The Quest for TB Biomarkers Discovery: the Journey from the Bench to the Bush; Introducing the Validation process

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TB Diagnostic Research: Beyond Basics
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Worldmapper Cartographs

(Gastner and Newman, PNAS 2004 Diffusion-based method for producing density-equalizing maps)

Total Global population:
The size of each territory shows the relative proportion of the world's population living there.

Global TB cases:
Territory size shows the proportion of worldwide TB cases found there.

http://www.worldmapper.org/
Worldmapper Cartographs

(Gastner and Newman, PNAS 2004 Diffusion-based method for producing density-equalizing maps)

Influenza Outbreaks:
The territory size shows the proportion of people worldwide living where there is an influenza outbreak, per week, between 2000 and 2005.

Global TB deaths:
Territories are sized in proportion to the absolute number of people who died from tuberculosis in one year.

http://www.worldmapper.org/
All great undertakings are achieved through mighty obstacles.
Outcome associated with exposure to *Mycobacterium tuberculosis*

Adapted from Parrish et al, 1998
**Definitions**

**BIOMARKER(S):** Characteristic(s) that is objectively measured and evaluated as an indicator of normal biological processes, pathological processes or physiological/pharmacological responses to an intervention.

**CORRELATES OF PROTECTION:** Measurable sign(s) in a host in response to an infectious agent indicating whether the individual is being protected against becoming infected and/or developing disease.

**SURROGATES OF PROTECTION:** Validated markers of correlates of protection.

**CLINICAL ENDPOINT:** Characteristic or variable that reflects the final outcome of disease in terms of function, effect, progress, recovery, survival or death.

**SURROGATE ENDPOINT:** Biomarker that is intended to substitute for a clinical endpoint, predicting clinical outcome in terms of benefit, or harm or lack of benefit or harm.

*Kaufmann and Parida, 2008*
Biomarkers in TB

• Differences in the immune response between individuals exposed to TB and protected from the disease to those who develops active disease.

• Particular attention on people coinfected with both *Mtb* and HIV with or without ART.

• Harness design and testing of new TB vaccines, drugs and diagnostics, especially in areas with high HIV infection rates.
Types of Markers

- Immunologic
  - Selection of antigens
- Transcriptomics
  - RNA
  - 100,000 transcripts
  - Differentially expressed genes which distinguish latent infection from active TB
- Proteomics
  - Proteins
  - 1,000,000 proteins
  - Differentially expressed proteins which distinguish latent infection from active TB
- Metabolomics
  - Biochemicals
  - 2,400 compounds
  - Metabolites which distinguish latent infection from active TB

Combinations
Biomarkers of protective immunity and surrogate markers of TB disease in Africa

**WP1:** Profiling of pathogen response - identification of antigen specific T cell responses

**WP2:** Profiling of host response to infection - identification of relevant host markers

**WP3:** Natural history studies of TB

**WP4:** Impact of HIV infection/AIDS and response to treatment

**WP5:** Protective immunity following BCG vaccination

**WP6:** Coordination, management

**African field studies** correlates of protection and disease

**Two year Follow-up**

- 966 HIV- TB+ patients
- 4521 HIV- LTBI
- 6363 LTBI Adolescents
- 862 HIV+ LTBI; 305+ TB
- 5663 neonates + 200 children following BCG vacc
WP 3: Natural protective immunity against TB
HIV -ve newly diagnosed Pulmonary TB patients
Household contacts

WP4: Impact of HIV-1/AIDS and response to treatment on immunity against TB
HIV +ve individuals

- Immunological markers
- Diagnosis
- Phenotyping

Exposure to TB 6 months 18 months 2 years

HIV infection Prophylactic treatment of LTBI ART treatment

Protected Not protected

Grand Challenges in Global Health #6-74
Mechanics of a TB Contact Study

Diagnose and Rx Index Case

Counsel and Enroll Household

Evaluate Household

F/U Evaluations

TST

Blood for Biomarkers

Data Management

Research Lab. and Storage
**Mtb genome**

- **~4,000 ORFs**
- **GC rich**


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**Dormancy:**

**Non-replicating Persistent (NRP) state**

- hypoxic stress
- nitrosative stress
- pH stress
- nutrient shift

- Alpha crystallin (acr) hspX: Rv2031c
- **Dormancy survival Regulator (DosR): Rv3133c**
- Fused nitrate reductase (narX): Rv1736v
- Nitrate/nitrite transporter (narK2): Rv1737c
- Isocitrate lyase (icl): Rv 0467

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**Enduring Hypoxic Response (EHR) genes:** 230

