Genetic Dissection of host resistance to *Mycobacterium tuberculosis*

Stefan H.E. Kaufmann
Max Planck Institute for Infection Biology

21st European Congress of Clinical Microbiology
and
27th Infectious Diseases International Congress of Chemotherapy

7 – 10 May 2011
Milan – Italy
Commercial relations disclosure

As speaker of the education activity “21st European Congress of Clinical Microbiology and 27th Infectious Diseases International Congress of Chemotherapy”, I declare that in the last two years I had the following commercial relationship with the below mentioned companies:

1) Intercell, Austria – Scientific Advisory Board member

2) Innate Pharma, France – Scientific Advisory Board member
125,000 infections / day

2,000,000,000 latently infected

27,000 new cases / day

10,000,000 new TB cases annually

5,000 deaths / day

2,000,000 TB deaths annually

Immunity, 2010
TB Vaccines, Diagnostics and Drugs

Targets of Stop TB Partnership:

- Reduce prevalence and mortality by half till 2015 as compared to 1990
- Eliminate TB (Reduce annual incidence by 2050 to < 1/1,000,000, ie 8000 new cases/year)
TB Vaccines, Diagnostics and Drugs

Targets of Stop TB Partnership:

• Reduce prevalence and mortality by half till 2015 as compared to 1990
• Eliminate TB (Reduce annual incidence by 2050 to < 1/1,000,000, ie 8000 new cases/year)

How can this be achieved?

• Pre-exposure vaccine: reduction by 39 – 52%
• Drugs with shorter treatment time plus against MDR/XDR-TB: reduction by 10 – 27%
• Rapid diagnosis: reduction by 13 – 42%
• Combined: reduction by 70%

(Abu-Raddad et al., PNAS, 2009
Kaufmann, Hussey & Lambert Lancet 2010)
From Gene to Metabolite

DNA → RNA → mRNA → Protein → Metabolite

Transcriptional control
Post-transcriptional control (alternative splicing, RNA editing, alternative polyadenylation)

Transcription

Processing

Translation

Translational control, frameshift

Translation activity

Post-translational modification (phosphorylation, glycosylation, lipid attachment, peptide cleavage, degradation, compartmentalization)
From Gene to Metabolite

Transcriptomics → Epigenetics → Proteomics → Metabolomics

DNA → RNA → mRNA → Protein → Metabolite

Transcriptional control
Post-transcriptional control (alternative splicing, RNA editing, alternative polyadenylation)

Translation
Translational control, frameshift
Post-translational modification (phosphorylation, glycosylation, lipid attachment, peptide cleavage, degradation, compartmentalization)

Enzymatic activity
Biomarkers: Concept

Differences in the host response between people exposed to TB who never become sick (ca 2 billion) and those who develop serious disease (ca 10 million annually).

Ideally both point of care and reverse translation.

Custom made biosignature composed of immune markers and biomics:

• Antigen specific T cell response (Luminex)
• Transcriptome
• Metabonome
Biomarkers: Goals

Biosignature for discrimination between active TB and LTBI (point of care diagnostic).

Biosignature for understanding biology of infection, immunity & pathogenesis (reverse translation).

Biosignature for monitoring of vaccine & drug trials (more sophisticated).

Biosignature for prediction of risk of disease reactivation (correlate of protection & surrogate clinical endpoint).

www.biomarkers-for-TB.net
Host biomarkers in PBMC from TB patients and healthy LTBI: (low endemic /caucasian)

Microarray comparison

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Jacobsen et al., JMM, 2007
Rab13, 24, and 33A expression analyses

Rab33A is predominantly expressed in CD8+ T cell

Importance of deconfounding

Jacobsen et al., J. Infect. Dis., 2005
Rab33A

- Small GTPase
- X-linked
- Expressed in brain, lymphocytes, melanocytes
- Expressed in dense-core vesicles
- Together with Rab3A, Rab27A and Rab37 involved in docking of dense-core vesicles leading to exocytosis
- Release of cytotoxic molecules from CD8 CTL?

