Risk of Tuberculosis Reactivation With Tofacitinib (CP-690550)

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Individuals with latent tuberculosis infection (LTBI) live with a risk of reactivation, and several treatments for chronic inflammatory conditions are highly associated with such reactivation. A new Janus kinase inhibitor, tofacitinib (CP-690550), has shown promising results for treatment of inflammatory disorders, thus raising concerns of risk of active tuberculosis. Our goal was to characterize the impact of tofacitinib on LTBI using a mouse model of contained tuberculosis. Our data indicate that tofacitinib reduces host containment of *Mycobacterium tuberculosis* and promotes bacterial replication in the lungs, suggesting tuberculosis reactivation. Tofacitinib may carry a significant risk for LTBI reactivation in humans.

Tuberculosis is a devastating human infectious disease responsible for the death of nearly 2 million people worldwide every year [1]. Even more alarming, the World Health Organization has estimated that nearly 2 billion individuals harbor a latent infection with *Mycobacterium tuberculosis*, the etiologic agent of tuberculosis [1]. Thus, approximately one-third of the world's population is at risk to develop active disease and contribute to the continued spread of *M. tuberculosis* within communities.

Although the detailed mechanisms by which *M. tuberculosis* infection transitions from latent to active disease are poorly understood, epidemiologic studies have identified clear risk factors for activation of latent tuberculosis infection (LTBI), of which the most well known are human immunodeficiency virus infection and treatment with tumor necrosis factor alpha (TNF-α) inhibitors [2]. The TNF-α antagonists etanercept, infliximab, adalimumab, certolizumab, and golimumab are commonly used for the treatment of chronic inflammatory conditions, including rheumatoid arthritis, psoriasis, and Crohn's disease. Compared with individuals with LTBI who do not receive TNF-α blockers, patients with LTBI who use these anti-inflammatory treatments are at a significantly higher risk to develop active tuberculosis [3]. The screening of patients for LTBI prior to anti–TNF-α treatment can reduce the risk for active tuberculosis by 90% if patients testing positive are first treated with isoniazid preventive therapy prior to TNF-α antagonist administration [4].

Tofacitinib, an oral Janus kinase (JAK) inhibitor (CP-690550, formerly tasocitinib), is a new anti-inflammatory agent developed by Pfizer. Tofacitinib displays properties similar to TNF-α inhibitors, and a recent clinical trial of this drug in patients with rheumatoid arthritis showed significant benefit [5]. The new JAK inhibitor has also shown promising results against other inflammatory conditions [6, 7]. As an oral agent (as opposed to the intravenous or injectable TNF-α antagonists), tofacitinib may become a commonly used anti-inflammatory agent in the coming years. However, this new drug has not yet been evaluated as a risk factor for LTBI reactivation. In this study, we examined the influence of tofacitinib in a published mouse model of chronic paucibacillary tuberculosis that was developed to represent human LTBI [8, 9].

METHODS

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The Johns Hopkins Animal Care and Use Committee approved all procedures described in this article.

Animals

Six-week-old BALB/c mice were obtained from Charles River Laboratories and maintained in an animal biosafety level 3 facility at all times. The paucibacillary mouse model of LTBI was used as previously described [9]. Mice were immunized with *Mycobacterium bovis* bacille Calmette-Guérin (BCG)

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Table 1. Experimental Design and Mouse Sacrifice Schedule

Treatment Group	Number of BALB/c Mice Sacrificed at Each Time Point				
	Week −12 (BCG Immunization)	Week −6 (H37Rv Infection)	Week 0 (Start Treatment)	Week 2	Week 4
BCG/ H37Rv Low Dose	4	4	4	4	4
BCG/ H37Rv High Dose				4	4
BCG/ H37Rv Untreated				4	4
H37Rv only	…	4	4	4	4
TOTAL	4	8	8	16	16

Week −12 represents the time of immunization with bacille Calmette-Guérin (BCG), and lung colony-forming units (CFUs) for BCG implantation were determined for these mice. For all other time points, lung CFUs for H37Rv were determined.

Copenhagen prior to infection with *M. tuberculosis* H37Rv, following the experimental design presented in Table 1.

Aerosol Infection and Colony Counting Procedures

Bacterial strains were prepared in standard 7H9 Middlebrook liquid medium containing 0.2% Tween-80, and aerosol infections were performed using the Glas-col Inhalation Exposure System per the manufacturer's instructions. At each time of death (noted in Table 1), mice lungs were dissected and total lung colony-forming unit (CFU) counts were determined as previously described [8]. Following infection with *M. tuberculosis*, mouse lung homogenates were serially diluted and plated on selective 7H11 agar supplemented with 4 mg/mL of 2-thiophenecarboxylic acid hydrazide (TCH) (Sigma); *M. tuberculosis* but not *M. bovis* BCG can grow in the presence of TCH [9]. Thus, all CFUs reported in this article for *M. tuberculosis*–infected mice (whether also infected with BCG or not) represent the CFUs for H37Rv only.

