Immunodiagnostic Tools for Leprosy: Exposure, Infection & Disease

Brussels, 19th September 2013

Plenary Session
(Facing the) Challenges in Leprosy

- prevalence ↓
- still **millions** patients suffer from leprosy (> MS)

- **NCD**: quite static number globally (2012)
  - 232,857
  - 21,349 children

- **Transmission** is ongoing
Early Diagnosis

Transmission

M. leprae = killed
95%

M. leprae survives
5%

Early diagnosis
Leprosy

Diagnostic test

Early treatment

2-6 yrs >10 yrs
Diagnosis 2013

- Based on **clinical signs**: # lesions, peripheral neuropathy
detection of *M. leprae*

  => No **early** detection

- Based on **immune responses**:
  1. Anti-PGL-I Ab: **HMI**: mostly detects MB not PB
     Positivity does **not** indicate leprosy
  2. Lepromin skin test: **CMI**: not leprosy-specific
Tests covering leprosy spectrum: combined HMI & CMI

Paucibacillary (PB) - Multibacillary (MB)

Longitudinal studies are lacking

Infected contacts

T-cells/mφ/neutrophils *marker?*

\[ \begin{array}{ccccccc}
& \text{CMI} & & \text{HMI} & & \\
\text{TT} & & & & & \\
\text{BT} & & & & & \\
\text{BB} & & & & & \\
\text{BL} & & & & & \\
\text{LL} & & & & & \\
\end{array} \]

\[ \begin{align*}
\alpha &- \text{PGL-I IgM} \\
\alpha &- \text{LID-1 IgG} \\
\alpha &- \text{Ag85 IgG} \\
\alpha &- \text{LAM IgG} \\
\end{align*} \]
### M. leprae-specific CMI

**Pre-genomic era**

<table>
<thead>
<tr>
<th>T-ESAT &amp; T-CFP10:</th>
<th>TB diagnostics: Quantiferon</th>
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<tbody>
<tr>
<td>L-ESAT</td>
<td>63% similarity to T-ESAT6</td>
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<tr>
<td>L-CFP10</td>
<td>40% similarity to T-CFP10</td>
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</tbody>
</table>

- Extensive recognition of L-ESAT-6 and L-CFP-10 by TB
- *No* application as diagnostic tools in TB endemic countries
Ag discovery (2001 – 2010)

Identification of *M. leprae* unique sequences

Analyse T cell reactivity in endemic regions
Surplus Value of T cell assay using *M. leprae* unique proteins:
1st T cell study IDEAL

Selection *M. leprae* proteins & peptides

- Immunogenic in exposed
- non-responsive in EC (Rio)

CSU Fiocruz LSHTM

IDRI

Pasteur

Brazil
Fiocruz/Goiás

Nepal
TLMN

Bangladesh
ICDDR,B

Pakistan
Aga Khan

Ethiopia
AHRI

Pakistan
Aga Khan

Ethiopia
AHRI
IFN-γ in LST

cumulative for 5 sites

ML 2283

IFN-γ (pg/ml)

0 100 200 1000 2000 3000 4000

12/43 0/7 9/50 10/50 10/46 3/50

EC Mlep+ EC Mlep- BL/LL BT/TT HHC TB

HHC
IFN-γ responses in EC

• Why IFN-γ responses in EC & TB to *M. leprae* unique antigens?

• Influence of leprosy endemicity of habitat?
IFN-γ & *M. leprae* unique protein in WBA allows detection of *M. leprae* exposure
IFN-γ to *M. leprae* unique Ag in EC\textsubscript{low} and EC\textsubscript{high} in one city
Biomarkers for *M. leprae* Exposure

*M. leprae* unique proteins can be used as tools to identify *M. leprae* exposed individuals using IFN-γ as a read out

What about tools for *M. leprae* infection?
Stages of *M. leprae* infection

**Infection stage:**

1. Elimination of *M. leprae* without priming of *M. leprae*-specific T cells
2. *M. leprae* infection eliminated in association with T cell priming
3. *M. leprae* replication maintained at a subclinical level by immune response
4. Pathogenic immune response to *M. leprae*

**Biomarkers for:**

- Innate immunity to *M. leprae*: IGRA\textsuperscript{neg}
- Adaptive immunity to *M. leprae*
- Early/Subclinical infection
- Leprosy disease

**Stages of *M. leprae* infection**

- EC\textsubscript{high}
- HC
- less HC

**Co-infections**

- Diabetes

Adapted from Young et al. *Trends Microbiol* 2009
T cell subsets/ Cytokines involved in leprosy

Activated T cell:

- Th1
  - IL-12/IFN-γ

- Th2
  - IL-4
  - IL-1β/IL-6
  - TGF-β, IL-23

- Th17
  - TGF-β

- Treg

(Possible) Role in:

- Protection
- TT/BT
- RR

Biomarkers

M. leprae

Naive T cell

M. leprae

APC

IFN-γ

IL-10

α-PGL-I

IL17A

IL-22

IL21

IFN-γ

TNF

IP-10

IL-4

IL-13

IL5

IL-1β/IL-6

TGF-β

IL-23

• LL/BL

• ENL

• TT/BT

• RR

• LL/BL

International Leprosy Congress Brussels, 16th-19th September 2013
Biomarkers for *M. leprae* infection

endemic vs not endemic (anymore) areas

**IP-10**

- Bangladesh
- South-Korea

**MIP-1β**

- Bangladesh
- South-Korea

*pg/ml*
Biomarkers for *M. leprae* infection

*endemic vs not endemic (anymore) areas*

**MCP-1**

- **Bangladesh**
  - TT/BT
  - HHC
  - EC
  - TB

- **South-Korea**
  - TT/BT
  - HHC
  - EC
  - TB

**IL-1β**

- **Bangladesh**
  - TT/BT
  - HHC
  - EC
  - TB

- **South-Korea**
  - TT/BT
  - HHC
  - EC
  - TB

*pg/ml*
New Biomarkers with Relevance to Leprosy Diagnosis Applicable in Areas Hyperendemic for Leprosy


