J Clin Tuberc Other Mycobact Dis 9 (2017) 5–9

Contents lists available at ScienceDirect

J Clin Tuberc Other Mycobact Dis

journal homepage: www.elsevier.com/locate/jctube

A mode of error: Immunoglobulin binding protein (a subset of anti-citrullinated proteins) can cause false positive tuberculosis test results in rheumatoid arthritis

Maria Greenwald*, JoAnn Ball, Atul Deodar

Maria Greenwald, MD, FACR, Desert Medical Advances, 72855 Fred Waring Dr., suite A6 Palm Desert, CA 92260, United States

ARTICLE INFO

Keywords:
Tuberculosis
IGRA
Rheumatoid arthritis
Interferon
Anti-citrullinated peptide antibody (ACPA)
Immunoglobulin binding protein (BiP)

ABSTRACT

Citrullinated Immunoglobulin Binding Protein (BiP) is a newly described autoimmune target in rheumatoid arthritis (RA), one of many cyclic citrullinated peptides(CCP or ACPA). BiP is over-expressed in RA patients causing T cell expansion and increased *interferon* levels during incubation for the QuantiFERON-Gold tuberculosis test (QFT-G TB). The QFT-G TB has never been validated where *interferon* is increased by underlying disease, as for example RA.

Of ACPA-positive RA patients ($n = 126$), we found a 13% false-positive TB test rate by QFT-G TB. Despite subsequent biologic therapy for 3 years of all 126 RA patients, none showed evidence of TB without INH. Most of the false-positive RA patients after treatment with biologic therapy reverted to a negative QFT-G test. False TB tests correlated with ACPA level ($p < 0.02$).

Three healthy women without arthritis or TB exposure had negative QFT-G TB. *In vitro*, all three tested positive every time for TB correlating to the dose of BiP or anti-BiP added, at 2 ug/ml, 5 ug/ml, 10 ug/ml, and 20 ug/ml.

BiP naturally found in the majority of ACPA-positive RA patients can result in a false positive QFT-G TB. Subsequent undertreatment of RA, if biologic therapy is withheld, and overtreatment of presumed latent TB may harm patients.

Introduction

The interferon-gamma release assays (IGRA) were developed about 20 years ago to screen for exposure to tuberculosis (TB) especially to screen patients with human immunodeficiency virus who had low resistance to TB. The interferon tests for TB were therefore specifically designed for immunosuppressed patients with low CD4 and low induction of interferon. These IGRA tests do not test for TB directly but measure interferon release as a surrogate marker. Actually proving TB infection involves obtaining sputum or biopsy materials to culture tuberculosis which can be difficult and subsequent culture to prove growth of tuberculosis can take months to complete. In the past decade, interferon-gamma release assays were approved, and these tests require one to two weeks for results due to complicated processing at two different laboratory facilities; three different plasma tubes are incubated for 16–24 h at the test site, then the specimens are transported to a central facility equipped for measurement of interferon release. (See supplement 1.) Alternatively, the more traditional tuberculin skin test using purified protein derivative (PPD) testing for TB requires 3 days with minimal personnel training and no equipment. Mantoux PPD tests exemplify classic type IV delayed type hypersensitivity, with no interferon involved for screening TB.

Background/Purpose: Rheumatoid arthritis (RA) is an autoimmune systemic disease that can spontaneously release interferon as well as other immune cytokines [1]. RA develops synovial disease in up to 68 diarthrodial joints, and also a systemic disease stimulating cytokines in the bone marrow, lung, pleural and pericardial tissue, blood vessels, skin, eye, salivary glands, central and peripheral neural tissues. Autoantibodies such as rheumatoid factor (RF) and anti-citrullinated protein (ACPA) together have a sensitivity for the diagnosis of RA of up to 85%. Though specificity for RA with RF only is 40%, specificity using serum ACPA rises to 98% [2]. The trigger to initiate symptoms with RA is unknown, but the fact that ACPA is present years before symptoms, has led to speculation that antigen targets of ACPA play important roles in the pathogenesis of RA [3].

In autoimmune disease, there is a breakdown of tolerance mechanisms, resulting in log-rhythmic escalation of autoimmune activity (including spontaneously released interferon). In RA specifically, ACPA

* Corresponding author.
E-mail addresses: dmaregulatory@gmail.com, greenwald.maria@gmail.com (M. Greenwald).

http://dx.doi.org/10.1016/j.jctube.2017.08.004
Received 3 December 2016; Received in revised form 16 May 2017; Accepted 14 August 2017

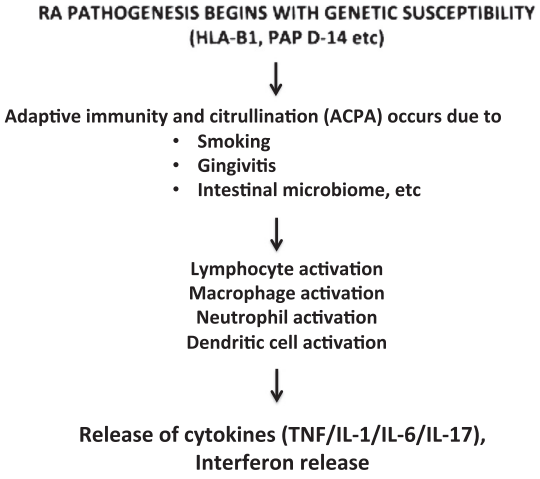

Fig. 1. Pathogenesis of rheumatoid arthritis and subsequent secretion of spontaneous interferon.

are produced in response to citrullination of native proteins. Although citrullination is a physiologic normal response to inflammation, in RA there are very high levels of BiP citrullination; triggers identified to induce citrullination include cigarettes, gum disease, and intestinal bacteria Prevotella copri [4,5,6]. Therefore, the current causal hypothesis for RA is a genetic predisposition (such as HLA-DRB1), followed by an inflammatory condition inducing citrullination (raising ACPA), promoting activated T cells, B cells, and antigen presenting cells. The activated immune cell proliferation results in cytokine and *interferon release*. (Fig. 1)

Active research into the pathogenesis of RA concerns antibody reactivity against antigens that are modified posttranslationally to contain citrulline rather than arginine. Recently, polymorphisms in PAD14 gene, which codes for protein citrullination have been shown to be associated with RA susceptibility [7]. Immunoglobulin binding protein (BiP) contains 27 arginine residues that can be replaced by citrulline [8].

