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Interferon Gamma Test for the Detection of *Mycobacterium tuberculosis* Complex Infection in *Macaca mulatta* and Other Nonhuman Primates

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Abstract

We have formatted an assay to detect *Mycobacterium tuberculosis* complex infections of nonhuman primates. Commercially available reagents were used to elicit a specific immune response that was measured by interferon-gamma release. Initial evaluation using blood samples from Rhesus macaques experimentally infected *with M. tuberculosis* distinguished infected versus uninfected animals.

Keywords

Interferon Gamma Release Assay (IGRA); Purified Protein Derivative-Tuberculin (PPD); Cell Mediated Immune Response (CMI)

Introduction

Mycobacterium tuberculosis (MTB) complex infections in nonhuman primates (NHPs) continue to be a serious threat to animal health and an occupational risk to the humans who have direct or indirect contact with them. The primary mode of transmission is respiratory, following inhalation of the infectious bacteria in aerosols from hosts with active infection. Infection continues to be found in both new incoming and established colony animals, albeit at a significantly reduced rate compared to previous years [1–5]. Primary infection results in a spectrum of disease from latent (noninfectious) to active (infectious) [3]. Because the triggers to progress through the continuum from latent to active are not all understood, ongoing surveillance to detect all infected animals is important. The *in vivo* intradermal tuberculin skin test (TST) using mammalian old tuberculin (MOT) has been the primary diagnostic tool for MTB infection in NHPs since the 1940's. The TST is a simple, inexpensive procedure, but requires training to perform accurately. The limited sensitivity

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and specificity, subjectivity of readout, and the periodic lack of MOT supply are documented deficiencies [5]. In 2018, repeatedly skin test negative but MTB complex infected animals were found in an established cynomolgus macaque colony [6]. Alternative *in vitro* interferon gamma (IFNy) release assays (IGRA) are now used widely in humans and cattle [7, 8]. A similar IGRA for nonhuman primates [1, 9] was discontinued by its manufacturer in 2015, leaving no readily available alternative or adjunct to TST for monitoring specific cell mediated immunity to MTB infection. The Primate Assay Laboratory at the California National Primate Research Center has formatted an IGRA using commercially available avian and bovine purified protein derivative tuberculin (aPPD, bPPD) for MTB complex specific stimulation of cells in whole blood and monkey IFNy ELISA reagents for detection. Assay performance characteristics were determined by testing blood samples collected and stimulated at regular time points from rhesus macaques (RM) experimentally infected with MTB at the Oregon National Primate Research Center (ONPRC).

Materials and Methods

Humane Care Guidelines: All Indian-origin rhesus macaques described in this study were used with the approval of the ONPRC Institutional Animal Care and Use Committee, under the standards established by the U.S. Federal Animal Welfare Act and the Guide for Care and Use of Laboratory Animals[10, 11].

Experimental infection and sample collection: Sixteen Indian-origin rhesus macaques received intrabronchial Mycobacterium tuberculosis (Erdman KO-1, obtained from BEI Resources (NR-1504). Doses ranged from 2 CFU/ml to 100 CFU/ml. Intravenous blood samples for GIFT assay analysis were collected post-infection day 0, 14, 28, 42, 56 (n=5), 70 (n=3), and 84 (n=2). Infection progression was confirmed by MTB-specific T-cell response assays. CT scans were performed at two week intervals post-inoculation to quantify pulmonary disease. Animals were euthanized and necropsied at 6–10 weeks post challenge for anatomic pathology studies and confirmation of infection.

Sample processing: Within 4 hours of collection, 1 ml of heparinized whole blood was added to each of four vials containing 1) negative control: PBS 2) positive control: pokeweed and concanavalin A mitogens (Sigma-Aldrich, St. Louis, MI) 3) aPPD 4) bPPD (both PPDs- Thermo Fisher, Lelystad, The Netherlands). The vials were mixed well and incubated overnight (16 +/−4 hours) at 37°C; and then the plasma was collected by centrifugation at 1000xg for 15 minutes and stored frozen at −80 °C until all the samples were collected (Figure 1).

The plasma samples were thawed and assayed for IFNγ using commercially available Monkey IFNy ELISA reagents as components or a complete kit (MabTech, Minneapolis, MN) according to the protocol as outlined in Figure 1. The manufacturer's literature claims specificity for human, macaque (cynomolgus, rhesus and pig-tailed), baboon, sooty mangabey, African green monkey, aotus, common marmoset, and squirrel monkey IFNy.

