Abatacept Treatment Does Not Exacerbate Chronic *Mycobacterium tuberculosis* Infection in Mice

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**Objective.** Treatment of rheumatoid arthritis and other autoimmune disorders with anti–tumor necrosis factor (anti-TNF) agents is associated with an increased risk of reactivation of latent *Mycobacterium tuberculosis*. While the mechanism of action of abatacept is fundamentally different from that of anti-TNF therapies, its effect on the protective response to latent tuberculosis is not known. We undertook this study to determine the effect of abatacept treatment in a murine model of chronic *M tuberculosis* infection.

**Methods.** Chronic *M tuberculosis* infection was established in C57BL/6 mice. Four months after infection, mice were treated for up to 16 weeks with abatacept, anti–murine TNF antibody, or vehicle. The primary end point was survival; body weight, bacterial load, histologic features, interferon-γ (IFNγ) production by T cells, and cellular infiltration were also assessed.

**Results.** Abatacept- and vehicle-treated groups both maintained control of *M tuberculosis* infection, with 100% survival after 16 weeks of treatment. These 2 groups had no significant differences in body weight, no clinically relevant differences in bacterial load in the lungs, lymph nodes, or spleen, and no differences in the mean percentage of total or activated T cells, macrophages, neutrophils, or B cells, or in IFNγ production in the lung or lymph nodes. In contrast, 100% mortality was seen in the anti-TNF antibody–treated group by week 9, with significant body weight loss and increased bacterial load in the lungs, lymph nodes, and spleen. Furthermore, the anti-TNF antibody–treated group had increased pathology consistent with the exacerbation of *M tuberculosis* infection.

**Conclusion.** Abatacept did not impair the ability of mice to control a chronic *M tuberculosis* infection. In contrast, mice treated with anti-TNF therapy showed increased pathology and bacterial load, with 100% mortality by week 9. The clinical significance of these findings has not yet been determined.

A number of recent reports have highlighted the infectious complications associated with anti–tumor necrosis factor (anti-TNF) therapies for rheumatoid arthritis (RA) (1–4). Of particular concern is the link between anti-TNF therapy and an increased risk of reactivation of latent *Mycobacterium tuberculosis* infection (5–8).

TNF is a proinflammatory cytokine that plays a central role both in the host inflammatory response to mycobacterial infection and in the immunopathology of tuberculosis (TB) itself (7). In vitro studies have demonstrated that TNF increases the ability of macrophages to phagocytose and kill mycobacteria (9,10), and in vivo TNF has been shown to play an important role in the formation and maintenance of granulomas, the focal accumulations of lymphocytes and macrophages that work together to control mycobacterial growth and limit the spread of infection (11). TNF-deficient mice infected with *M tuberculosis* are highly susceptible to disease (12), and granuloma formation is impaired in these mice (11). Additionally, neutralization of TNF in mice chronically infected with *M tuberculosis* has been shown to exacerbate infection, induce granuloma structural deficiencies, and cause increased mortality (12,13). Accordingly, it is...
to be expected that the use of anti-TNF agents might exacerbate recently acquired TB or reactivate latent *M. tuberculosis* infections. Indeed, the advent of therapeutic agents that target TNF has led to clinical reports of TB in patients receiving these agents (1–4).

Abatacept, a fully human soluble selective costimulation modulator, is the first in a new class of agents for the treatment of RA that selectively modulates the CD80/CD86-mediated CD28 costimulatory signal required for full T cell activation (14). Abatacept is a fusion protein that consists of the extracellular domain of human CTLA-4 linked to the modified Fc (hinge, C1,C2, and C3 domains) portion of human IgG1 (15). Abatacept has demonstrated efficacy in patients with active RA and an inadequate response to methotrexate, as well as in those with an inadequate response to anti-TNF therapy (14,16). While abatacept has a fundamentally different mechanism of action from anti-TNF therapies, it is an immunomodulator and may therefore affect the host response to opportunistic infections, including TB. Abatacept has been shown not to impact innate immunity (14), and it does not completely inhibit costimulation, perhaps due to the redundancies in the system provided by alternate costimulatory pathways (17). The effect of abatacept on the protective response to latent TB, however, is not known.