Citrullinated BiP is a newly described subgroup of anti-cyclic citrullinated peptide (ACPA). Citrullinated BiP in both serum and synovial fluid is over-expressed in RA patients and correlates with local lab measurements of ACPA [8]. Peripheral T cells from RA patients show high levels of proliferation in response to BiP, whereas healthy donor T cells do not [8]. T Cells from RA patients have a strong proliferation response to BiP and this response correlates significantly with clinical measures of RA disease activity . In response to BiP, RA peripheral blood mononuclear cells release tumor necrosis factor, IL-6, IL-17 and *interferon-gamma* [9].In one study, in response to BiP, RA synovial T cells produced *interferon-gamma* at >5000 pg/ml [10]. Depletion of CD8 cells further increases cytokines, including *interferon-gamma*. Many of the biologic therapies in use today change the composition of immune cell subsets in RA [11]. This makes interpreting interferon testing in RA extremely complicated since subsets of immune cells vary both with RA disease and with biologic therapy. There are no "control" values for interferon validated in RA. Depending on the immune cell types found in an individual RA patient, stimulation of *interferon-gamma* is widely variable. Also antibody to BiP (anti-BiP) are frequent in RA and higher than in the normal population [10]. Use of IGRA testing in autoimmune diseases where interferon is easily stimulated should be critically suspect and if possible avoided as a screening test for TB.

We postulated that one way to manipulate QFT-G TB testing would be to vary *in vitro* serum BiP and anti-BiP (both naturally found in elevated levels in RA). Either BiP or anti-BiP would cause T cell expansion and increase interferon during incubation of the QuantiFERON-Gold tuberculosis in-tube test (QFT-G TB). Since the test is incubated for 16–24 h, the autoantigen BiP found in RA sera could spontaneously produce interferon due to auto-immune activity, and thus result in a false positive TB test, regardless of the TB antigen in the tube. The interferon spontaneously produced in a untreated active RA patient may be extremely high, then resolve as biologic immune treatment controls the RA autoimmunity (see the follow up data from interferon tests in Fig. 2.) Certainly, after RA patients are treated with biologic immune-modulators, the reproducibility of interferon-gamma levels would be even more unpredictable . Although part of our hypothesis, the effects of biologic immune therapy on QFT-G TB was not tested by specific biologic therapies due to small numbers of subjects in this study, beyond showing that biologic treatment caused the QFT-G TB tests in many cases to revert to normal.

Methods: ACPA-positive active RA patients (n = 126) meeting the ACR-EULAR 2010 Classification Criteria for RA were tested with QFT-G TB (sourced Cellestis) prior to initiating biologic therapy [12]. Active RA disease were defined as patients with a minimum of 6 tender and 6 swollen joints, and a dermal joint temperature of over 97 F [13]. RA patients were allowed MTX at 15–25 mg/week along with folic acid 1 mg/day but no other traditional disease modifying conventional therapy. No use of tobacco was permitted. All patients had full TB evaluation at baseline and annually for three years with medical and travel history, physical examination, standard hematology and chemistry laboratory testing, chest PA and lateral radiographs, PPD and control skin testing (The IGRA test QFT-G was drawn before the Mantoux PPD skin test was performed.) [14,18] RA patients had ACPA, RF,

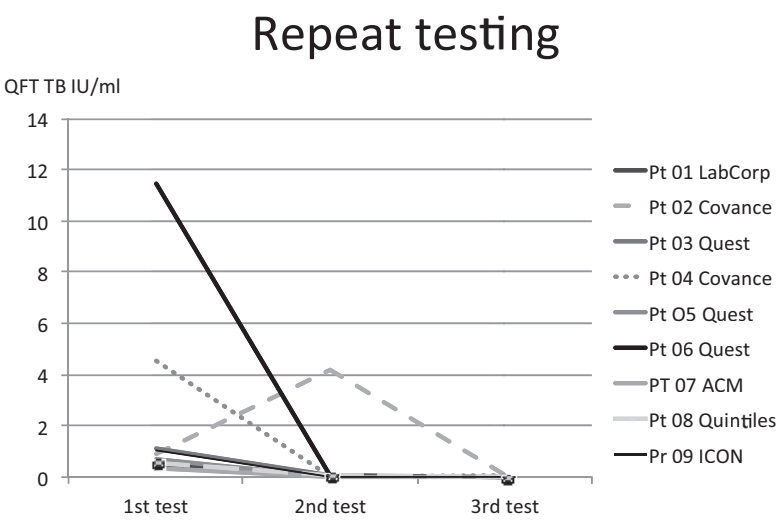

Fig. 2. QFT-G test was repeated in 9 positive ACPA (CCP) RA patients with negative repeat testing. The second test was performed at 1 year after biologic treatment began, and the third test was repeated at 2 years. No patient received INH therapy.