Tuberculosis and opportunistic infections: Relevance to biologic agents

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Abstract

With the expanding use of biologic agents, in particular TNF inhibitors, tuberculosis and other opportunistic infections have become an important and growing concern in rheumatology. Clinicians using these therapies should have an understanding of the scope of the problem, the underlying scientific rationale, as well as the optimal approaches to screening, monitoring and treatment.

Introduction

The introduction of biologic agents, particularly inhibitors of the key pro-inflammatory cytokine tumor necrosis factor (TNF), spawned a new therapeutic era in rheumatology. These agents have proven effective not only in controlling the signs and symptoms of rheumatoid arthritis (RA), but also in improving patients’ functional status and attenuating structural damage. Accompanying the excitement regarding their clinical efficacy in RA and other autoimmune diseases has been caution concerning potential safety issues. Because these agents are potent modulators of the immune response, they may adversely impact host immunosurveillance, potentially increasing susceptibility to infection, among other sequelae. Though patients taking inhibitors of TNF are at increased risk for many potential pathogens, there has been particular concern regarding tuberculosis (TB).

TB, an anathema since antiquity, remains a potent pestilence in the 21st century. In recent decades, as its incidence decreased in the developed world, TB was considered a nearly conquered contagion. However, the emergence of factors such as increasing population density and mobility, antibiotic resistance, the HIV pandemic, and the introduction of TNF inhibitors has renewed interest in this pernicious pathogen (1-3).

Tuberculosis

With over 8 million new cases per year and a proclivity for latent infection, TB infects about a third of the world’s population. Untreated, roughly a third of patients with active TB die within a year, and half die within 5 years. Due largely to its association with HIV, TB remains a leading infectious killer, causing 2 million deaths annually. The incidence of TB varies worldwide. In 2003 the incidence of TB in the USA was 5.1 cases per 100,000 population, representing the smallest decline in rate since 1992 (4). It is higher in some European countries, for example, approximately 25/100,000 in Spain. In sub-Saharan African populations, prevalences near 300/100,000 population are seen (1, 2).

The consequences of infection with Mycobacterium tuberculosis depend mainly upon the immunocompetence of the host. Within 2 years of exposure, 5% of persons cannot control the infection and develop symptomatic TB. Among HIV patients, two-thirds present with extra-
pulmonary involvement. In contrast to those who become symptomatic, about 95% of exposed adults are able to control the initial infection, containing the organisms within granulomas (the so-called 'tubercles'). However, although its growth can be inhibited, TB is typically not eradicated. Sometime during their lives, approximately 10% of patients with this latent TB infection (LTBI) experience a reactivation of the previously dormant infection and become symptomatic. In areas of low endemicity, most cases of active TB result from reactivation. Reactivation of LTBI often occurs concomitant with ill health and weakened immune function. Among HIV patients, rates of reactivation may exceed 10% each year (1). As will be discussed, many cases of TB related to the use of TNF inhibitors appear to be reactivation of LTBI. Interestingly, among cases of TB thought to be due reactivation of LTBI, infection may actually be a second primary infection. In a series including both HIV and non-HIV patients, 33% of TB cases in previously exposed patients were shown instead to be infection with a different strain of TB (5). As is the case for primary infections, manifestations of reactivation of LTBI depend on the competencies of the immune system. Pulmonary manifestations are most common, with a predilection for apical and posterior segments, due to their higher oxygen concentration. While any organ can be involved with extrapulmonary TB, the most common sites are lymph nodes, pleura, genitourinary tract, bones and joints, meninges, and peritoneum.