Rustad et al, Cell Micro 2009
LUMC activities

Production of recombinant proteins

- panel of 86 TB antigens for screening
  (0.5-5 mg)

- set of ~20 TB antigens for cohort studies
  (~150mg+)

- QC of antigen batches

Kees Franken, Michel Klein, Tom Ottenhoff
Immunogenicity screening of 86 TB antigens by 6-day whole blood culture (IFNγ)
Antigen Screening Results from Mining Exercise – Top 5
Screening of 86 antigens

Screening of 42 cytokines

5 antigens

3 cytokines

Combinatorial approach: 5 x 3 = 15 possible biomarkers
Biomarkers to distinguish TB patients and healthy contacts

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Antimicrobial

Inflammation

Chemokines

Vesicle trafficking

Jacobsen et al., JMM, 2007
Deconfounding of microarray data

Differential gene expression

• Differences in cell type proportions
• Cell type specific gene expression

Deconfounding: Mathematical dissection of heterogeneous tissue into its components (cell types)

Dirk Repsilber et al., Univ. of Dummerstorf
Deconfounding of microarray data

Proof of principle *

- Current deconfounding algorithm has been shown to be successful in mathematically dissecting heterogeneous tissue.
- Improved validity in detecting differentially expressed genes.

Limitation of current algorithm:

Current deconfounding algorithm limited to a single marker gene for T cells and monocytes/macrophages

→ New experimental setup to identify cell type specific gene expression profiles

Improving current deconfounding algorithm

Expression profiling of all major cell types in blood

RNA isolation

Microarray

Deconfounding algorithm
Improving current deconfounding algorithm

Expression profiling of all major cell types in blood

Power of deconfounding can be greatly improved:

- Dissect tissue into all major components (cell types)
- Multiple markers per cell type

- Differentially expressed genes can be identified with greater validity (less false-positive and false-negative hits)
- Differentially expressed genes can be assigned to a specific cell type
Host biomarkers in disease and protection

Disease ↔ Protection

TB patients

Infection → Disease

X

TST+ contacts

Infection → Protection

Y

TST− contacts
Random forest analysis of gene subsets discriminating between TB and LTBI.
Quest for Immune Correlates of Protection in TB

Current Status:
- Recruitment completed – follow-up ongoing (would end in October 2010)
- Additional recruitment initiated and ongoing to obtain more Sec. Cases
- Assays: ELISA for IFNγ – assay qualification at all site being completed
  - Luminex assay for Multicytokine studies
  - Transcriptomics – Microarray; MLPA
- Antigens: Large scale Production achieved

Next Plans:
To do analysis centrally on all secondary cases and matched controls (4 x progressors) at the end of the follow-up period
  Validation of the patterns showing association with protection:
  - 1. Soluble cytokines
  - 2. T cell cytotoxic molecule expression
  - 3. Gene expression profiles
  Complementary analysis to delineate soluble cytokine expression patterns from longer term assays that associate with protection
Thinking out of the box!

Plausible paths:

- **Fine map the immune responses** –
  T cell resp  Ag-specific frequency
  Phenotypes – specific subsets
  Cytokines, proliferative potential
  Cytotoxic potential, functionality
  Treg
  relevant host markers,
  pathogen markers
  different combinations of markers and platforms (biosignatures)

- **Unbiased global profiling** – differential gene expression / multiple cytokines

- **Integrated approach** of all composite data sets over time

- Further **longer term assay** comparisons

- **Robust bioinformatics** – systems biology approach

- **Validation**, validation, validation
Biomarkers in TB

Immunologic

- Material
  - Blood
  - Tissue

- Application
  - Diagnose disease
  - Monitor vaccine trial
  - Predict susceptibility
  - Treatment outcome
  - Monitor drug trial
Biomarker Needs in context of TB!

- Surrogate markers of immune protection – need for assessing potential vaccine candidates
- Surrogate marker of bacterial clearance (clinical end-point) – need for assessing potential drug candidates
- Markers of relapse
- Markers of treatment failure (drug resistance)
- Diagnostic Markers
- Markers for infection
- Prognostic markers for reactivation/disease
The long and winding road from Bench to the Bush... and back from Bush to Bench
As I address myself to the young immunologists, my message is still one of the encouragement. There are plenty of uncharted territories for you to explore, numerous mysteries to unravel and revelations to behold.

Stay away from the beaten track.

Don’t be afraid to question established dogma, to dare the impossible, or to seek the unexpected.

Nature rewards and yields its magic secrets to the most daring imaginative.

Hard work and dedication, while essential are not enough.

-- Kyoto, August 1983