Results

The delta bPPD-aPPD optical density values for the RM experimentally inoculated with MTB are shown in Figure 2. The pre-inoculation values ranged from −0.018 to 0.026. In 15 monkeys with confirmed infection, values for all day 28 and subsequent time points ranged from 0.030 to 2.071. Day 14 values were intermediate. At day 14, 7/16 monkeys were more reactive with aPPD as compared to bPPD before developing greater bPPD reactivity at the next time point. Infection was not confirmed and the IFNy response did not exceed baseline in one monkey that received 2 CFU/ml.

Discussion

The GIFT assay accurately detected MTB infection in 15 RM beginning at day 28 post experimental infection. Infection was attempted but failed in one additional animal; it was confirmed GIFT negative. Since IFNy levels can be a marker of non-specific stimulation and vary widely across individuals, mock stimulation with PBS was used as a baseline to normalize reactivity for each animal. MTB infection can result in immunosuppression; thus, stimulation with the mitogens concanavalin A and pokeweed were used to ensure that the animal was immune competent enough to rule out a false negative response. aPPD was originally included as a specificity control to rule out infection by environmental *Mycobacterium*. The finding of reactivity to aPPD at day 14 is intriguing and needs to be further investigated to determine if it is an assay artifact or a useful marker in early infection. Since *M. bovis* is a member of the MTB complex group it should elicit an IFNy response from infected animals. Although this study does not include enough animals to do a complete statistical analysis of any power, it is an important proof of concept. We are now testing additional uninfected colony animals to establish the normal range of reactivity and confirm specificity. It is difficult to obtain samples from naturally occurring MTB complex infections; therefore, accumulating GIFT results from such animals to determine sensitivity is difficult. To expedite this important validation, we are sharing the assay protocol widely (particularly to institutions in areas of the world where naturally occurring tuberculosis in NHPs is common) and cumulating GIFT results for confirmed infected animals. The GIFT assay can be a useful screening tool in what will likely be a multi-assay algorithm for improved detection of MTB complex infection.

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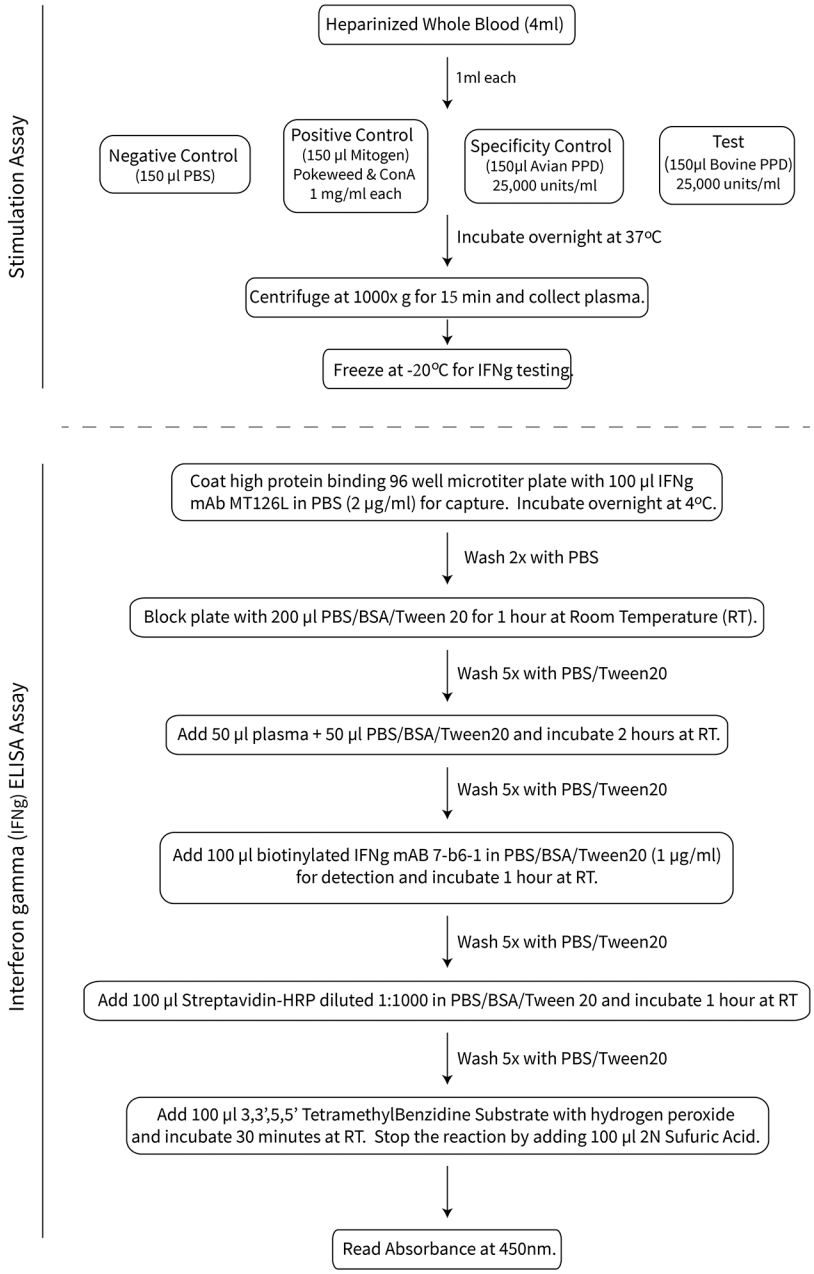