Typically, C57BL/6 mice are fairly resistant to *M. tuberculosis* and control the infection with stable numbers of bacteria in the lungs, liver, and spleen (18). They generally have no obvious impairments, such as weight loss or visible signs of distress; however, the disease is never eliminated, and eventually, all mice progress to fatal TB (18). Nonetheless, control of an initial low-dose infection can be maintained for up to 1 year. Thus, in the mouse, TB is a chronic infection, and this murine system has been used to identify host factors that are important in the control of TB, including TNF (12,13).

Because *M. tuberculosis* infection may affect as much as one-third of the world’s population (19), understanding the role of antiinflammatory agents in its activation has important clinical relevance. Thus, this mouse model of chronic TB was chosen to better understand the potential role of abatacept in the immune response to latent TB infection. Doses of abatacept used in this study were ~20 mg/kg, 2-fold higher than the dose that results in pharmacologically significant immunomodulation in humans (14,16). Furthermore, subcutaneous injection of abatacept or related CTLA-4Ig versions at doses of ≤10 mg/kg have demonstrated safety and efficacy in murine models of lupus (20), suppression of a primary antibody response (21), cardiac allograft rejection (22), human pancreatic islet cell xenograph rejection (23), graft-versus-host disease (24), and skin allograft rejection (25). Understanding what effect, if any, abatacept might have on the immune response to *M. tuberculosis* may shed greater light on its clinical safety profile.

**MATERIALS AND METHODS**

**Suppression of antibody response.** Abatacept or vehicle was administered subcutaneously once weekly for 7 weeks to female CD-1 mice (n = 10 per group). Keyhole limpet hemocyanin (KLH), 0.25 ml/animal of 1 mg/ml solution, was administered intraperitoneally prior to the fifth dose of abatacept or vehicle (day 29). KLH-specific antibodies were detected by an enzyme-linked immunosorbent assay using a cocktail of alkaline phosphatase (AP)–conjugated donkey anti-mouse IgG and AP-conjugated donkey anti-mouse IgM. Antibody titers were defined as the reciprocal of the interpolated dilution that resulted in an absorbance reading equal to 5-fold the mean plate background, with the absorbance measured in the absence of serum. Results are expressed as the percentage suppression of individual animal titers compared with the group mean titer in vehicle-treated control mice. Negative percentage suppression values were set at zero.

**TB infection and treatment.** Female C57BL/6 mice were infected intranasally with a low dose (15 colony-forming units [CFU]) of *M. tuberculosis* strain Erdman, as described previously (12). At 4 months postinfection, during the chronic infection state, 12 mice were euthanized for baseline values. Remaining animals were randomly assigned to 3 study groups (see below). Abatacept and Dulbecco’s phosphate buffered saline were administered subcutaneously into the pectoral region (the lateral, cranial-thoracic, ventral site) once weekly at a dose of 0.5 mg/25 gm mouse (~20 mg/kg). This dose is greater than that required for immunomodulation in humans (16,18) and mice (14,16,20–25) and has been shown to suppress the anti-abatacept antibody response in mice (25). Rat anti-murine TNF antibody, clone MP6-XT22 (provided by DNAX [Palo Alto, CA] and prepared by National Cell Culture Center [Minneapolis, MN]), was administered intraperitoneally twice weekly at 0.5 mg/mouse, a dose known to exacerbate chronic TB in mice (12). Four mice per group were euthanized 1, 2, 3, 4, 6, and 8 weeks posttreatment. All remaining mice (15 per treatment group) were treated for an additional 8 weeks (total of 16 weeks) to assess survival. Six mice per group, if surviving, were euthanized 16 weeks posttreatment.