### Immune defense against TB

The initial response to TB infection entails innate immunity, with ingestion of organisms by pulmonary macrophages, mediated in part via recognition of mycobacterial constituents via toll-like receptors (6, 7). The predominant host defense against TB involves cell-mediated immunity, orchestrated by CD4+ T cells. The key processes that come into play are cytokine-mediated activation of macrophages, and cytolytic killing of infected cells. Activated macrophages ingest and kill bacilli utilizing reactive oxygen intermediates and products of inducible nitric oxide synthase (iNOS), such as NO and nitrotyrosine (8, 9). Granulomas, formed by organized activated macrophages along with lymphocytes, effectively contain organisms and prevent their spread. Cytokines serving an important role in granuloma formation and maintenance include TNF-α, IFN-γ, IL-12, IL-15 and LT-α (6, 10, 11). IL-10 plays an inhibitory role, increasing susceptibility to TB (12). Direct T cell cytolysis of infected phagocytes also eliminates bacilli and can deprive TB of an intracellular sanctuary, particularly when macrophage activating responses are suboptimal. However, excessive cytokine responses may result in the destruction of normal tissue, resulting in cavitation and damage to surrounding parenchyma, vessels, and airways. Indeed the host response to both acute as well as latent TB requires constant balanced immune-driven inflammation. Insufficient responses allow spread of infection, while excessive responses can cause tissue destruction. While information concerning the role of mediators including IL-12 and IFN-γ in the host response to TB comes from immunodeficient patients (13), much evidence derives from animal studies. Data from numerous models have established that TNF-α plays an essential role in granuloma formation and in control of TB (Table I) (14-20). Data from these studies indicate that inhibition of TNF by any of a variety of approaches increases the frequency and severity of TB infection. It has been suggested that transmembrane forms of TNF may be particularly important (21). Lymphotoxin-α may also play a role in

### Table I. The role of TNF in defense against TB: animal models.

<table>
<thead>
<tr>
<th>Model</th>
<th>Reference</th>
<th>Findings</th>
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</thead>
<tbody>
<tr>
<td>P55 TNF-R KO mice</td>
<td>(14) J Pathol 1999; 189: 127-37</td>
<td>Newly formed pulmonary granulomas acutely disintegrated, with apoptotic cell death and neutrophil influx; mice succumbed to infection just beyond the stage of granuloma initiation</td>
</tr>
<tr>
<td>Pentoxifylline (TNF inhibitor) treated C57BL/6 mice</td>
<td>(15) Immunology 2001; 102: 248-53</td>
<td>Treated mice had accelerated tissue damage compared to controls.</td>
</tr>
<tr>
<td>TNF-α KO mice injected with virulent M. TB strain or avirulent BCG.</td>
<td>(16) Lab Invest 1999; 79: 379-86</td>
<td>The number of mycobacterial colonies per unit weight of lung and spleen increased in KO mice</td>
</tr>
<tr>
<td>TNF-RI KO mice treated with soluble TNF-RI</td>
<td>(17) J Immunol 1996;157:5022-6</td>
<td>Treated mice developed fewer and smaller granulomas than controls</td>
</tr>
<tr>
<td>Nramp1+/- and Nramp1+ mice treated with anti-TNFα mAb</td>
<td>(18) J Immunol 1999; 162: 6122-31</td>
<td>Nramp1 influences TNF and IL-10 levels; macrophage survival decreased during M.Tb infection</td>
</tr>
<tr>
<td>P55 TNF-R KI mice</td>
<td>(19) Eur J Immunol 1997; 27: 3182-90</td>
<td>Mice overexpressing sTNFR1 had increased sensitivity to M. Tb and inhibition of granuloma formation.</td>
</tr>
<tr>
<td>Mice treated with an anti-TNF mAb</td>
<td>(20) Infect Immun 2001; 69: 1847-55</td>
<td>Treated mice developed fatal reactivation of M. TB</td>
</tr>
</tbody>
</table>