Drug Preparation and Administration

Tofacitinib was purchased from Selleck Chemicals, and stock solutions were prepared weekly using 0.5% methylcellulose in water as vehicle and stored at 4°C. Tofacitinib was administered twice daily to the mice by oral gavage to achieve 1.5 or 15 mg/kg in volumes of 0.2 mL.

Data Analysis

Lung CFU counts were $\log_{10}$-transformed. Friedman's 2-way analysis of variance by ranks was used for statistical analyses, with significance set at $P < .05$.

RESULTS

Paucibacillary Model

Mice were immunized with aerosol BCG infection yielding a day 1 (T = −12 weeks) implantation of mean 3.75 (standard deviation [SD] = 0.05) $\log_{10}$ CFUs. Six weeks following immunization (T = −6 weeks), BCG lung counts were mean 3.20 (SD = 0.17) $\log_{10}$ CFUs, at which point the immunized mice and a group of nonimmunized mice were infected with a low dose of H37Rv, with an implant of mean 1.45 (SD = 0.12) $\log_{10}$ CFUs. When tofacitinib therapy was initiated 6 weeks later (T = 0 weeks), the H37Rv lung counts had reached mean 4.13 (SD = 0.06) and mean 6.02 (SD = 0.04) $\log_{10}$ CFUs for immunized and nonimmunized mice, respectively. The immunized mice not treated with tofacitinib experienced a conserved 4 $\log_{10}$ CFU plateau, whereas the nonimmunized mice had 1–2 $\log_{10}$ more CFUs (Figure 1*A*), thus validating the paucibacillary model in this experiment.

Tuberculosis Reactivation With Tofacitinib

After 14 days of tofacitinib treatment, both the low- and high-dose groups exhibited mean lung H37Rv CFU counts similar to the immunized, nontreated group (Figure 1*B*). However, after 28 days of treatment, the low-dose tofacitinib group had higher but nonstatistically significant lung CFU levels compared with the untreated mice, and the group receiving the high-dose tofacitinib treatment had a significantly higher lung H37Rv burden than the nontreated mice (mean, 5.04 [SD = 0.25] vs mean, 4.01 [SD = 0.26]; $P = .045$) (Figure 1*B*). These results demonstrate that 4 weeks of tofacitinib treatment promotes bacterial growth in a dose-dependent manner in the mouse lungs.

Gross pathology indicated that the lungs of the nonimmunized mice had more and larger lesions than the lungs of the BCG-immunized mice (Figure 1*C*). Lungs from the immunized, tofacitinib-treated mice exhibited intermediate gross lesions, which were indistinguishable between the low or high dose. Consistently, the most severe lesions, containing collapsed alveolar sacs and leukocyte aggregation, were found in the nonimmunized mouse lungs, whereas no obvious histopathological lesions were noted in the BCG-immunized, untreated mouse lungs (Figure 1*D*). Tofacitinib-treated mouse lungs had limited leukocyte aggregation on histopathology examination.

DISCUSSION

These data indicate that caution is needed when using the new JAK inhibitor tofacitinib in patients with chronic inflammation. Screening for LTBI should be required in all settings (ie, in both low- and high-burden tuberculosis regions) where tofacitinib is approved for use. Using the paucibacillary