**Bacterial load determination.** At necropsy, bacterial load was determined for each individual animal by plating dilutions of lung, mediastinal lymph node, and spleen homogenates on 7H10 plates (Difco, Detroit, MI). CFU were counted after 3 weeks of incubation at 37°C in a CO2 incubator (18). CFU were calculated per organ.

**Histopathology.** Tissue samples from lung and spleen were fixed in 10% formalin, sectioned, and stained with hematoxylin and eosin (H&E). Evaluation of sections was performed by one of the authors (JLF), who was blinded to the treatment the mice received.
T cell responses by enzyme-linked immunospot (ELISpot) assay for interferon-γ (IFNγ). Single-cell suspensions of lung and lymph node tissue from each individual animal were plated in ELISpot plates coated with anti-IFNγ antibody, as previously described (26). Cells were added at 80,000/well for lung and 150,000/well for lymph node. Murine dendritic cells (uninfected and infected with M tuberculosis) served as stimulators. IFNγ was detected as described and recorded as spot-forming units per well, representing the frequency of cells producing IFNγ in each tissue (26).

Determination of cellular infiltration into the lungs. Single-cell suspensions of lung and lymph node tissue from each individual animal were plated in ELISpot plates coated with anti-IFNγ antibody, as previously described (26). Cells were added at 80,000/well for lung and 150,000/well for lymph node. Murine dendritic cells (uninfected and infected with M tuberculosis) served as stimulators. IFNγ was detected as described and recorded as spot-forming units per well, representing the frequency of cells producing IFNγ in each tissue (26).

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Statistical analysis. Experimental groups (n = 15 animals per group) were evaluated for survival with respect to the percentage of animals that were alive at the end of the observation period using Fisher's exact test. Numbers of bacteria in the organs were log-transformed and compared among groups using Student's 2-tailed t-test. P values less than 0.05 were considered significant. For immunologic assays, Student’s 2-tailed t-test was also used to compare groups (26).

RESULTS

Antibody suppression by abatacept. To confirm the immunomodulatory effect of abatacept in mice (20–25) at the dose, regimen, and route of administration used in this study, the ability of abatacept to inhibit a primary antibody response was tested. Abatacept or vehicle control was administered subcutaneously once weekly for 7 weeks at doses up to 65 mg/kg. Prior to the fifth dose (on day 29), the immunogen KLH (27) was administered intraperitoneally to initiate a primary antibody response. KLH-specific antibody titers were measured on day 50. Abatacept suppressed the antibody response to KLH, with 60% suppression observed at the 10 mg/kg dose and 100% suppression observed at doses ≥20 mg/kg (Figure 1).

Survival and bacterial loads. In order to examine the effect of abatacept and anti-TNF therapies on the murine response to M tuberculosis, latent infections were established as shown in Figure 2. Mice were then treated with abatacept, anti-TNF antibody, or vehicle. All abatacept-treated and vehicle-treated mice survived to week 16. All mice in the anti-TNF–treated group were judged to be moribund from M tuberculosis infection and were euthanized before week 9, with a group mean survival time of 44 days (P < 0.0001 compared with vehicle-treated mice) (Figure 3A). No significant body weight losses were observed in the abatacept- or vehicle-treated groups throughout the 16-week course of the study, whereas significant body weight loss was observed in the anti-TNF–treated group by weeks 7 and 8 (P < 0.0001 versus vehicle-treated group) (Figure 3B). Body weight loss in the anti-TNF–treated group corresponded to worsening of disease.

There was a slight increase in M tuberculosis CFU in the lungs for the abatacept-treated group at week 8, but this value did not differ significantly from that in the vehicle-treated group. At all other time points,
Abatacept-treated mice had bacterial loads similar to those in vehicle-treated controls. Compared with the vehicle-treated control group, at week 4 in the anti-TNF–treated group there was a 16-fold increase in *M. tuberculosis* CFUs in the lungs (\(P < 0.0001\) versus vehicle-treated mice). B, Change in body weight following treatment with abatacept, anti-TNF antibody, or vehicle. Surviving mice were evaluated at regular intervals during a period of 16 weeks. Values are the mean ± SD. Compared with vehicle-treated mice, significant body weight loss was observed in the anti-TNF–treated group by weeks 7 and 8.