Leprosy is not eradicable with currently available diagnostics or interventions, as evidenced by its stable incidence. Early diagnosis of Mycobacterium leprae infection should therefore be emphasized in leprosy research. It remains challenging to develop tests based on immunological biomarkers that distinguish individuals controlling bacterial replication from those developing disease. To identify biomarkers for field-applicable diagnostics, we determined cytokines/chemokines induced by M. leprae proteins in blood of leprosy patients and endemic controls (EC) from high leprosy-prevalence areas (Bangladesh, Brazil, Ethiopia) and from South Korea, where leprosy is not endemic anymore. M. leprae-sonicate-induced IFN-γ was similar for all groups, excluding M. leprae/IFN-γ as a diagnostic readout. By contrast, ML2478 and ML0840 induced high IFN-γ concentrations in Bangladeshi EC, which were completely absent for South Korean controls. Importantly, ML2478/IFN-γ could indicate distinct degrees of M. leprae exposure, and thereby the risk of infection and transmission, in different parts of Brazilian and Ethiopian cities. Notwithstanding these discriminatory responses, M. leprae proteins did not distinguish patients from EC in one leprosy-endemic area based on IFN-γ. Analyses of additional cytokines/chemokines showed that M. leprae and ML2478 induced significantly higher concentrations of MCP-1, MIP-1β, and IL-1β in patients compared with EC, whereas IFN-inducible protein-10, like IFN-γ, differed between EC from areas with dissimilar leprosy prevalence. This study identifies M. leprae-unique Ags, particularly ML2478, as biomarker candidates that may be applicable in regions with high leprosy-prevalence.
Biomarkers for *M. leprae* infection (mycobacterial disease)

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<th>IFN-γ</th>
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**ROC curve**
TT/BT vs EC
MCP-1, MIP-1β, IL-1β

Area 0.9900
Std. Error 0.01616
95% confidence interval 0.9583 to 1.022
P value 0.0002145
Summary I

- *M. leprae* unique Ag can identify *M. leprae exposed* individuals using IFN-γ; importance of proper reference group as EC (same socio-economic background, same part of town)

- Combinations of additional cytokines & chemokines can discriminate between between *M. leprae infected vs. uninfected* (but exposed) healthy individuals in highly endemic areas

- **Longitudinal** follow-up studies in HC from leprosy-endemic areas will be essential for evaluation (intra-individual comparison)
Early Diagnosis 2

1. Early diagnosis
   - M. leprae infection

2. Early diagnosis
   - M. leprae infection

Transmission

M. leprae = killed
95%

M. leprae survives
5%

2-6 yrs >10 yrs

Diagnostic test

Early treatment
Biomarkers for Leprosy Reactions
## Biomarker for T1R

<table>
<thead>
<tr>
<th>Country</th>
<th>Institution</th>
<th>2007-</th>
<th>2008-</th>
<th>2010-</th>
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Biomarkers for T1R

Overall objective:
• Identify novel biomarker signatures for early diagnosis of leprosy reactions

Working hypothesis:
• The development of leprosy reactions coincides with changes in the:  
  - immunological profile and/ or
  - gene expression profile
Biomarker Study Set-up: *prospective* cohort

- **t = 0:**
  - at recruitment
  - before MDT
  - no signs of T1R

- **t = x:**
  - onset of T1R

- **t = end:**
  - end of therapy

**T1R**

**MB**

**EC**

1. CMI
2. Serum
3. RNA
Cellular Biomarkers: Cytokine 4

Nepal

Bangladesh

pg/ml

RR RR RR

t=0 t=x t=end

pg/ml

RR RR RR

t=0 t=x t=end
Biobanking:
1. WBA samples
2. RNA samples

Longitudinal trial Nilphamari
Test development: UCP-LF

- A **field-friendly**, LF is developed for **cellular** (cytokines) and **humoral** (PGL-I) IR to *M. leprae*.
- Th1 (IFN-γ) & Th2 cytokines (IL-10)
- More cytokines under development
- Evaluated in Africa (AE-TBC/ EDCTP)
UCP-LF Diagnostic Test

Samples

Flow

Strip 1

Strip 2

Flow

Value
LUMC, NL
Jolien vd Ploeg-Schip
Elisa Tjon Kon Fat
Kees Franken
Louis Wilson
Marielle Haks
Paul Corstjens
Tom Ottenhoff

Fiocruz, Brazil
Euzenir Sarno
Roberta Olmo Pinheiro
Cristina Pessolani
Geraldo Pereira
Marcia Brandao
Milton Moraes

KIT: Linda Oskam
CSU: John Spencer
Yonsei: Ray Cho

LSHTM
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Anandaban Hospital, Nepal
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Prathiba Thapa
Chhatra B. Kunwar
Murdo McDonald
Deanna Hagge

UFU, Brazil
Luiz & Isabela Goulart
Janaina Lobato

AHRI
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ICDDR,B
Sayera Banu
Senjuti Kabir

Q.M. Gastmann-Wichers Foundation