Alpha-crystallin: A Marker of Latent TB Infection

Fred Quinn
Department of Infectious Diseases
College of Veterinary Medicine
University of Georgia, Athens

fquinn@uga.edu
http://www.vet.uga.edu/id
"the single most valuable diagnostic test for use in the United States would be one that could predict which latently infected individuals are most at risk of developing active tuberculosis." As further stated in the report "........because so many of the cases in the United States are not the result of recent transmission of tuberculosis, but rather are the result of reactivation of latent infection, the greatest needs in the United States are new diagnostic tools for the more accurate identification of individuals who are truly infected and who are at risk of developing tuberculosis."
TB Diagnostics

Chest Radiograph

- Abnormalities often seen in apical or posterior segments of upper lobe or superior segments of lower lobe
- May have unusual appearance in HIV-positive persons
- Cannot confirm diagnosis of TB

AFB smear

Arrow points to cavity in patient's right upper lobe.

AFB (shown in red) are tubercle bacilli

Culture

- Results in 4 to 14 days when liquid medium systems used

Colonies of *M. tuberculosis* growing on media
TB Diagnostics
Tuberculin Skin Test

- Inject 0.1 mL PPD intradermally
- Should produce wheal of 6–10 mm

- Read 48–72 hrs after placement
  - If HCW returns after >72 hrs, place and read another TST*
  - Do not let HCWs read their own results
- Find and measure induration
  - Measure diameter of induration across the arm
  - Do not measure redness
TB Diagnostics
QuantiFERON®-TB Gold Test

Stage One – Blood Stimulation and Harvesting

1. Dispense 1 mL of subject's heparinized whole blood into 4 wells of a 24-well culture plate.
2. Add 3 drops of the appropriate stimulating antigen to each well.
4. Harvest at least 200 µL plasma from each well. Store in racked microtubes or uncoated microplates.

Stage Two – Human IFN-γ ELISA

1. Add 50 µL of conjugate solution to each well. Add 50 µL of plasma or standard.
2. Shake covered plate for 1 min. Incubate for 120 minutes at Room Temperature.
3. Wash plate 6 times. Add 100 µL of substrate. Incubate 30 min at Room Temperature.
4. Add 50 µL of stop solution. Read absorbance within 5 min at 450 nm (620-650 nm ref).
5. Calculate Results using standard analysis programs (QFT-Gold Analysis Software available soon).
- non-human primates (PrimaTB STAT-PAK™)
- white tail deer, reindeer, and elk (CervidTB STAT-PAK™)
- cattle (BovidTB STAT-PAK™)
- badgers (BrockTB STAT-PAK™)
- camels, llamas, and alpacas (CamelidTB STAT-PAK™)
- elephants (ElephantTB STAT-PAK™)

- antibody detection assays that employ cocktails of recombinant antigens from *M. bovis* and *M. tuberculosis*
- The tests can use serum, plasma, or whole blood samples and yield a result in 20 minutes
Phase 1: Alternative Approach

- High specificity in a test for latent TB could be achieved by detecting the presence of a specific latency-associated bacterial antigen(s), rather than measuring the host immune response.
- Look in the granuloma…

Many of these tests and others have been used to detect both LTBI and active TB, but cannot discriminate.
Early infection

- Endothelium
- T-Lymphocyte
- Fibroblast
- Dendritic cell
- Blood monocyte
- Capillary
- Air Space
- Type I pneumocyte
- Type II pneumocyte
- Alveolar macrophage
- Bacillus
- Basement membrane
Extravasation and Granuloma Formation

Suspected/Confirmed Conditions within TB Granuloma
- Within granuloma: low $O_2$, pH and nutrient levels; high levels of lipids
- Bacilli are non or slowly replicating though metabolically active

Models that may mimic these conditions
- hypoxic chamber
- *in vitro* granuloma
- *in vivo* lung granuloma
*M. tuberculosis* bacilli can enter a dormant state *in vitro*

- **Aerobically growing *M. tb.***
- **Gradual O$_2$ depletion**
  - Cells elongate, cell walls thicken
- **Further O$_2$ depletion**
  - DNA synthesis and cell division ceases, increased resistance to antibiotics
  - >99% of cells die
  - (≥7 days)

- **Addition of O$_2$**
  - DNA synthesis resumes, cells divide. Sensitivity to antibiotics increases.
**Total protein analysis**

