Host Response In Tuberculosis

Systems Approach to Understand the Immune Response in Tuberculosis

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Therapeutic Vaccines:
Reprogramming Immunity in Infectious diseases, Allergy and Cancer

Organised by The Merieux Foundation
"les Pensières" in Annecy, France

25th - 27th March, 2013
Caused by infection with *Mycobacterium tuberculosis* (*Mtb*)

Predominantly affects lung

**Active TB**: 9.4 million cases/1.4 million deaths/year:
50% untreated mortality

*Diagnosis difficult; vaccine variable; treatment arduous*

**Latent TB**: 2 billion infected - asymptomatic - skin test/IGRA+

10 - 20% - *subclinical disease/will reactivate to active TB* - *No test*

*Also cannot easily distinguish active TB from latent (except by Mtb culture & symptoms)*

Difficulties in Dealing With Tuberculosis (i)

What constitutes a protective immune response is not fully understood

Factors involved in pathogenesis/development of chronic infection unclear

Host-pathogen interaction is extremely complex
The Human Immune Response In Tuberculosis

LUNG INFECTION

Infected cells form a granuloma

Anti-TNF (human)
TNFα -/- (mouse)

Infected macrophage

Mtb intracellular pathogen

TNF
IL-12
iNOS / ROS

HIV (human)
CD4+ -/- (mouse)

Infected dendritic cell

IL-12

CD4+ T cell

MSMD (human)
IFNγ -/- (mouse)

CD8+ T cell

IFNγ
TNFα

IL10

MOST VACCINE STUDIES AND DIAGNOSTICS
Difficulties in Dealing with Tuberculosis (ii)

Diagnosis difficult:
- *Mtb* culture takes weeks (30% no culture)
- Smear bacilli positivity in sputum
- New *Mtb* gene test (GeneXpert) in sputum/BAL
- Sputum not always available
- Extra-pulmonary forms of TB – need invasive procedures for diagnosis
- Confounding diseases eg sarcoid, pneumonias, lung cancer
- No biomarkers for monitoring TB treatment

BCG vaccine variable – new vaccines in trials but:
What is protective immune response??
What factors in pathogenesis/promote chronic infection??
Treatment arduous (many pills for 6 months, MDR, side effects)
Two Billion people are estimated to have “Latent Tuberculosis”

Active TB 10 - 20% lifetime risk

Latent TB

Symptoms  Fever/Cough/sweats/Wt loss

No Symptoms
No Test to Determine which “Latent TB” will Develop Active TB Disease

Only 10 - 20% progress - Which ones?

Heterogenous: *(Barry et al., NRM, 2009)*

- Cleared infection
- Controlled / persistent infection
- Subclinical active disease
- False positives

Defined by Immune reactivity – cannot differentiate these groups

Skin test induration

or

*Mtb* Antigen specific IFN-γ production (IGRA)

or

Both?

**BUT:**

CANNOT DISTINGUISH ACTIVE TB FROM LATENT
CANNOT DETERMINE WHICH WILL DEVELOP ACTIVE
TB DISEASE
Why do latent individuals remain healthy? - Protection
Why do individuals develop active TB? - Pathogenesis

A broad unbiased survey: Genomics?

Human Blood Transcriptional Signatures In Latent And Active Tuberculosis
‘London the tuberculosis capital of Europe’ (The Telegraph, Dec 2010)

UK: 9,153 cases in 2009
London 2009: 38% of national total (3,476 cases)
Incidence of 44 cases/100 000
HPA, 2010

20/100 000 in 1987

Two tuberculosis sufferers speak out to raise awareness

TB has ruined my life but I’m fighting back

Doctors say I am lucky to be alive
TB is a Global Disease

Are our findings in London representative?
Study Design

### Study Design

**PRE-TREATMENT**

1. **Active Disease**
2. **Latent Disease**
3. **Unvaccinated Controls**
4. **Vaccinated Controls**