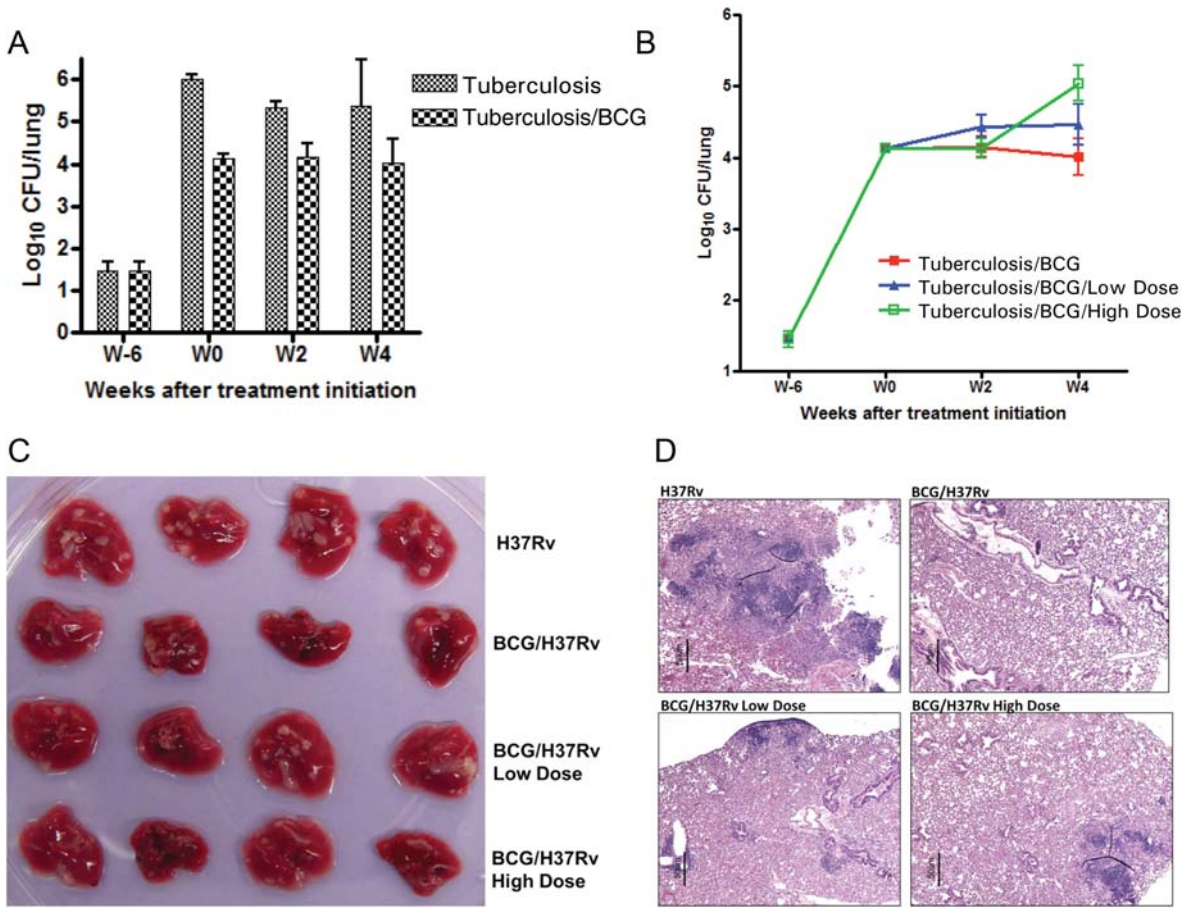

Figure 1. Tofacitinib treatment was associated with enhanced bacterial growth and pathology in mouse lungs. *A*, The paucibacillary model of latent tuberculosis. *Mycobacterium tuberculosis* H37Rv $\log_{10}$ colony-forming unit (CFU) counts in the lungs of bacille Calmette-Guérin (BCG)–vaccinated (Tuberculosis/BCG) and unvaccinated (Tuberculosis) mice. Error bars represent standard deviation. *B*, The impact of tofacitinib treatment on *M. tuberculosis* growth. *M. tuberculosis* H37Rv $\log_{10}$ CFU counts in the lungs of mice from vaccinated mice that were untreated (Tuberculosis/BCG), treated with 1.5 mg/kg/day (Tuberculosis/BCG/Low Dose), or treated with 15 mg/kg/day (Tuberculosis/BCG/High Dose). Error bars represent standard deviation. *C*, Gross pathology of mouse lungs from each treatment group. *D*, Representative histopathology sections from mouse lungs from each treatment group, stained using hematoxylin and eosin.

chronic mouse model of LTBI, we have shown that tofacitinib administration leads to increased bacterial replication, indicating a risk of tuberculosis reactivation. The 1.5 and 15 mg/kg/day doses used in this study have been previously reported in BALB/c mice to have an anti-inflammatory effect, and plasma levels of tofacitinib in mice receiving 15 mg/kg/day were reported to average 61.2 ± 5.75 ng/mL, which is similar to the levels reported in humans receiving a twice-daily 15 mg/kg dose of tofacitinib [10, 11]. Thus, our study conditions reflected those seen in human tofacitinib administration, and our results indicated that this drug promotes *M. tuberculosis* replication in a dose-dependent manner. These data are consistent with a recent report on tofacitinib phase 3 trial that found 2 cases of tuberculosis related to the use of the drug [12].

Tofacitinib signaling pathways involve both the adaptive and innate immune responses by inhibiting interleukin 4–dependent Th2 cell differentiation and interfering with Th17 cell differentiation [13]. Additionally, the fact that tofacitinib promotes bacterial growth indicates that this drug could be useful as host-directed adjunctive therapy in combination with tuberculosis antibiotics because replicating *M. tuberculosis* are readily killed by most first-line tuberculosis drugs and are also less likely to be sequestered within granulomas [14].

These findings have important clinical implications. Tofacitinib has been demonstrated to be an effective oral treatment for several inflammatory diseases for which other treatments are frequently inadequate or uncomfortable [15]. Our results show that like existing TNF-α inhibitors, tofacitinib reduces the ability of the host to contain LTBI and stimulates tuberculosis reactivation. Although this study hasn't evaluated the isoniazid prophylaxis prior to tofacitinib therapy, it strongly suggests that screening for LTBI will be valuable for patients

prior to initiation of the therapy. If a patient is found to have LTBI, isoniazid prophylaxis may be considered. Further studies are needed to evaluate the benefit of isoniazid prophylaxis with tofacitinib.

Notes

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