Flow Cytometric Immune Profiling in Infliximab-Associated Tuberculosis

Clinical Medicine Insights: Case Reports
Volume 10: 1–3

DOI: 10.1177/1179547617724776

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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 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

RECEIVED: March 28, 2017. **ACCEPTED:** July 13, 2017.

PEER REVIEW: Two peer reviewers contributed to the peer review report. Reviewers' reports totaled 243 words, excluding any confidential comments to the academic editor.

TYPE: Case Report

FUNDING: The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This research was supported by internal Mayo Clinic grants (The 2009 and 2011 Lucille Nelson Clinical Career Development Award in Pulmonary Research; the 2011 Mayo Clinic Center for Clinical and Translational Sciences (CCaTS) Career Transition Award; the 2012 Mayo Clinic CCaTS Novel Methodology Award; the 2014 Mayo Clinic Department of Medicine Career Development Time for Scholarly Physicians Award; and the 2010 Clinical Immunology and Immunotherapeutics Program Award (P.E.). This research was also supported by K23CA159391 (T.P.). Part of this project was also supported by Grant Number UL1 TR000135 from the National Center for Advancing Translational Sciences (NCATS). This paper's contents are solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health or Mayo Clinic. No other financial or material support for this work was provided to the authors and participants.

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: Drs. Escalante and Peikert and their institution have filed two patent applications related to immunodiagnostic laboratory methodologies for latent tuberculosis infection. To date, there has been no income or royalties associated with those filed patent applications. None of the authors have any other conflicts of interest to declare.

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Introduction

Tumor necrosis factor α antagonists (TNFAs) 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 formation[2–4]; 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 patients[5,6] 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 2α receptor) and CD134 (a TNF-α receptor superfamily member) 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 without improvement. She had no known TB exposure including prior travel to endemic TB areas.

Physical examination revealed an afebrile woman in mild distress. Vital signs were notable for mild hypoxia (SpO_2 = 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

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 09-003253 00). 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 2×10^5 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]

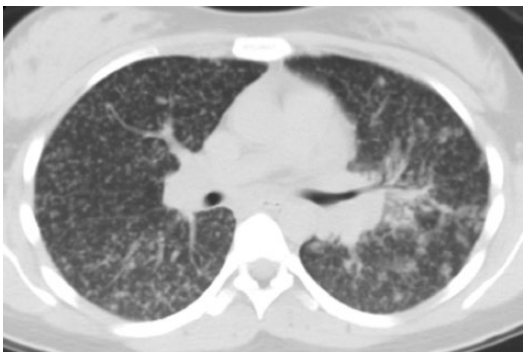

Figure 1. Bilateral diffuse miliary nodular infiltrates with mediastinal and bilateral hilar adenopathy.

$CD25^+CD134^+$ 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,

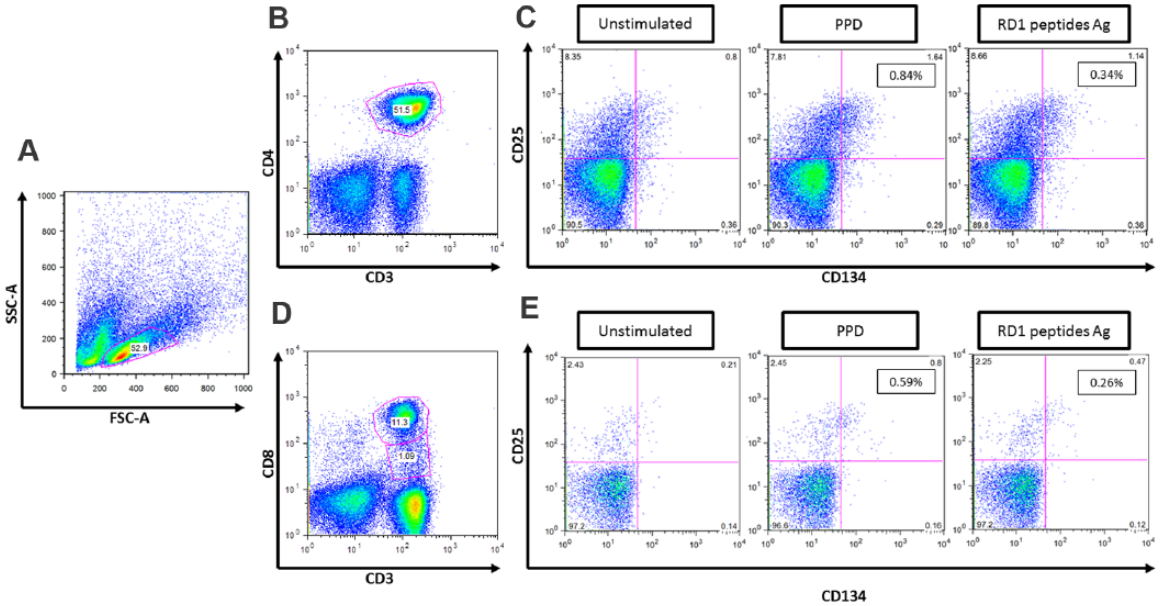

Figure 2. Flow cytometric gating strategy for detection of percentage of activated T cells ($CD3^+CD4^+$ and $CD3^+CD8^+$) co-expressing $CD25^+CD134^+$ markers. (A) Viable lymphocyte gate using side and forward scatter, (B) gate on $CD3^+/CD4^+$, (C) $CD3^+CD4^+/CD25^+CD134^+$ co-expression after 48 hours incubation with an unstimulated sample, PPD, and ESAT-6/CFP-10 peptides mix (RD1 peptide antigen), (D) gate on $CD3^+/CD8^+$, (E) $CD3^+CD8^+/CD25^+CD134^+$ co-expression after 48 hours incubation with an unstimulated sample, PPD, and RD1 peptide antigen. Percentages (boxes) indicate the calculated distribution of $CD25^+CD134^+$ among $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells after the subtraction of background (nil). FCS-A indicates forward scatter; PPD, purified protein derivatives; RD1, region of difference 1; SSC-A, side scatter.

respectively, of TB cases in patients receiving TNFA leading to many atypical presentations.[8] 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.[5] 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.[9–11] 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.[7] Furthermore, this strategy has been used to identify patients with LTBI with HIV co-infection.[12] 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.

Acknowledgements

This case was reported at the September 2016 European Respiratory Society Meeting in London, England, as a poster presentation.

Author Contributions

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

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