Baseline (Time 0)

- 2 weeks
- 2 months
- 3 months
- 6 months
- 12 months

<table>
<thead>
<tr>
<th>Active</th>
<th>Latent</th>
<th>Healthy (non-vaccinated)</th>
<th>Healthy (vaccinated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptoms</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Exposure</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Skin test</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IFN-γ assay</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Culture</td>
<td>+</td>
<td>- / ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

### Locations

- **London, UK**: NIMR
- **BIIR Dallas**

### Laboratory Protocols

- Whole Blood
- Separated Cells
- Antigen Stimulated Whole Blood
- Serum
- RNA
- Microarray
- FACS
- Luminex
10 - 20% of Latent TB individuals cluster with Active TB

Berry et al., 2010,
*Nature.* 466, 973-77
An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis

Matthew P. R. Berry¹, Christine M. Graham¹*, Finlay W. McNab¹*, Zhaohui Xu⁶, Susannah A. A. Bloch³, Tolu Oni⁴,⁵, Katalin A. Wilkinson²,⁴, Romain Banchereau⁹, Jason Skinner⁶, Robert J. Wilkinson²,⁴,⁵, Charles Quinn⁶, Derek Blankenship⁷, Ranju Dhawan⁸, John J. Cush⁶, Asuncion Mejias¹⁰, Octavio Ramilo¹⁰, Onn M. Kon³, Virginia Pascual⁶, Jacques Banchereau⁶, Damien Chaussabel⁶ & Anne O’Garra¹

Transcriptional signature differentiated between active TB and most latent TB patients (potential diagnostic biomarker)

10-20% of latent patients transcriptional response was similar to active TB patients (potential prognostic biomarker)

Similar Results in Two Independent Studies in Africa and in Indonesia


The transcriptional signature of Active TB correlates with radiographic extent of disease

Berry et al., 2010, *Nature*. 466, 973-77
Transcriptional signature diminished by successful anti-TB treatment (potential treatment response biomarker)
Detectable Changes in The Blood Transcriptome Are Present after Two Weeks of Antituberculosis Therapy

Chloe I. Bloom¹*, Christine M. Graham¹, Matthew P. R. Berry¹,³, Katalin A. Wilkinson²,⁴, Tolu Oni⁴,⁵, Fotini Rozakeas¹, Zhaohui Xu⁶, Jose Rossello-Urgell⁶, Damien Chaussabel⁶,⁷, Jacques Banchereau⁶, Virginia Pascual⁶, Marc Lipman⁸, Robert J. Wilkinson²,⁴,⁵, Anne O’Garra¹

Also reported by Cliff, Dockrell et al., J.Infect.Diseases, 2013

Improvement for monitoring TB treatment and testing new drugs:
Current test sputum-conversion >2 months; only in <50% patients
TB Signatures for Treatment Monitoring and Have Potential Use as Diagnostics and Prognostics

Treatment monitoring
Diagnostics

COST: Reduced number of genes with high specificity & sensitivity: Simplify technology platform

Factors in Pathogenesis??

Proactive immune factors for vaccines

Can the TB Signature predict which Latent individuals will develop Active TB?

Active TB

Latent TB

Reactivation

Infection

Infection
Modular signature of Active TB: UK vs. South Africa

PTB UK Training

PTB UK Test

PTB SA Test

Interferon Inducible

Functional Interpretation

Berry et al., 2010, *Nature.* 466, 973-77
Type I IFNs:
Protect against viruses & cancer:
Exacerbate bacterial infections

Berry et al., 2010, Nature. 466, 973-77
Many bacterial pathogens can induce the production of Type I interferons: eg. *Listeria monocytogenes*.