Figure 3. A, Survival curves for *Mycobacterium tuberculosis*–infected mice treated with abatacept, anti–tumor necrosis factor (anti-TNF) antibody, or vehicle. Mice were tracked for survival for 16 weeks. Abatacept and vehicle lines overlap. Anti-TNF–treated mice had a group mean survival time of 44 days (\(P < 0.0001\) versus vehicle-treated mice). B, Change in body weight following treatment with abatacept, anti-TNF antibody, or vehicle. Surviving mice were evaluated at regular intervals during a period of 16 weeks. Values are the mean ± SD. Compared with vehicle-treated mice, significant body weight loss was observed in the anti-TNF–treated group by weeks 7 and 8.

Figure 4. Colony-forming units (CFU) of *Mycobacterium tuberculosis* found in the lungs (A), lymph nodes (B), or spleen (C) of infected mice. Mice were treated with abatacept, anti–tumor necrosis factor (anti-TNF) antibody, or vehicle as indicated. Tissue samples were tested at scheduled intervals during a period of 16 weeks. Values are the mean ± SD. Compared with the vehicle-treated control group, at week 4 in the anti-TNF–treated group there was a 16-fold increase in *M. tuberculosis* CFU in the lungs.

Surviving mice were tracked up to week 16, and there were no significant differences between the groups in terms of bacterial load in the lungs, spleen, or lymph nodes. This finding is consistent with previous studies in which anti-TNF–treated mice did not necessarily have
exceptionally high numbers of bacteria in the lungs, but succumbed to *M tuberculosis* infection as a result of pulmonary pathology (26). TNF does play an important role in the clearance of pathogens; thus, the increases in bacterial burdens in the lymph nodes and spleen are consistent with the requirement for TNF to control infection (26).

**Histopathology findings.** *M tuberculosis* persists in macrophages within a granuloma in the organs of infected hosts (28). The granuloma is a cellular accumulation of macrophages and lymphocytes that serves to control mycobacterial infection, limit dissemination of the infection from the lungs, and protect alveolar tissue (11). In comparison with the localized granulomas containing activated macrophages and T cells in the lungs of wild-type mice, TNF-deficient mice have been shown to have poorly formed granulomas, with extensive regions of necrosis and neutrophilic infiltration of the alveoli (13).
Blinded evaluation of H&E-stained tissue sections revealed no differences between the lungs and spleens of abatacept-treated mice and vehicle-treated mice 4 weeks posttreatment. In contrast, compared with control mice, anti-TNF–treated mice showed an increase in pathology, with increased infiltration of cells (Figure 5) and unorganized granulomas in the lungs. Furthermore, compared with control mice, anti-TNF–treated mice also showed increased infiltration of cells and more granulomas in the spleen. These findings are consistent with the exacerbation of \textit{M tuberculosis} infection, as previously reported (18,26).

**IFN\(\gamma\) production by T cells.** IFN\(\gamma\) is a key cytokine responsible for the control of \textit{M tuberculosis} infection (28), and it is produced by both CD4\(^+\) T cells and CD8\(^+\) T cells in response to \textit{M tuberculosis} (29,30).

The central role of IFN\(\gamma\) in the pathogenesis of TB is highlighted by the high susceptibility to mycobacterial infections, including \textit{M tuberculosis}, of individuals defective in genes for IFN\(\gamma\) or the IFN\(\gamma\) receptor (31). Mice deficient in IFN\(\gamma\) are extremely susceptible to \textit{M tuberculosis} (32,33). Additionally, increased production of IFN\(\gamma\) in mice has been noted to be an indicator of the immune response to mycobacterial infection and disease progression (18,34).

