Evaluation of DPPD, a Recombinant *Mycobacterium tuberculosis* Skin Test Antigen

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Background

- 300 million people worldwide are infected with M. tuberculosis (M. Tb)
- Accurate and affordable tests for both latent tuberculosis infection (LTBI) and active disease are an essential tool to reduce the burden of TB worldwide
- Despite over 100 years of test development, significant gaps remain in access to optimal TB testing
Current Gaps in Tuberculosis Testing

- PPD has limitations to sensitivity and specificity
  - Specificity reduced in the setting of prior BCG vaccination and exposure to other mycobacterial species
  - Sensitivity reduced in the setting of immunocompromised state and active disseminated disease
- PPD production is marked by heterogeneity of final product and severe supply limitations
- Interferon-gamma release assays (IGRAs) are expensive, not widely available in many areas where TB is endemic, and have limited sensitivity in immunocompromised hosts and persons with active disease
Product Profile of an “Ideal” TST

- **Acceptable**
  - Safe / reduced toxicity
- **Accurate**
  - High sensitivity among high-risk groups, including persons with HIV
  - High specificity among persons in areas where TB is endemic (prior BCG vaccination and exposure to environmental mycobacteria)
- **Available**
  - Locally-producible in high quantity with minimal lot-to-lot variability
- **Agile**
  - Able to be used in a variety of clinical settings, including LTBI and active TB infection
- **Annual**
  - Does not sensitize for annual tests (lower dose of protein)
- **Affordable**
DPPD, a Novel Recombinant Protein Tuberculin Skin Test Antigen

Recombinant protein derived from a gene unique to M. Tb

Named for 1st four amino acids of N-terminus of HPLC fraction (DPPD)

Shown to elicit delayed-type hypersensitivity in guinea pigs infected with M. Tb but not other members of the genus Mycobacterium

Excellent safety profile in Phase 1 and 2 studies
Cloning of a \textit{Mycobacterium tuberculosis} Gene Encoding a Purified Protein Derivative Protein That Elicits Strong Tuberculosis-Specific Delayed-Type Hypersensitivity

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The purified protein derivative (PPD) skin test has been used for the diagnosis of tuberculosis for more than 75 years. However, the test lacks specificity because all mycobacteria share antigens present in PPD. Therefore, sensitization with nontuberculous pathogenic or with environmental nonpathogenic mycobacteria can lead to positive skin tests. This communication describes a novel PPD protein present only in tuberculous complex mycobacteria. A recombinant protein was obtained and named DPPD on the basis of the first 4 amino acids of its N-terminus sequence. DPPD elicited delayed-type hypersensitivity (DTH) in 100\% of \textit{Mycobacterium tuberculosis}-infected guinea pigs but in no animals sensitized with several organisms representative of all members of the \textit{Mycobacterium} genus. Preliminary results indicate that DPPD induces strong and specific DTH in humans. This work points to the definition of a single recombinant \textit{M. tuberculosis} protein that may be an alternative to the PPD test.
How was DPPD Selected / history

- Tuberculin (PPD) fractionated by HPLC
- Fractions Evaluated in Guinea Pig
- N terminal sequence determined (DPPD)
- Full length gene cloned, expressed
- Protein purified, prepared for clinical evaluation
Phase 2 Study in High-Risk Populations and Active Disease

Study Design
- Prospective, Open-label, clinical trial conducted over 1 year in Bahia, Brazil

Study Population
- Brazilian adults who received BCG vaccination at birth
- Group 1: Asymptomatic, HIV-negative volunteers
- Group 2: HIV-negative persons with active TB
- Group 3: HIV-positive persons with active TB

Intervention
- All ppts received intradermal injections of both DPPD and PPD simultaneously in opposite arms

Definitions
- BCG vaccination verified by scar
- Microbiologic and clinical criteria for active TB infection

Endpoint
- Proportion of participants in each group with positive TST, stratified by type of TST, HIV status, presence of active disease
Evaluation of DPPD, a single recombinant Mycobacterium tuberculosis protein as an alternative antigen for the Mantoux test (Tuberculosis 2001. 81:353)

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Summary  We have recently described a recombinant antigen (DPPD). The gene is unique to the M. tuberculosis complex organisms. Here we present results of a clinical investigation using DPPD. Mantoux test using both PPD and DPPD was initially performed in 26 patients with confirmed pulmonary tuberculosis and in 25 healthy PPD negative individuals. PPD and DPPD elicited DTH in 24 out of the 26 individuals. No DTH was observed in any of the PPD negative individuals. A clinical trial was performed in a population of 270 clinically healthy individuals. Because the DPPD gene is not present in non-tuberculous bacilli, these results suggest that this molecule can be an additional tool for a more specific diagnosis of tuberculosis. &2001Harcourt Publishers Ltd
**Study Cohort**

- **375 Screened**
  - **202 Excluded**
    - 137 asymptomatic without BCG scar
    - 65 without microbiologic confirmation of active TB
  - **173 Enrolled**
    - **95 healthy controls**
    - **78 Persons with Active TB**
      - 40 HIV-negative
      - 38 HIV-positive
Distribution of Induration, by TST and Risk Group

- Asymptomatic BCG-Vaccinated
- HIV-Negative Active TB
- HIV-Positive Active TB
Definitions for Positive TST

• Asymptomatic, without HIV, previous history of BCG, from TB endemic area: **10 mm induration**

• Suspicion of active TB or HIV positive, G positive or negative: **5 mm induration**
Distribution of Induration, by TST and Risk Group

- Asymptomatic BCG-Vaccinated
  - PPD
  - DPPD
- HIV-Negative Active TB
  - PPD
  - DPPD
- HIV-Positive Active TB
  - PPD
  - DPPD

False Positives
False Negatives
Proportion of Participants with a Positive Test

Asymptomatic BCG Immunized: 53.7%, 9.5%
HIV-Negative Active TB: 100.0%
HIV-Positive Active TB: 50.0%, 71.1%
Conclusions

- A novel TST using low-dose of a recombinant protein from M. Tb (DPPD) displayed:
  - **Improved specificity** compared with PPD in asymptomatic / healthy persons previously vaccinated with BCG in a TB-endemic region
  - **Better sensitivity** compared with PPD for detecting active TB among HIV-positive individuals
  - **Excellent safety** with no reported hematomas or injection-site reactions
- Future studies should evaluate the DPPD in:
  - HIV-positive persons with previous BCG vaccination but without active TB
  - HIV-negative persons with active TB
  - Close-contacts to known TB cases
- The DPPD may represent an excellent alternative to the PPD for diagnosing LTBI in endemic regions or for assisting with the diagnosis of active TB in high-risk individuals
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