Animal models of infection with *L. monocytogenes* have shown that type I IFNs have a negative effect:


**Mtb infection and Type I IFN**

- Hypervirulent strains of *Mtb* (e.g. HN878) induce high levels of type I IFN which associated with decreased Th1 immunity against *Mtb* and shorter survival times of mice (B6D2/F1 mice)
  

  IFNαβR/- mice have longer survival times than WT following infection with two different strains of *Mtb* (HN878, and CDC1551) (A129 and A129 mice carrying a targeted disruption (knockout) in the IFNAR gene (IFNAR KO)


**Increasing Levels of Type I IFN During Mtb infection exacerbates disease**

- Intranasal Poly-ICLC treatment in *Mtb*-infected mice causes 2-fold increase in lung *Mtb* bacterial load, but not in IFNαβR/-
  
  - Antonelli, Sher, *JCI*, 2010

- Innate and adaptive interferons suppress IL-1α and IL-1β production by distinct pulmonary myeloid subsets during Mycobacterium tuberculosis infection.
  
Type I IFN Can be Induced By Hypervirulent Strains of M. tuberculosis

Hypervirulent Mtb

Epithelial cell

Type I IFN

pDC

Virus???
Adjuvant eg MVA???

Early

Infected Macrophage

Type I IFN

IFN-γ

CD4+ T-cell

CD8+ T-cell

Late
Enhanced protection to *Mtb* in IL-10-/— mice is accompanied by earlier and enhanced Th1 responses in the lungs

Blockade of IL-10 Receptor during BCG vaccination of CBA/J mice leads to increased levels of IFNγ, IL-17A and on Mtb challenge.

Lung

Overview: the roles of IL-10 in immune-regulation during *Mtb* infection

Redford, O'Garra et al., *EJI* 2010; Redford, O'Garra. *Muc. Imm.* 2011.
Strategies for Elucidating Determinants of Protection or Disease in Tuberculosis: An Iterative Process Between Human Disease and Experimental Models

**Human Disease**
- Blood Microarray
- TB drug treatment

**Mouse models**
- Blood Microarray
- MTb infection

**Modular analysis**
- Whole mice

**Mechanistic studies**
- Neutrophil
- Dendritic cell
- Lymphocyte
- Macrophage

Transcriptome analysis of human TB to enhance mouse models of *MTb* infection for in-depth mechanistic studies

TB Signatures for Treatment Monitoring and Have Potential Use as Diagnostics and Prognostics

- Treatment monitoring
- Diagnostics
- COST: Reduced number of genes with high specificity & sensitivity: Simplify technology platform
- Factors in Pathogenesis
  - Immune therapies
  - ?Adjuvants in vaccines in LTBI?
- Reactivation
- Prognosis
  - Can the TB Signature predict which Latent individuals will develop Active TB?
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Table 1
Tuberculosis vaccines currently in the clinical pipeline.

