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Original article

Use of QuantiFERON-TB Gold In-tube assay in screening for neutralizing anti-interferon-γ autoantibodies in patients with disseminated nontuberculous mycobacterial infection

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ABSTRACT

Objective: Anti-interferon- γ (IFN-γ) autoantibodies (anti-IFN-γ Abs) have been increasingly recognized as an important cause of disseminated nontuberculous mycobacterial (DNTM) infection, and identification of this immunodeficiency impacts clinical management. However, the protean disease manifestations and inaccessibility to diagnostic tests in clinical settings hamper its early diagnosis. Here, we sought to determine whether QuantiFERON-TB Gold In-tube (QFT-GIT), a commercialized IFN-γ release assay, could be used to screen for neutralizing anti-IFN-γ Abs among previously healthy adults with DNTM infection.

Methods: Non-HIV patients with DNTM infection were prospectively enrolled for the QFT-GIT assays. We measured their plasma concentration of anti-IFN-γ Abs and their neutralizing capacity through enzyme-linked immunosorbent assay and flow cytometry. We then analysed the correlation between QFT-GIT results and the presence of neutralizing anti-IFN-γ Abs among patients with and without previously recognized immunosuppression, respectively.

Results: Irrespective of the autoantibody concentration or disease activity, all patients with neutralizing anti-IFN-γ Abs (100%, 30/30) had indeterminate QFT-GIT results because of extremely low or undetectable IFN-γ levels in the mitogen tubes. None of the four DNTM patients who were previously healthy and tested negative of anti-IFN-γ Abs had an indeterminate QFT-GIT result, and their IFN-γ levels in the mitogen tube were significantly higher than those of the patients with anti-IFN-γ Abs (8.28 IU/mL vs. 0.05 IU/mL, p 0.001).

Conclusion: An indeterminate QFT-GIT result because of undetectable or extremely low IFN-γ level in the mitogen tube suggests the presence of neutralizing anti-IFN-γ Abs in a previously healthy patient with DNTM infection. **U.-I. Wu, Clin Microbiol Infect 2018;24:159**

Introduction

The presence of anti-interferon-γ (IFN-γ) autoantibodies (anti-IFN-γ Abs) have been increasingly recognized as an adult-onset immunodeficiency which confers enhanced susceptibility to a number of systemic opportunistic infections, particularly those caused by nontuberculous mycobacteria (NTM) [1–3]. As the autoantibodies potently interfere with downstream activities induced by IFN-γ, including signal transducer and activator of transcription 1 (STAT1) phosphorylation, production of interleukin-12 and tumour necrosis factor-α [1,3], patients are often refractory to antimicrobial therapy and relapses occur frequently [3–5]. Although several investigators had advocated that anti-IFN-γ Abs should be examined in a previously healthy person with an unexplained course of disseminated NTM (DNTM) infection [1,4,6–9], delay in diagnosis has often been reported because of the highly diverse manifestations and inaccessibility to diagnostic assays in

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general clinical settings. This could result in patients undergoing excessive examinations and invasive diagnostic procedures or even receiving unwanted medications, because the manifestations of disseminated opportunistic infections could mimic those of malignancies and autoimmune disorders [6,10,11].

QuantiFERON-TB Gold In-tube (QFT-GIT), a US Food and Drug Administration-approved and commercially available IFN-γ release assay (IGRA), has been widely used in clinical settings for diagnosis of *Mycobacterium tuberculosis* (Mtb) infections [12]. This assay is performed by collecting whole blood into three separate tubes serving as a negative control (Nil tube), positive control (mitogen tube) and a TB antigen tube containing Mtb complex-specific recombinant antigens, respectively, and then measuring the concentration of IFN-γ secreted by the peripheral blood mononuclear cells (PBMCs) in each tube. We hypothesized that the presence of biologically active anti-IFN-γ Abs in a patient's blood might substantially neutralize IFN-γ secreted in the mitogen tube and in turn provide an indeterminate result. Thus, QFT-GIT could be used as a readily accessible and reasonable assay in general clinical settings to screen for the presence of neutralizing anti-IFN-γ Abs in an otherwise healthy person with DNTM infection.

Methods

Participants and definitions

We prospectively enrolled non-HIV adult patients with disseminated NTM infections regardless of the presence of comorbidities from six medical centres located in northern, middle, and southern Taiwan from March 2015 to December 2016. Disseminated NTM infection was defined as the isolation of NTM from the blood, bone marrow, or at least two noncontiguous organs [5]. Isolated bloodstream infection caused by central catheter use was excluded. Patients were grouped on the basis of the presence or absence of recognized immunosuppression for comparisons. Immunosuppression included classical inherited or acquired immune defects such as transplantation, malignancies, and systemic immunosuppressive therapy, which have been well recognized to predispose to DNTM infection [1,13,14]. Healthy volunteers 20 years of age or older were solicited from advertisements posted at the National Taiwan University Hospital. They were excluded if they had known immunodeficiency (e.g. HIV infection, malignancy, autoimmune disorder, use of immunosuppressive agents), fever or symptoms of acute infection within 7 days prior to enrolment. Whole blood was collected from each patient and a healthy volunteer on the same day for each experiment. Participants' demographics, medical comorbidities, laboratory results at disease onset, all microbiology and histopathology data, and details of past medical history suggestive of immunosuppressed status were recorded. All patients and healthy volunteers provided written consent for the study protocols approved by the research ethics committee of National Taiwan University Hospital (REC No. 201412163RIN).