Cheng et al., J Invest Derm, 2006;
Tsuboi and Fukuda, J Cell Sci, 2006
Two-way clustered heat-map of 988 genes differentially regulated in both comparisons of TB vs. LTBI and of TB vs. NID

TB patients vs LTBI vs NID: high endemic South Africa

Validation by RT-PCR

F_{\gamma}R1B = F_{\gamma} receptor 1B
GBP = guanylate binding protein
DEFA3 = defensin \alpha3
HP = haptoglobin
CASP1 = caspase 1
GZMA = granzyme A

**FcγR I**: Antibody Fc binding receptor

**Lactoferrin**: Iron uptake

**Guanylate-binding proteins**: Large GTP binding protein, IFN (type 1 & 2), IL-1, TNF inducible

**Granzyme A**: Cytotoxic molecule

**Defensin-α1 & α4**: Antimicrobial (PMN, MP)

**Rab33a in CD8 T cells**: Small GTPase; release of killer granules
Low endemic: FcγR, lactoferrin and Rab33a (88%/91%)

High endemic: FcγR1, lactoferrin, guanylate-binding protein-5, granzyme A (94%/97%; 91%/72%)

Further improvement: defensin-α1, defensin-α4 (>95%/>90%)

Rab33a (selected CD8 T cells) valuable in low endemic but less so in high endemic.
Comparison between TB cohorts

“TB signature”
393 transcripts (Berry MP et al. Nature 2010)

Genes differentially expressed between TB patients and healthy infected individuals (whole blood cells)
Biomarkers: Goals

Biosignature for discrimination between active TB and LTBI (point of care diagnostic).

Biosignature for understanding biology of infection, immunity & pathogenesis (reverse translation).

Biosignature for monitoring of vaccine & drug trials (more sophisticated).

Biosignature for prediction of risk of disease reactivation (correlate of protection & surrogate clinical endpoint).
What about selected cells? Can we gain information about function? Can we increase sensitivity and specificity? Selected CD3, CD4, CD8 T lymphocytes
JAK/STAT pathway-related candidate genes in T cells: high endemic South African

SOCS = suppressor of cytokine signaling
CIS = cytokine inducible SH2-containing protein
JAK = Janus kinase
STAT = signal transducers and activators of transcription

Gene expression in T cells; T cell regulation in susceptibility to TB

Interactions of suppressor of cytokine signaling 3 (SOCS3) with the JAK/STAT pathway and induction/regulation of cytokine signaling (IFN, IL2, IL6)

Functional protein interaction network
Red nodes – up-regulated expression in TB versus LTBI
Grey nodes – no differential expression between TB and LTBI

Jacobsen M et al. Clin Microbiol Infect 2010
Classification of candidate genes from the JAK/STAT regulatory network by linear discriminant analysis

Results from the training step leave-one-out cross-validation are shown as horizontal bar charts.

Prediction accuracy TB vs. LTBI: 98%

IL-2 RA/JAK3/SOCS3 (+ Myc/PIM1/CSH)

Prediction accuracy LTBI vs. NITD: 100%

PIM1/IL-2/RA/JAK3/SOCS3/CSH/Myc

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Biosignature for discrimination between active TB and LTBI (point of care diagnostic).

Biosignature to understand biology of infection, immunity & pathogenesis (reverse translation).

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Biosignature for prediction of risk of disease reactivation (correlate of protection & surrogate clinical endpoint).
Vaccine induced gene expression

Phase I trial: BCG versus rBCGΔureC-\textit{hly}+ (Sponsor: VPM)

**Trial groups**

<table>
<thead>
<tr>
<th>Naïve (TST-neg) / pre-exposed (TST-pos)</th>
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</thead>
<tbody>
<tr>
<td>Parental BCG (10 volunteers)</td>
</tr>
<tr>
<td>Recombinant BCG (3 doses, 10 volunteers each)</td>
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</table>

PBMCs isolated at 0, 28, 56 and 180 days

- Safety data / immune analysis (VPM)
- mRNA expression profiling / microarray (MPI-IB)

<table>
<thead>
<tr>
<th></th>
<th>Up</th>
<th>Down</th>
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<tr>
<td><strong>Day 28</strong></td>
<td>489</td>
<td>432</td>
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<tr>
<td><strong>Day 56</strong></td>
<td>240</td>
<td>133</td>
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<tr>
<td><strong>Day 180</strong></td>
<td>382</td>
<td>428</td>
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</table>
BCG induced regulation of several immune-related genes

