reports indicate favorable therapeutic responses to anti-TNF mAbs in patients with life-threatening paradoxical responses that had not responded adequately to corticosteroids (93,94). Favorable responses to the immunomodulator thalidomide have also been reported (95); however, a randomized placebo-controlled of adjunctive thalidomide in pediatric TB meningitis was halted prematurely due to excess mortality in the thalidomide arm (96). Prevention of IRIS by CCR5 inhibition has also been proposed, based the role of CCR5 in tissue homing of effector T cells (97,98). However, a recent study to test this hypothesis found that the addition of maraviroc to standard therapy in high-risk patients had no effect on TB-IRIS incidence (99).

Summary

Aspects of the cellular host response contribute to both protection and immunopathology in tuberculosis. Optimal TB treatment may include adjunctive immune suppression during its initial phase to accelerate bacillary clearance and limit lung damage, and immune enhancement during its final phase to prevent recurrence. Chemokine inhibition may also help to prevent host-mediated tissue damage in AIDS patients with TB. TB treatment may ultimately be shortened and improved by strategies that target both the host and microbe.

Figures

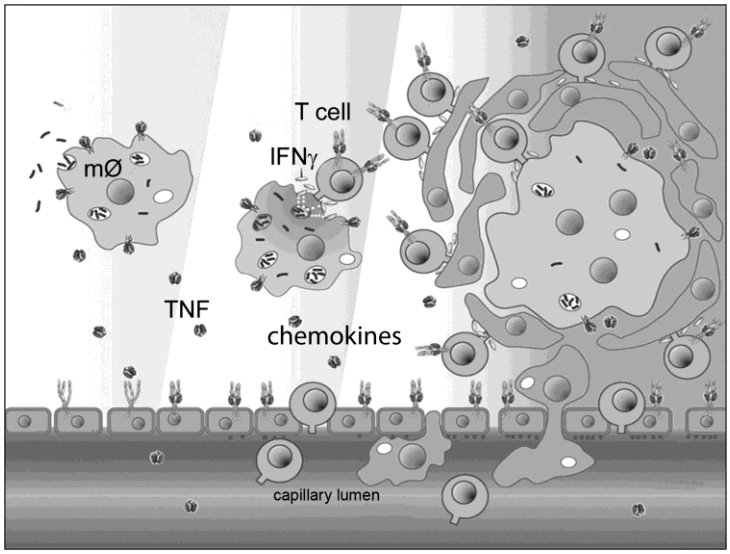

Figure 1. Granuloma formation in the lung. The central region of multinucleated giant cells, mycobacteria, and necrotic debris (right) is surrounded by concentric rings of tightly apposed epithelioid cells and lymphocytes, with smaller numbers of neutrophils, plasma cells, and fibroblasts.

Tables

	HIV+	HIV–
	Rate (95% CI)	
Recurrence >2 years after initial episode:	24.4 (17.2–34.8)	4.3 (2.2–8.3)
Recurrence (all):	19.7 (16.4–23.7)	7.7 (6.1–9.8)
Initial episode:	3.7 (3.3–4.1)	0.75 (0.67–0.84)

Table 1. Rates per 100 person years at risk (PYAR) and 95% confidence intervals (CI) of initial and recurrent episodes of pulmonary TB in South African gold miners classified according to HIV-1 status. Recurrence rates were determined from cohorts of 342 HIV-infected and 321 HIV-uninfected subjects. Recurrent episodes >2 years after initial episodes are mainly due to reinfection. These data indicate persons with a prior TB episode, regardless of HIV status, are highly susceptible to recurrence due to reinfection. From reference (77).

	1: Immune suppression	2: Transition	3: Immune restoration
Immunotherapy:	Anti-TNF mAb	—	Vaccine
Chemotherapy:	Intensive phase	Sterilization phase	—
Goals:	Reduce inflammation, facilitate drug entry, enhance drug action, prevent resistance, accelerate cure	Ensure resolution of inflammation and eradication of infection	Prevent recurrence

Table 2. Three phases of proposed combined TB immuno/chemotherapy.

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