<table>
<thead>
<tr>
<th>Candidate</th>
<th>Description</th>
<th>Vaccine strategy</th>
<th>Status</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Viral vectors boost</strong></td>
<td></td>
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<tr>
<td>MVA85A (Aeras-485)</td>
<td>Ag85A expressed by modified vaccinia virus vector</td>
<td>Boost response to BCG, also considered as post-exposure vaccine</td>
<td>Phase IIb</td>
<td>[189–191]</td>
</tr>
<tr>
<td>Cruxell Ad35 (AERAS-402)</td>
<td>Ag85 and Tb10.4 expressed by adenovirus vector</td>
<td>Boost response to BCG</td>
<td>Phase IIb</td>
<td>[193,196,197]</td>
</tr>
<tr>
<td>AdAg85A (McMaster U)</td>
<td>Ag85A expressed by adenovirus vector</td>
<td>Boost response to BCG, also considered as post-exposure vaccine, and a BCG replacement</td>
<td>Phase I</td>
<td>[198,231]</td>
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<tr>
<td><strong>Protein + adjuvant</strong></td>
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<tr>
<td>M72 + AS01 (GSK)</td>
<td>Recombinant fusion protein of <em>Mtb</em> antigens Rv1196 and Rv0125 in adjuvant</td>
<td>Boost response to BCG, also considered as post-exposure vaccine</td>
<td>Phase IIa</td>
<td>[201–203]</td>
</tr>
<tr>
<td>H-1 + IC31</td>
<td>Ag85B-ESAT-6 fusion protein in adjuvant</td>
<td>Boost response to BCG, also considered as post-exposure vaccine, and a BCG replacement</td>
<td>Phase IIa</td>
<td>[204]</td>
</tr>
<tr>
<td>H-1 + CAR01</td>
<td>Ag85B-ESAT-6 fusion protein in adjuvant</td>
<td>Boost response to BCG, also considered as post-exposure vaccine, and a BCG replacement</td>
<td>Phase I</td>
<td></td>
</tr>
<tr>
<td>HyVac4 (Aeras-404) + IC31</td>
<td>Ag85B–Tb10.4 fusion protein in adjuvant</td>
<td>Boost response to BCG</td>
<td>Phase I</td>
<td>[205]</td>
</tr>
<tr>
<td>H56 + IC31</td>
<td>Ag85B-ESAT-6-Rv2660 fusion protein in adjuvant</td>
<td>Boost response to BCG, also considered as post-exposure vaccine, and a BCG replacement</td>
<td>Phase I</td>
<td>[177]</td>
</tr>
<tr>
<td>ID93 + GLA-SE</td>
<td>Fusion protein of 4 <em>Mtb</em> antigens in adjuvant</td>
<td>Boost response to BCG, also considered as post-exposure vaccine</td>
<td>Phase I</td>
<td>[206]</td>
</tr>
<tr>
<td><strong>Modified BCG</strong></td>
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<tr>
<td>rBCG30</td>
<td>Recombinant BCG overexpressing Ag85</td>
<td>Enhance BCG immunogenicity, also considered as post-exposure vaccine</td>
<td>Phase I completed</td>
<td>[175,178]</td>
</tr>
<tr>
<td>rBCGΔ<em>ureC:Hly</em> (VPM1002)</td>
<td>Rec. BCG with Listeriolysin to enhance MHC I presentation</td>
<td>Replace BCG or enhance BCG immunogenicity</td>
<td>Phase IIa</td>
<td>[176]</td>
</tr>
<tr>
<td><strong>Whole cell preps</strong></td>
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<tr>
<td><em>M. indicus pranii</em></td>
<td>Whole cell saprophytic environmental mycobacterium</td>
<td>Chemotherapy adjunct in HIV-infected individuals</td>
<td>Phase III</td>
<td>[208,209]</td>
</tr>
<tr>
<td><em>M. vaccae</em> (SRL172)</td>
<td>Inactivated environmental mycobacterium</td>
<td>Chemotherapy adjunct in HIV-infected individuals</td>
<td>Phase III completed</td>
<td>[210]</td>
</tr>
<tr>
<td>Rutti</td>
<td>Liposome prep made from <em>Mtb</em></td>
<td>For treating latent TB post chemotherapy</td>
<td>Phase IIa</td>
<td>[244]</td>
</tr>
</tbody>
</table>

Safety and efficacy of MVA85A, a new tuberculosis vaccine, in infants previously vaccinated with BCG: a randomised, placebo-controlled phase 2b trial

Summary Background BCG vaccination provides incomplete protection against tuberculosis in infants. A new vaccine, modified Vaccinia Ankara virus expressing antigen 85A (MVA85A), was designed to enhance the protective efficacy of BCG. We aimed to assess safety, immunogenicity, and efficacy of MVA85A against tuberculosis and Mycobacterium tuberculosis infection in infants.

Interpretation MVA85A was well tolerated and induced modest cell-mediated immune responses. Reasons for the absence of MVA85A efficacy against tuberculosis or M tuberculosis infection in infants need exploration.

The Lancet.com Published online February 4, 2013 http://dx.doi.org/10.1016/S0140-6736(13)60177-4