Flow Cytometric Immune Profiling in Infliximab-Associated Tuberculosis

Kelly Pennington1, Humberto C Sasieta1, Guimerme P Ramos2, Courtney L Erskine3, Virginia P Van Keulen3, Tobias Peikert1 and Patricio Escalante1,4

1Division of Pulmonary and Critical Care Medicine, Department of Medicine, Mayo Clinic, Rochester, MN, USA. 2Department of Internal Medicine, Mayo Clinic, Rochester, MN, USA. 3Division of Immunology, Department of Medicine, Mayo Clinic, Rochester, MN, USA. 4Mayo Clinic Center for Tuberculosis, Mayo Clinic, Rochester, MN, USA.

ABSTRACT: Tumor necrosis factor α antagonists are increasingly used to treat inflammatory and autoimmune disorders and are associated with increased risk of active tuberculosis. Diagnosis of active tuberculosis in patients taking tumor necrosis factor α antagonists can be challenging owing to increased incidence of extrapulmonary manifestations and false-negative results on current available diagnostic tests. We present a case of a young woman on infliximab for ulcerative colitis who presented with disseminated tuberculosis. As part of a research study, we performed flow cytometric immune profiling, which has previously not been reported in patients with active tuberculosis taking tumor necrosis factor α antagonists. The flow cytometry results were within the positive thresholds for tuberculosis infection. Flow cytometric immune profiling may be a valid diagnostic tool for patients taking tumor necrosis factor α antagonists.

KEYWORDS: Tumor necrosis factor alpha antagonists, active tuberculosis, inflammatory bowel disease, flow cytometry

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CORRESPONDING AUTHOR: Patricio Escalante, Department of Pulmonary and Critical Care Medicine, Mayo Clinic, 200 First St. SW, Rochester, MN 55905, USA. Email: Escalante.patricio@mayo.edu

Introduction

Tumor necrosis factor α antagonists (TNFαAs) are increasingly used to treat connective tissue diseases and inflammatory bowel disease. Tumor necrosis factor α antagonists are associated with a high incidence of active tuberculosis (TB) that can reactivate despite chemoprophylaxis on some reports.1 The role of tumor necrosis factor α (TNF-α) in the immune response to Mycobacterium tuberculosis (MTB) is important but not entirely clear. In animal models, it appears to play a significant role in disease containment and granuloma formation2–4; which may explain the increased risk of patients on TNFA to develop TB, including disseminated disease.

Moreover, current diagnostic tests, tuberculin skin testing and interferon gamma release assays, have false-negative results in immunosuppressed patients5,6 and cannot distinguish between active and latent TB infections.6 Combinatorial immunoassay profiling using flow cytometric (FC) detection of co-expression of surface markers CD25 (interleukin 2 receptor) and CD134 (a TNF-α receptor) can identify antigen-specific effector CD4+ and CD8+ T-cell activation in latent TB infection (LTBI), and early studies suggest that it can distinguish between unexposed subjects, untreated subjects with LTBI, and treated patients with LTBI.7 This diagnostic strategy has not been used in active TB or in immunosuppressed patients receiving TNFA. We present a case of a patient on infliximab for ulcerative colitis who presented with disseminated TB. As part of a research study, we performed FC immune profiling.

Case Report

A 19-year-old US-born college student with a past medical history significant for ulcerative colitis treated with infliximab for the past 3 years and negative tuberculin skin test at initiation of TNFA was evaluated for a 3-month history of fever, night sweats, weight loss, productive cough, and abdominal pain. Several weeks prior to evaluation, she was treated for community-acquired pneumonia with azithromycin without symptomatic improvement. She was additionally treated with a short course of ciprofloxacin and prednisone for possible ulcerative colitis exacerbation. She had no known TB exposure including prior travel to endemic TB areas.

Laboratory evaluation revealed an afebrile woman in mild distress. Vital signs were notable for mild hypoxia (Spo2 = 92% on room air). She had no palpable lymphadenopathy. Bilateral rhonchi were present on pulmonary auscultation. Remainder of physical examination was unremarkable.