KO: knock out; mAb: monoclonal antibody; BCG: Bacillus-Calmette-Guérin; KI: knock in.
controlling mycobacterial infection (22, 23). In summary, the breadth of animal data defining a key role for TNF in host defense against TB provides biologic plausibility for the potential association between the use of TNF inhibitors and TB in patients. In addition to CD4+ and CD8+ T cells, natural killer (NK) T cells and gamma-delta T cells that utilize non-classical MHC molecules such as CD1 to recognize glycolipid mycobacterial antigens, may also play a role in host defense against TB (6). Other factors may be important in tuberculosis susceptibility. The capacity to respond to mycobacterial antigens is known to be partly inheritable (24). The natural resistance-associated macrophage protein 1 gene (Nramp; now designated SlC11A1) is heritable (24). The prevalence of TB, particularly with infliximab use, appears to exceed that of both the general population and of RA patients. For example, among RA patients treated with infliximab in the USA, the prevalence of TB has been estimated to range from 24.4/100,000 (based on cases reported to the FDA as of May 2001; reference 37) to 52.5/100,000 (based on a survey of RA patients from January 2000 to June 2002; reference 31). In Spain, analysis of data from 1,540 RA patients treated with TNF inhibitors (86% infliximab) revealed 17 cases of tuberculosis among patients treated with infliximab (36). This represented a risk ratio of 11.7 to 19.9 (2001, 2000 respectively) compared with RA patients not receiving such treatment, and a risk ratio of 53 to 90 (2001, 2000 respectively) compared with the overall Spanish population. These data led to regulatory changes in the package inserts for TNF inhibitors, highlighting the potential association and defining the need for consideration of TB and for appropriate screening.

The prevalence of TB among RA patients treated with etanercept approximates that of the general population, and appears to be less than that for infliximab (Table II). This suggests that there may be differences in the risk of TB with these two agents. For adalimumab, the availability of data solely from clinical trials and the fact that its development program was later than that of the general population, appears to be less than that for infliximab (Table II). This suggests that there may be differences in the risk of TB and for appropriate screening. The prevalence of TB among RA patients treated with etanercept approximates that of the general population, and appears to be less than that for infliximab (Table II). This suggests that there may be differences in the risk of TB and for appropriate screening.
of TB in the general population impacts any association with the use of TNF inhibitors.

With infliximab and adalimumab, the majority of cases were noted relatively soon after the initiation of therapy (Table II). This suggests that most cases represent reactivation of LTBI, although some primary infections did occur. With etanercept, there was a more variable time interval preceding cases. Interestingly, although the rate of TB varied among agents, TB cases associated with all agents have been associated with a substantial and similar incidence of extrapulmonary TB. The observed rate of about 50% far exceeds the approximate 18% incidence seen in the non-HIV general population (38). This suggests either a generalizable effect of all TNF inhibitors or a characteristic of altered immune function in this patient population. In one series of 33 patients with various rheumatic conditions who developed TB, none of whom were taking TNF inhibitors, 66% of patients developed extrapulmonary TB (39). However, in another series of TB cases among RA patients from Spain, 5 of 7 (71%) patients had pulmonary disease; 1 had pleural and 1 had gastrointestinal tract involvement (32). The effects of the modulations in immune function inherent in autoimmune diseases and those related to treatments remain to be fully defined.

Although explanations underlying any differential association with TB among the TNF inhibitors have not been identified, there are several potential contributors. Factors unrelated to mechanism, such as differences in the populations of patients receiving each agent (e.g. country of origin, age, concomitant medications, comorbidity) may have contributed. From a mechanistic standpoint there are several distinctions among the TNF inhibitors. Interestingly, while all agents have been shown to have comparable efficacy in controlling the signs and symptoms of RA, they appear to be variable in efficacy in other conditions. For example, infliximab is effective as a treatment for Crohn’s disease, a condition in which etanercept was ineffective. Adalimumab is presently being studied in this condition. Thus, the differences in mechanisms described below may be relevant to some aspects of efficacy as well as toxicity.

While all of the 3 approved macro-molecule TNF inhibitors have volumes of distribution consistent with intravascular dissemination, there are pharmacokinetic differences. Infliximab, which is administered intravenously, has a large peak followed by a steady state serum concentration; the subcutaneously administered agents do not have this initial peak. Both monoclonal antibodies target TNF-α specifically, whereas the soluble receptor construct binds both TNF-α as well as the related cytokine LT-α (previously known as TNF-β). While all agents bind TNF-α with high affinity, the avidity of the antibodies may be higher than that of the soluble receptor, leading to more long-lasting inhibition. Also, the antibodies are very effective at binding both membrane bound and soluble forms of TNF, whereas the soluble receptor binds the soluble form better. (40). Ex vivo studies have suggested differential immunologic effects between infliximab and etanercept.