Increased IFN\(\gamma\) production from T cells in response to mycobacterial antigens was observed in all groups by week 1 of treatment. Abatacept-treated mice showed a trend toward lower IFN\(\gamma\) production in the lungs by week 1 and slightly higher IFN\(\gamma\) production in the lymph nodes by week 3, but levels were not significantly different from those in the vehicle-treated group.
at later time points. Anti-TNF–treated mice showed an increase in IFNγ production in the lungs and lymph nodes at later time points, as expected due to increased bacterial load, although this increase was not statistically significant (Figure 6). There was a slow increase in IFNγ production between week 3 and week 8 in the lungs and lymph nodes from both the abatacept-treated and the vehicle-treated mice, and this increase persisted in the lung to week 16, likely due to the increasing pathology that accompanies prolonged infection (in this case 8 months).

**Cellular infiltration into the lungs.** Because the _M. tuberculosis_ pathogen lives within cells, usually macrophages, the cell-mediated immune response elicited by T cells is required to control or eliminate bacteria (28). In response to infection, both CD4+ and CD8+ T cells migrate to the lungs, where they can interact with macrophages in the formation of granulomas. Typically, during infection ~50% of CD4+ and CD8+ T cells in the lungs are also CD69+, indicating that they are active and interacting with antigen-presenting cells to challenge infection (28).

Compared with vehicle-treated mice, abatacept-treated mice showed no significant differences in the percentage of CD4+ cells or CD8+ cells (data not shown) or in the percentage of CD4+,CD69+ (activated) cells or CD8+,CD69+ (activated) cells in the lymph nodes (data not shown) and lungs (Figure 6C and D) during 8 weeks of treatment. Anti-TNF–treated mice, however, demonstrated a significant increase in the percentage of CD4+ cells, CD8+ cells, and CD8+,CD69+ (activated) cells in the lungs during 4 weeks compared with vehicle-treated mice (115% [P = 0.019], 101% [P = 0.030], and 194% [P = 0.032], respectively) (Figure 6). This increase likely reflects an increase in bacterial burden in these mice. Lymph nodes from anti-TNF–treated mice did not demonstrate significantly increased percentages of any of these 4 cell types, likely reflecting the movement of newly primed T cells from the lymph nodes to the lungs as the bacterial burden increased there. No appreciable differences were observed between abatacept-treated, anti-TNF–treated, and vehicle-treated mice in the percentages of CD11b+,GR1−,CD11c− cells (macrophages), CD11b+,GR1+,CD11c− cells (neutrophils), or B220+ cells (B cells) in the lungs or lymph nodes.

**DISCUSSION**

The risk of reactivation of latent _M. tuberculosis_ infections is an important clinical concern in the treatment of RA (1-4), particularly with some agents targeting TNF (5-8). The clinical use of infliximab, a monoclonal antibody that targets and inactivates TNF, has been implicated in several studies in the reactivation of recent or remotely acquired TB infection (1,2). Etanercept, a dimeric soluble form of the TNF receptor, binds and sequesters TNF and has also been associated with the development of TB (35). Although the rates of _M. tuberculosis_ reactivation between infliximab and etanercept are not directly comparable, it appears that infliximab treatment carries a greater risk of TB-related complications than does etanercept (6-8). It is possible that differences in the underlying patient population, dosing schedules, concomitant prednisone use, or other mechanistically unrelated factors may be responsible for some of the difference in TB risk between agents (8).

However, the differences in the mechanism of action between the 2 drugs may provide a biologic rationale for the greater risk of TB with infliximab (8,36). Another anti-TNF antibody in clinical use, adalimumab, has also been linked with an increased risk of TB complications at higher doses (4).

The clinical risk of TB reactivation corresponds to data obtained in murine models of chronic TB, in which treatment with anti-TNF antibody has been demonstrated to fatally reactivate latent TB (12). Abatacept is the first in a new class of biologic agents for the treatment of RA with a mechanism of action that is fundamentally different from that of anti-TNF agents (14,16). Since abatacept is still relatively new to the market, a definitive clinical assessment of the effect of abatacept treatment on latent TB cannot be made at this time. Therefore, studies with abatacept in a murine model of chronic TB increase the understanding of this clinical concern.