Laboratory evaluation revealed a normal complete blood count and inflammatory markers. Human immunodeficiency virus (HIV) testing was negative. Quantiferon-TB Gold in-Tube (QFT) test was positive (2.62 IU/mL). Transbronchial lung biopsy and bronchoalveolar lavage showed acid-fast-positive mycobacteria. Transbronchial lung biopsy and bronchoalveolar lavage showed acid-fast-positive mycobacteria.
bacilli, and subsequent cultures grew pan-sensitive MTB. Computed tomography of the chest, abdomen, and pelvis revealed miliary pulmonary pattern, patchy nodular infiltrates, and mediastinal lymphadenopathy with peritoneal and omental involvement (Figure 1). She did well after completion of 6 months of anti-TB therapy.

**FC Immunoprofiling**

In addition to the clinical QFT test, peripheral blood mononuclear cells (PBMCs) were analyzed by FC as part of a research study. This research study was approved by Mayo Clinic Institutional Review Board (Mayo IRB number 09-00325300). Peripheral blood mononuclear cells were isolated by Ficoll-Paque separation from 40 mL of heparinized blood within 1 hour of collection and cryopreserved in liquid nitrogen until stimulation. Multiparameter antigen stimulation with costimulatory antibodies (MTB-purified protein derivatives (PPD), region of difference 1 (RD1) peptide antigen [ESAT-6/CFP-10 peptide mix or specific MTB antigens], positive and negative controls) was completed. The PBMC sample and antigens were incubated for 48 hours at 37°C and then stained with fluorescent dye–conjugated anti-CD3, anti-CD4, anti-CD8, anti-CD25, and anti-CD134 antibodies and isotype controls. About $2 \times 10^5$ cells were analyzed by fluorescence-activated cell sorting (FACS) (BD FACSCanto) and gated using FlowJo software and Kaluza FC software. Detailed methods have been reported previously.7

CD25^+CD134^+ co-expression was detected on 0.34% and 0.84% of RD1 peptide and PPD-stimulated CD3^+CD4^+ T cells, respectively (Figure 2). In addition, upregulation of CD25^+CD134^+ was present on 0.26% and 0.59% of RD1 peptide and PPD-stimulated CD3^+CD8^+ T cells, respectively. These results were in the range of untreated LTBI associated with an increased risk of TB reactivation, as previously described,7 and suggest possible active TB infection in an immunosuppressed and symptomatic patient.

**Discussion**

Tumor necrosis factor α antagonists are associated with an increased risk of development of active TB. Extrapulmonary TB and disseminated TB represent one-half and one-quarter,
respectively, of TB cases in patients receiving TNFA leading to many atypical presentations. Moreover, current diagnostic tools, tuberculin skin tests and interferon gamma release assays, can be false negative in this population and do not distinguish between active and latent infections. The FC-based assays of T-cell markers could potentially provide an additional diagnostic tool to identify patients on TNFA with latent and active TB. Combinatorial interferon gamma release assays and FC assays assessing TB antigen–induced T-cell CD25 (interleukin-2 receptor α chain) and CD134 (TNF-α receptor superfAMILY member) co-expression were recently described as a method to risk stratify patients with LTBI. Furthermore, this strategy has been used to identify patients with LTBI with HIV co-infection. However, FC has not been used to clinically identify active TB, and the effects of TNFA on the FC detection of CD134 have not been described.

Our patient was on infliximab for several months prior to presenting with a clear diagnosis of disseminated TB in an immunosuppressed host. Flow cytometric immune profiling was completed as a research tool to determine the feasibility of this strategy in immune compromised patients with active TB on TNFA therapy. The result demonstrated that this FC immune profiling strategy can detect antigen-specific T-cell activation in an immunosuppressed patient with disseminated TB receiving TNFA; however, further validation is warranted.

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Author Contributions
All authors contributed to the research and construction of this manuscript.

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