Assessment of stimulated T cells from ankylosing spondylitis patients showed that infliximab treatment resulted in a significant decrease in cells positive for intracellular TNF-α and IFN-γ, whereas etanercept treatment resulted in a significant increase in these cytokines (41, 42). In vitro, anti-TNF antibodies better utilize effector mechanisms related to Fc receptor interaction, including complement mediated lysis of TNF over-expressing target cells, as compared to soluble receptor constructs. While this does not appear to be a relevant mechanism in vivo, induction of apoptosis in the gut and in T cells and monocytes from patients with Crohn’s disease has been demonstrated with infliximab treatment (43, 44). Interestingly, although cellular infiltration in the rheumatoid synovium is reduced with infliximab treatment, this does not relate to the induction of apoptosis (45). The involvement of any of these differences in mechanisms as regards potential association with TB remains speculative at present.

Approach to RA patients considered for treatment with TNF inhibitors

Screening

A recent systematic analysis defined the benefits of screening for and treating LTBI among various populations (46). For patients with rheumatic diseases, as a result of the reports of TB in patients treated with TNF inhibitors, screening for LTBI prior to therapy and maintenance of a high index of suspicion for TB infection during treatment have become the standard of care. Importantly, there is data from a variety of sources suggesting that this approach has been successful in reducing the incidence of TB among RA patients receiving TNF inhibitors (Fig. 1) (36). The USA Centers for Disease Control and Prevention (CDC) and the American Thoracic Society have recently established guidelines on TB testing and the treatment of LTBI (47). Although much of the data upon which they were based derives from HIV patients, and although there are regional differences in medical practice that may affect various components, these guidelines are germane to patients for whom treatment with a TNF inhibitor is being considered. The “gold standard” in the detection of LTBI remains delayed type hypersensitivity (DTH) testing with purified protein derivative (PPD), a diagnostic test dating from the latter 19th century. In order to exclude false negative responses, many clinicians were trained in the use of additional antigens as ‘positive controls’ for DTH testing. However, because of difficulties in the standardization of the other antigens, potential variations in DTH responses over time, and evidence that such testing lacks utility as far as increasing the reliability of a negative PPD or providing a rationale for treatment, anergy testing is currently not recommended (48). Of note, it has been demonstrated that cutaneous anergy is more common among RA patients than among controls (37% compared to 0%), and that anergy may correlate with some measures of disease severity (49). A recent study from an endemic TB region demonstrated far less PPD reactivity among RA patients (20%) than among controls (70%), unrelated to treatment or dis-
ease activity (50). Thus, the possibility that PPD testing may be falsely negative should be borne in mind in RA patients.

One means by which false negative PPD tests may be minimized is ‘booster’ or ‘two-step’ testing (51). Repeated testing 1 to 5 weeks after a negative PPD may evoke waned recall DTH responses, and has been recommended in some settings. However, booster testing may lead to a larger number of false positive tests, particularly for persons previously vaccinated with Bacille Calmette-Guerin (BCG) in an attempt to prevent TB infection (52). Booster PPD testing has not become a standard for patients being considered for TNF inhibitor therapy.

Criteria for what constitutes a positive PPD response are somewhat different in the most recent CDC guidelines from previous practice. Patients are stratified according to their risk for TB based upon comorbid conditions (47, 53). RA patients being considered for TNF inhibitor therapy would probably be most appropriately classified in the high risk group, which includes “immunosuppressed patients receiving the equivalent of ≥15 mg of prednisone per day for ≥1 month” in addition to HIV patients, close contacts of active TB cases, and those with CXR evidence of past disease. For such high risk patients, induration of ≥5 mm is considered a positive reaction.