Figure 1.
Outline of GIFT assay steps

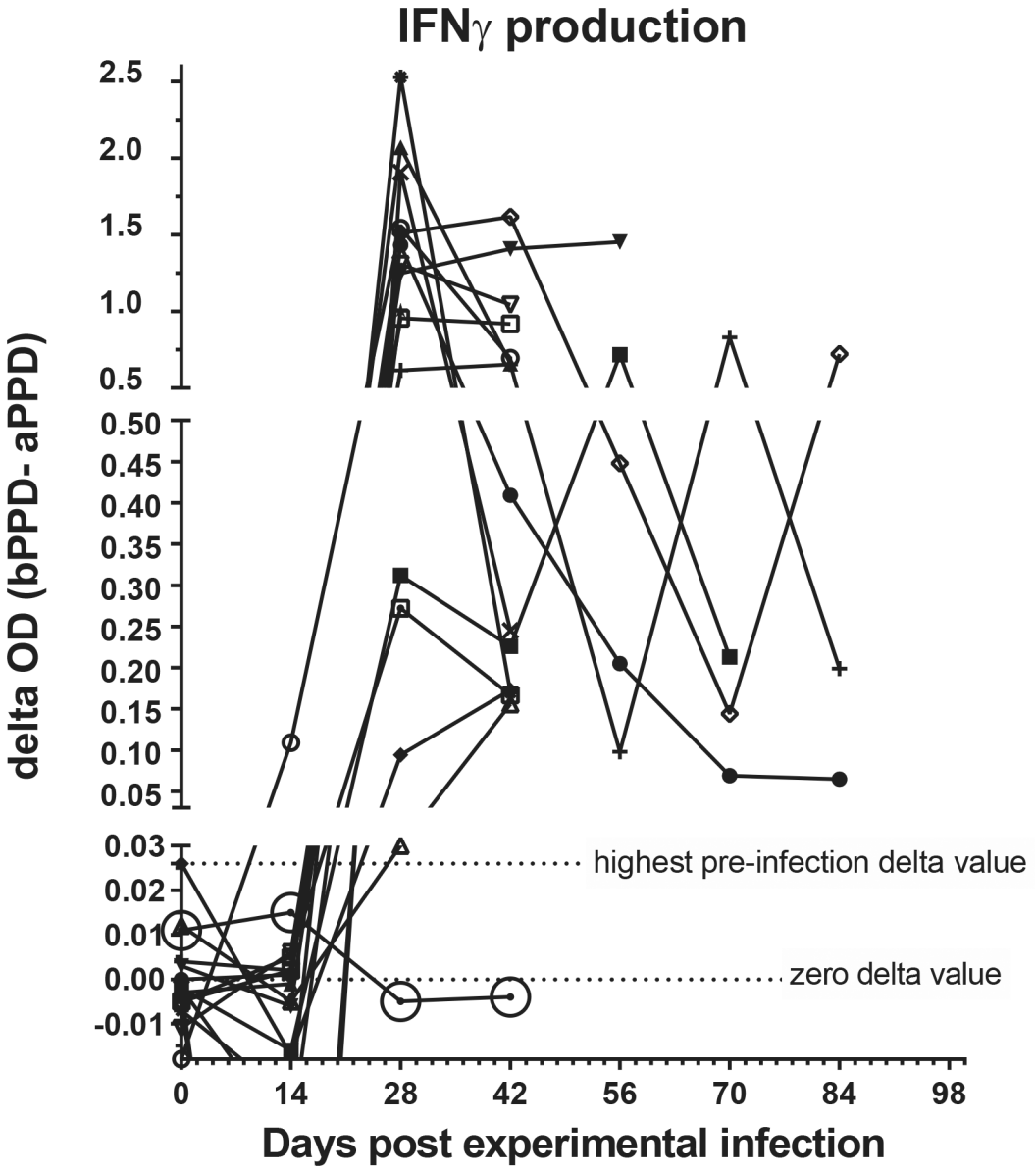

Figure 2.
GIFT Assay Results from Rhesus macaques experimentally infected with tuberculosis