Gene expression profiling of the host immune response to infection with *Mycobacterium tuberculosis*

Identification of host biomarkers in tuberculosis
The need for biomarkers

- Tuberculosis (TB) disease is still a major health problem.
- 1/3 of the world population is infected, about 2 million people die of disease each year.
- Vaccination is not very successful → global search for new vaccination strategies.

but …

- How to determine success of vaccination?
- Potentially long latent phase before disease development, not all infected persons develop disease.
- Need for surrogate endpoints that “predict” (lack of) protection early after vaccination.
The need for biomarkers in tuberculosis

- Identify host biomarkers that can be used as surrogate endpoints and “predict” (lack of) protection early after vaccination.

- Identify host biomarkers that predict protective host cellular immunity in household contacts.

- Identify host biomarkers that identify latently infected household contacts that are developing active TB.

- Identify host biomarkers that predict (in)adequate responsiveness to therapy in active TB patients.
Current status of biomarkers in tuberculosis

- Very little is known about markers that are specifically regulated early after infection or vaccination.
- IFNγ is frequently used as an indicator of immune activity but not a true biomarker.
- Probably single biomarkers are not sufficient, need for multicomponent signatures.
- Preferably use assays that can measure multiple potential biomarkers simultaneously.
- Assays should be robust, easy to perform and transferable to developing countries.
<table>
<thead>
<tr>
<th>Method</th>
<th>Selection of Genes</th>
<th>RNA Requirement</th>
<th>Special Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microarray</td>
<td>Thousands of genes.</td>
<td>± 2 µg</td>
<td>No selection of genes.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 0.2 µg</td>
<td>Expensive. Enormous amount of data. Less quantitative.</td>
</tr>
<tr>
<td>RT-Multiplex Ligation-dependent Probe Amplification (RT-MLPA)</td>
<td>± 60 genes.</td>
<td>± 0.05 µg</td>
<td>Selection of genes of interest. Genes can be changed easily. (Semi) quantitative.</td>
</tr>
<tr>
<td>Quantitative PCR (Taqman)</td>
<td>Single gene.</td>
<td>± 0.2 µg</td>
<td>Selection of gene of interest. Genes can be selected easily. Quantitative.</td>
</tr>
</tbody>
</table>

- **Broad screen for new biomarkers on few samples.**
- **Testing a set of candidate biomarkers in larger groups.**
- **Testing single candidate biomarkers in larger groups.**
RT-MLPA (Multiplex Ligation-dependent Probe Amplification)

Hybridization

Ligation

PCR amplification

Fragment run

Fragment length analysis

Fluorescence intensity

Fragment length (bp)

PCR primer sequence

Probe 1

Probe 2

cDNA target A

cDNA target B
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Hybridization
- PCR primer sequence
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- cDNA target A
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- Fluorescence intensity
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RT-MLPA (Multiplex Ligation-dependent Probe Amplification)

Hybridization

PCR primer sequence

Probe 1  Probe 2

cDNA target A

cDNA target B

Ligation

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RT-MLPA as tool to identify biomarkers in tuberculosis

- Identify host biomarkers that predict protective host cellular immunity in household contacts - Gambia cohort.
  - 37 TST-Elispot- HHC.
  - 13 TST+Elispot+ HHC.

- Identify host biomarkers that predict (in)adequate responsiveness to therapy in active TB patients - Gambia cohort.
  - 10 TB patients - recruitment.
  - 12 TB patients - 2 months treated.
  - 12 TB patients - 4 months treated.
  - 5 TB patients - 6 months treated.
Direct ex vivo RNA isolation out of blood: PAXgene

PAXgene tube

- 9 ml tube, contains 6.5 ml fixative and is filled with 2.5 ml blood.
- Can be used with standard vacutainer system.
- Mixing ensures immediate fixation of the RNA profile.
- Fixation is longlasting, stable for more than 24h at room temperature, 1-2 weeks at 4°C, at least 2 months at -20°C, and indefinately at -80°C.
- RNA isolation is completely standardized using a kit.
- 1 tube contains ± 4 µg RNA, sufficient for > 20 assays.
RT-MLPA as tool to identify biomarkers in tuberculosis

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Gambia cohort: TST- versus TST+ HHC

Gene A
(P<0.0001)

Gene B
(P<0.0002)

Gene C
(P<0.0001)

Gene D
(P<0.0018)

Gene E
(P<0.0079)

Gene F
(P<0.0143)

Gene G
(P<0.0668)

Gene H
(P<0.0002)

Peak area

TST - Elispot -
TST + Elispot +
Gambia cohort: TST- versus TST+ HHC

Gene A
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Peak area

TST - Elispot -
TST + Elispot +
Secondary cases
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RT-MLPA as tool to identify biomarkers in tuberculosis

- Identify host biomarkers that predict (in)adequate responsiveness to therapy in active TB patients - Paraguay cohort.
  - 8 Acute TB (<4 days of therapy).
  - 12 TB treatment (received between 6-8 weeks treatment).
  - 16 Health care workers.
  - 4 Healthy controls.

↓

Can biomarkers identified in a West-African population be applied to other populations in Africa or maybe even world-wide?
Conclusion: Follow up of active TB patients in Africa and South-america identifies comparable potential biomarkers.
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