Interestingly, as the use of TNF inhibitors expands into other indications, the optimal definition of a positive test may have to be revisited. For example, patients with psoriasis or ankylosing spondylitis who are candidates for TNF inhibitor treatment may be younger, healthier, and on fewer concomitant medications than RA patients. Lacking data to the contrary, it would seem prudent to still consider these patients as ‘high risk’ in terms of PPD interpretation. For patients with lesser risk ≥10 mm induration is positive, while for “normal” individuals it is ≥15 mm. Reactivity to PPD is not specific for Mycobacterium tuberculosis, as it can be seen in relation to Mycobacterium bovis and various soil mycobacteria. This raises the potential for false positive PPD tests, an issue that is of particular relevance among individuals previously vaccinated with BCG, which is derived from attenuated M. bovis. BCG vaccination can cause a positive PPD, the size of which varies indirectly with the time between vaccination and testing. Approximately 80% of patients become PPD reactive within 6 weeks of BCG vaccination. Interestingly, however, there is no correlation between PPD reactivity due to vaccination and protection against developing TB infection (54). By 10 years, 15-25% of patients will still be PPD positive; this decreases to less than 10% by 15 years (53, 55).

As noted, repeated PPD testing can have a booster effect among BCG recipients, making the interpretation of results more difficult. BCG vaccination, although rare in the United States, is common worldwide, particularly in countries with a high prevalence of TB. Because BCG vaccination is less effective in endemic areas, this further confounds PPD interpretation. Given these considerations, current recommendations call for ignoring previous BCG exposure when interpreting PPD. This would seem relevant for RA patients being considered for TNF inhibitor therapy, as most of those who are being considered for treatment would have received BCG many years prior.

Some clinicians obtain a chest radiograph (CXR) to screen for LTBI. Although evidence of prior TB infection such as nodules and fibrotic or scarred lesions may be observed, the CXR is normal in most patients with LTBI, thereby limiting its utility as a screening test (53).

Given the constraints of current testing approaches, there has been substantial interest in developing non-PPD based TB specific testing. In vitro assays have been developed, based upon specific T cell responses to antigens present in Mycobacterium tuberculosis but not in soil mycobacteria or in Mycobacterium bovis (e.g. early secretary antigen target-6; ESAT-6). Such assays, which have received regulatory approval, may allow the distinction between exposure to TB versus BCG vaccination, and therefore offer greater accuracy in LTBI identification (56, 57).
As larger numbers of patients receive treatment with TNF inhibitors, the issue of repeated testing for TB after the initiation of therapy will become increasingly important. At present, there are no guidelines for TB testing at regular intervals, although screening after potential TB exposure would seem prudent. While the effect of TNF inhibitor therapy on PPD reactivity has not been specifically addressed, there is data suggesting that these agents do not appear to inhibit DTH responses or T cell reactivity to mitogens or recall antigens (58, 59). Interestingly, there are data suggesting that TNF inhibitor therapy, by removing the inhibitory effect of chronic TNF exposure, may actually enhance T cell responses (60, 61).

**Treatment**

In patients with a positive PPD, a CXR is obtained to exclude the presence of active TB. If the CXR is normal, appropriate treatment for LTBI is initiated (Table III) (47, 53). The standard regimen suggested is isoniazid for 9 months, although alternatives may be substituted if indicated. A relevant issue for RA patients, many of whom are on concurrent therapy with DMARDs, particularly methotrexate, is the potential hepatotoxicity of anti-TB medications (62). After LTBI therapy is begun, treatment with a TNF inhibitor may be started. While some clinicians wait days or weeks between LTBI treatment and TNF inhibitor therapy, at present there are no data supporting any particular interval. If the CXR shows evidence of active TB, the general consensus has been to withhold TNF inhibitor therapy until completion of an adequate TB treatment. In cases where CXR has been abnormal but not suggestive of active TB, it may be prudent to collect sputum samples for culture and possibly PCR testing to exclude active TB. When these are negative, TNF therapy may be initiated. Clearly, more data on the utility of these various approaches are needed.