At subcutaneous weekly doses of abatacept at ~20 mg/kg for 16 weeks, all abatacept-treated mice (as well as vehicle-treated mice) were able to contain chronic _M. tuberculosis_ infection and did not succumb to the disease. After 16 weeks of treatment, no clinically significant differences in mycobacterial load in the lungs, lymph nodes, or spleen were observed between abatacept-treated mice and vehicle-treated mice. Furthermore, by week 16, there were no differences in IFNγ production in the lungs or lymph nodes of abatacept-treated mice compared with vehicle-treated mice. In addition, through week 8, there were no significant differences in the numbers or percentages of CD4+ and CD8+ T cells and no significant differences in the numbers or percentages of CD69+ (activated) T cells in abatacept-treated mice compared with vehicle-treated mice.
mice. Finally, no differences were observed in the histologic features of the lungs and spleen of abatacept-treated and vehicle-treated mice. Thus, selective costimulation modulation of T cell activation through the CD80/CD86 and CD28 pathway in the chronic phase of infection did not impair the ability of mice to control *M tuberculosis* infection. The lack of impact of abatacept treatment on both the number and percentage of activated T cells highlights both the highly selective mechanism of action of abatacept and the complex nature of T cell activation, which involves several known costimulatory pathways (17).

It is theoretically possible that in this mouse model, abatacept is being inactivated by murine anti-abatacept antibody. This is unlikely, however, because abatacept is able to completely inhibit the potent immunoinactivator KLH at 20 mg/kg (Figure 1), and previous mouse studies have demonstrated the efficacy of abatacept at doses less than and equal to the dose used in this study (14,16,20–25). Furthermore, at the dose used in this study, abatacept has been previously demonstrated to suppress the antibody response to itself in mice (25).

In contrast to the abatacept-treated mice, all of the anti-TNF–treated mice succumbed to the infection before week 9. There was a trend toward higher bacterial load in the lungs, lymph nodes, and spleen, with an ~10-fold increase in IFNγ production by T cells in the anti-TNF–treated group compared with the vehicletreated group. In addition, anti-TNF–treated mice showed an increase in the numbers and percentages of CD4+ and CD8+ T cells as well as CD69+ (activated) T cells in the lungs, consistent with higher bacterial loads in these animals. Finally, histopathologic evaluation demonstrated an increase in cellular infiltration (both macrophages and T cells) in the lungs and spleen, less well-organized granulomas in the lungs, and more granulomas in the spleen, consistent with the reactivation and dissemination of *M tuberculosis* infection. These findings correlate with the known effects of anti-TNF treatment in murine models of chronic TB (12).

While the clinical relevance of these findings is not known, there may be some biologic rationale for these observations. In a mouse system, TNF is known to play a central and required role in the host response against TB (37), and in humans, treatment with anti-TNF agents has been associated with increased rates of TB (7,8). Abatacept selectively modulates T cell activation, and while T cells are important in the immunobiology of TB infections (28), alternate costimulatory pathways exist (17), perhaps providing a rationale for the resistance of abatacept-treated mice to TB reactivation.

Specifically, abatacept does not directly inhibit TNF production by macrophages in vitro (Nadler SG: unpublished data). Clinical data from observational studies are needed to assess the relevance of these findings to humans.

**AUTHOR CONTRIBUTIONS**

Dr. Flynn had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Study design.** Bigbee, Gonchoroff, Vratsanos, Nadler, Haggerty, Flynn.

**Acquisition of data.** Bigbee, Flynn.

**Analysis and interpretation of data.** Bigbee, Gonchoroff, Vratsanos, Nadler, Haggerty, Flynn.

**Manuscript preparation.** Bigbee, Gonchoroff, Nadler, Haggerty, Flynn.

**Statistical analysis.** Bigbee, Flynn.

**Study monitor and study report.** Gonchoroff.

**ROLE OF THE STUDY SPONSOR**

Bristol-Myers Squibb had no role in the study design or in the collection, analysis, or interpretation of the data.

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