Preliminary results

Parental BCG and rBCG induce shared and distinct patterns of gene expression

Individual comparisons: eg, IL17, TLR4 interactor upregulated in rBCG group
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Biomarker der protektiven Immunität gegen TB im Kontext mit HIV/AIDS in Afrika

LUMC
Leiden, The Netherlands

MPIIB (Coordinator)
Berlin, Germany

STANFORD UNIVERSITY
Stanford, USA

SSI
Copenhagen, Denmark

EHNRI
Addis Ababa, Ethiopia

AHRI
Addis Ababa, Ethiopia

MAK
Kampala, Uganda

KPS
Karonga, Malawi

CWRU/TBRU
Cleveland, USA

AERAS
Rockville, USA

LSHTM
London, UK

UC/T/SATVI
Cape Town, South Africa

SUN
Tygerberg, South Africa

MRC
Banjul, The Gambia

UMCU
Utrecht, The Netherlands

https://gc6.biomarkers-for-tb.net
Index: 850 HIV - newly diagnosed pulmonary TB patients
Household contacts: 4500
Recruitment completed Q2 2010
Analysis 2 years later (Q2 2012)

Expected household contacts with TB after 2 years follow-up:
112 (0% loss) TB cases
91 (20% loss) TB cases
Current status: 75 TB cases

Protected >97%
Not protected <3%
Biomarkers: Concept

Differences in the host response between people exposed to TB who never become sick (ca 2 billion) and those who develop serious disease (ca 10 million annually). Ideally both point of care and reverse translation.

Custom made biosignature composed of immune markers and biomics:

• Antigen specific T cell response (Luminex)
• Transcriptome
• Metabonome
Metabolite Summary (Metabolon)

80% overlap in the number and identity of metabolites detected between these studies.

<table>
<thead>
<tr>
<th>Compound Classification</th>
<th>Study 1</th>
<th>Study 2</th>
<th>Study 3</th>
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<td>No. of Cmp</td>
<td>No. of Cmp</td>
<td>No. of Cmp</td>
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<tr>
<td>Total</td>
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<td>370</td>
<td>428</td>
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<td>Named / Identified</td>
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<tr>
<td>Unnamed</td>
<td>235</td>
<td>243</td>
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Metabonome as Biosignature of TB
Metabolomic Profile Differences

Confusion Matrix

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<th>Clinical Status</th>
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<tr>
<td>Latent TB</td>
<td>Latent TB</td>
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<td>Active TB</td>
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<tr>
<td>Active TB</td>
<td>1</td>
<td>37</td>
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4% Error Rate

Mean Decrease Accuracy

Random forest analysis indicates strong differences between the metabolomic profiles of patients with active or latent TB.
Differential abundance of metabolites between groups (J. Weiner et al. unpublished)

<table>
<thead>
<tr>
<th>Control</th>
<th>LTB</th>
<th>TB\text{active}</th>
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![Heatmap image showing differential abundance of metabolites between groups](image-url)
Prediction error and number of most predictive compounds used
Global Metabonome Analysis in TB

- Metabonomics allows differential diagnosis of Mtb latently infected healthy individuals and active TB patients.
- **15-20** metabolites are needed for robust discrimination (>95%).
- Many metabolites of differential abundance belong to distinct metabolic clusters.
- Cluster cognates are often correlated or counter-correlated

  - Hypoxia-induced inosine and methionine metabolism with anti-inflammatory activity
  - Tryptophan metabolism with immunosuppressive activity
  - Fibrinopeptides indicative for fibrotic lesions
  - Lysophosphatidylcholines indicative for massive cell death (apoptosis and necrosis)
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Online Lecture Library

Slide withheld at request of author
Slide withheld at request of author
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Slide withheld at request of author
Hans Mollenkopf
Jeroen Maertzdorf
January Weiner
Marc Jacobsen
Shreemanta Parida
January Weiner
**Collaborators:**

Gerhard Walzl, Martin Ota, Willem Hanekom & the whole GC-6 team

Dirk Repsibler, Potsdam

Robert Mohney, Metabolon

**Funding:**

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