As noted, BCG vaccination has been used as a potential means to prevent TB infection. However, its efficacy is quite variable, with reported rates of prevention ranging from 0-85% (54, 63). The efficacy of vaccination appears to be highest in the prevention of disseminated disease in children, and lower in endemic areas. There are no data at present supporting a role for BCG vaccination in preventing TB among RA patients being considered for TNF inhibitors. Of note, disseminated infection with the normally innocuous *M. bovis* strain used in BCG can be seen, particularly in the setting of immunosuppression (64, 65).

**The risk of TB with other biologic agents**

Anakinra (recombinant IL-1 receptor antagonist; IL-1ra), an inhibitor of IL-1, has also been approved for the treatment of RA. Many other novel immunomodulatory agents are currently under development. For those agents targeting TNF, the above considerations regarding TB should be relevant. For agents targeting other inflammatory cytokines, or other components of the immune response, it remains to be determined whether they will cause an increased proclivity to TB. Given the interplay of various components of the immune system, and the role of factors other than TNF in host defense against TB, such a risk is certainly possible. With anakinra, there has been one case of TB reported with more than 19,000 patient-years of exposure (through May 2003; data on file; Amgen). Long term pharmacovigilance data will provide critical information regarding the risk of TB and other opportunistic infections in patients treated with novel immunomodulatory agents.

**Other opportunistic infections**

In addition to TB, other opportunistic infections that have been observed among patients treated with TNF inhibitors include atypical mycobacteria, listeriosis, histoplasmosis, aspergillosis, candidiasis, coccidiomycosis, pneumocystis pneumonia, and others. Considerations discussed above regarding TB are largely relevant to these other pathogens. The most important points for clinicians are: 1) be aware of endemic infections in one’s locale, and 2) maintain a high index of suspicion for unusual pathogens when caring for patients receiving TNF inhibitors.

**Summary and conclusions**

Animal models demonstrating biologic plausibility, along with pharmacovigilance data concerning prevalence and temporal association, support associations of TB with TNF inhibitor therapy in patients with RA. There appear to be differential risks of TB with the currently available inhibitors, although the specific mechanisms underlying this difference remain to be delineated. Screening for LTBI with PPD is indicated for patients being considered for

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**Table III. Recommended drug regimens for treatment of latent TB infection.**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Interval and duration</th>
<th>Rating* (Evidence)**</th>
<th>HIV-</th>
<th>HIV+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid</td>
<td>Daily for 9 months or Twice weekly for 9 months^</td>
<td>B (II)</td>
<td>B (II)</td>
<td></td>
</tr>
<tr>
<td>Isoniazid</td>
<td>Daily for 6 months or Twice weekly for 6 months^</td>
<td>B (II)</td>
<td>C (I)</td>
<td></td>
</tr>
<tr>
<td>Rifampin plus pyrazinamide</td>
<td>Daily for 2 months or twice weekly for 2-3 months^</td>
<td>C (II)</td>
<td>C (I)</td>
<td></td>
</tr>
<tr>
<td>Rifampin</td>
<td>Daily for 4 months</td>
<td>B (II)</td>
<td>B (III)</td>
<td></td>
</tr>
</tbody>
</table>

*Strength of recommendation: A = preferred; B = acceptable alternative; C = offer when A and B cannot be given.

†Quality of evidence: I = randomized clinical trial data; II = data from clinical trials that are not randomized or were conducted in other populations; III = expert opinion.

^directly observed therapy must be used with twice weekly dosing
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treatment, and seems to have been effective in reducing the occurrence of TB in treated patients. However, the large number of disseminated and atypical presentations of TB observed, the potential for false negative screening tests, and the possibility of acquisition of primary TB while on therapy highlight the need for continuous vigilance on the part of the treating clinician for the possibility of TB in patients treated with these agents. Further information regarding the optimal diagnostic and therapeutic approaches will prove important to RA patients receiving TNF inhibitor therapy and to their physicians.

References
47. AMERICAN THORACIC SOCIETY, CENTERS FOR DISEASE CONTROL AND PREVENTION: Targeted tuberculin testing and treatment of latent tuberculosis infection. Am J Respir Crit Care Med 2000; 161 (Suppl.): S221-47.