Anti-IFN-γ abs enzyme-linked-immunosorbent assay (ELISA)

Plasma was collected from fresh heparinized blood of each participant and stored at −80°C until analysis. Microtitre plates were coated with 100 μL of IFN-γ (10 μg/mL; R&D Systems, Minneapolis, MN, USA) per well, incubated at 4°C overnight, and then washed three times with 200 μL of PBST (0.1% Tween 20/PBS, Sigma, St Louis, MO, USA). Recombinant mouse anti-human IFN-γ antibodies (R&D Systems) were used as standards with serial dilution. Plasma was diluted 10^{-5} times, and 100 μL of the diluent buffer (blank), samples, standards, positive and negative controls were added in duplicate to each well and incubated at room temperature for 3 hours. After washing three times with PBST, the standards were incubated with 100 μL of HRP-conjugated donkey anti-mouse IgG (Thermo Scientific, Rockford, IL, USA), while the patient plasma and control wells were incubated with 100 μL of HRP-conjugated sheep anti-human IgG (Thermo Scientific) for 1.5 hours. After three washes, 100 μL of TMB substrate (Thermo Scientific) was added to each well and incubated for 30 minutes, and 100 μL of stopping solution (Thermo Scientific) was added before determining the optical density at 450–550 nm. The normal range for the anti-IFN-γ Abs concentration was defined by the 99th percentile for healthy volunteers. Concentrations higher than the normal range were classified as positive for anti-IFN-γ Abs.

Detection of IFN-γ-induced STAT1 phosphorylation

To determine whether a patient's plasma contained neutralizing anti-IFN-γ Abs and showed interference in IFN-γ signalling, fresh PBMCs were isolated from heparinized whole blood of a healthy volunteer through density-gradient centrifugation using lymphocyte separation medium (GE Healthcare Uppsala, Sweden) and resuspended in complete RPMI 1640 medium (Gibco BRL, Grand Island, NY, USA) containing penicillin and streptomycin (Life Technologies, Carlsbad, CA, USA) and 20 mM HEPES with 10% of a patient's plasma. Cells were unstimulated or stimulated with IFN-γ (50 ng/mL; R&D Systems) for 15 minutes at 37°C, and stained with FITC-conjugated anti-CD14 (BD Harlingen, San Diego, CA, USA). After washing, cells were fixed and permeabilized with 100% ice cold methanol for intracellular staining with the anti-phosphorylated signal transducer and activator of transcription antibody (Anti-STAT1 [pY701] conjugated with Alexa Fluor 647; BD Biosciences, San Jose, CA, USA) [5]. The same experiment was repeated using PBMCs from the healthy volunteer with autologous plasma, as well as using the patient's PBMCs with plasma from the same healthy volunteer as controls (Fig. S1). Data were collected using FACSCalibur (BD Biosciences), analysed using FlowJo (Treestar), and graphed with Prism6 (Graphpad). A participant was considered to have biologically active anti-IFN-γ Abs if an elevated concentration of anti-IFN-γ Abs was detected in plasma, as measured through ELISA, which could inhibit IFN-γ-induced STAT1 phosphorylation in monocytes (Fig. S1).

QFT-GIT assay

The QFT-GIT assay (Qiagen, Carnegie, Australia) was performed strictly according to the manufacturer's instructions [15] by a technician who was blinded to the clinical information. In brief, fresh whole blood specimen was injected into the three tubes separately and incubated for 18 h (overnight) at 37°C in a humidified atmosphere. Plasma was collected and stored at −20°C until analysis. The optical density of IFN-γ released in each tube was determined at 450–620 nm on an ELISA reader (DS2, Dynex Technology Inc., Chantilly, VA, USA). All tests were performed in duplicate. The IFN-γ concentration in each tube and the results of the assay were determined using the QuantiFERON-TB Gold analysis software and interpreted as specified by the manufacturer. The result was considered positive if the IFN-γ level after stimulation with TB antigens minus the negative control was ≥0.35 IU/mL and ≥25% of the negative control. The test was considered negative if the IFN-γ level was <0.35 IU/mL (after subtraction of the negative control). The test result was considered indeterminate if (1) the negative control was ≥8.0 IU/mL or (2) the positive control was <0.5 IU/mL (after subtraction of the negative control).