*Mtb* bacilli from various *in vitro* growth stages

<table>
<thead>
<tr>
<th>Lane</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Marker</td>
</tr>
<tr>
<td>2</td>
<td>Aerobic</td>
</tr>
<tr>
<td></td>
<td>5 days (mid-log)</td>
</tr>
<tr>
<td>3</td>
<td>Latent</td>
</tr>
<tr>
<td></td>
<td>11 months (NRP2)</td>
</tr>
<tr>
<td>4</td>
<td>Anaerobic</td>
</tr>
<tr>
<td></td>
<td>7 days (NRP1)</td>
</tr>
<tr>
<td>5</td>
<td>Reactivated</td>
</tr>
<tr>
<td></td>
<td>30 hours</td>
</tr>
<tr>
<td>6</td>
<td>Marker</td>
</tr>
</tbody>
</table>

17Kda (Acr)
Acr regulation

- Acr protein secretion
- MTB growth
- hspX mRNA synthesis

Time

3d 7d

Acr status in MTB growth curve
Alpha-Crystallin homolog (Acr)

- encoded by \textit{hspX/Rv2031c} – first gene in four gene operon
- small 144 amino acid protein
- DosR regulated
- possesses heat shock and chaperonin characteristics
- undetectable in log-phase cultures
- predominantly produced in stationary-phase
- correlates with cell wall thickening
- localized with in cell wall
- expression induced during \textit{in vitro} infection of macrophages
- mutant strain \textit{ΔhspX} produces hypervirulent phenotype in aerosol mouse infections
- function unknown
Aerosol-infected Guinea Pig lung granuloma - 3wk.
Aerosol-infected rabbit lung granuloma-10wk.

αAcr IHC stain
In vitro granuloma

(Birkness, et al., Microbiol. 2006)
In vitro Granuloma 5-day

Acid-fast stain

αAcr IHC stain
Immunoblot analysis of *Mtb* culture supernatants probed with anti-Acr rabbit polyclonal antiserum

Lane 1, 12-month hypoxic culture
Lane 2, host cell control lysate
Lane 3, 9-day in vitro granuloma
Lane 4, 14-day stationary phase culture


## Phase I: Preliminary Acr Serum Diagnostic
probed with anti-Acr monoclonal antibodies (2 of 30)

<table>
<thead>
<tr>
<th>Test material</th>
<th>IT-4-probed Western blot</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS + 1 pM rAcr</td>
<td>+</td>
</tr>
<tr>
<td>NHS + 10 pM rAcr</td>
<td>+</td>
</tr>
<tr>
<td>NHS control</td>
<td>-</td>
</tr>
<tr>
<td>Normal guinea pig sera</td>
<td>-</td>
</tr>
<tr>
<td>Sera from 3-week infected guinea pigs</td>
<td>5/5</td>
</tr>
<tr>
<td>Group 1 human test sera</td>
<td>4/5</td>
</tr>
<tr>
<td>Group 2 human test sera</td>
<td>3/5</td>
</tr>
<tr>
<td>Group 3 human test sera</td>
<td>0/5</td>
</tr>
</tbody>
</table>

- **Group 1**, individuals with well-documented active disease, and in the early stages of chemotherapy
- **Group 2**, possibly latently-infected individuals, positive on the tuberculin skin test, but show no evidence of active infection, but in close contact with active TB cases
- **Group 3**, healthy, skin test negative individuals with no known contact with TB cases

Lane 1, MW marker
Lane 2, 10 femtomolar (fM) rAcr
Lane 3, 100 fM rAcr
Lane 4, 1 picomolar (pM) rAcr
Lane 5, 10 pM rAcr

Lane 1, MW marker
Phase II: Clinical Screen

- In the process of screening sera by ELISA for Acr
  - 35 human patients with active/acute tuberculosis,
  - 31 PPD-positive, asymptomatic close contacts
  - 50 PPD-negative controls
Future Directions

- Additional *Mtb* latency-specific proteins to be included in the assay
- Continue to screen additional antibodies and methodologies to enhance sensitivity
- Screen urine samples
- Collect samples from more “complicated” populations
  - e.g. HIV+, BCG-vaccinated
- Identify latent infections in animal patients
- Determine efficacy of latency therapies
  - Animals and humans
The Cast

**UGA**
Russ Karls
Deb Haas
Shelly Helms

**Emory**
Hank Blumberg
Jane Tapia

**CDC**
Kris Birkness
Peter King
David Beall
Jeannette Guarner
Libby White

**Texas A&M**
David McMurray
Susan Phalen

**Johns Hopkins**
Yuka Manabe

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College of Veterinary Medicine