Systems approaches to new vaccines and diagnostics for bovine tuberculosis

Johnjoe McFadden
Vaccine Development
The Traditional approach

Live cells
Dead cells
Cellular subunits

Protective immune response
Vaccine Development
The Traditional approach

- Live cells
- Dead cells
- Cellular subunits

→ Protective immune response

Been very successful for:
- Smallpox
- Diphtheria
- Measles
- etc
Vaccine Development
The Traditional approach

Live cells
Dead cells
Cellular subunits

Protective immune response

Hasn’t worked for
• HIV
• Malaria
• Tuberculosis
• Etc

❖ Need to open the box!
Vaccine Development
The Traditional approach

- Live cells
- Dead cells
- Cellular subunits

→ Protective immune response
Vaccine Development
The Traditional approach

Live cells
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Protective immune response
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The Traditional approach

Protective immune response
Vaccine Development
The Systems Approach

How do you measure this complexity?
How do you make sense of this complexity?

Protective immune response
Systems Biology

- “To understand a single life, you have to swallow the world”
- To understand a single gene, you have to swallow the organism
Vaccine Development
The Systems Approach
Measurement – big data

- Genome Sequencing
- Transcriptomics
- Proteomics
- Metabolomics
  - Statistics
Vaccine Development
The Systems Approach
Making sense – modelling

- Intuition fails for complex systems
- Need to instantiate knowledge in mathematical models
  - Integrate data
  - Generate hypotheses
  - Generate predictions
  - Test predictions
Vaccine Development
The Systems Approach
Example Approaches

- High-throughput screening of responses to infection/vaccination to identify molecular signatures of protection.
  - Which tissue/cells to sample?
  - Statistical or mechanistic models?

Gain insights into molecular mechanisms of protection.
Statistical Approaches

- Yellow fever vaccine YFP trial in humans
- Transcriptome analysis of peripheral blood of vaccines and controls
- Found gene expression responses in innate and adaptive immune system that correlated with protective immunity
- Led to hypothesis that induction of the integrated stress response in the innate immune system might play a key role in shaping the CD8+ T cell response to YF-17D.

Modelling Approaches

• “Actually, the orgy of fact extraction in which everybody is currently engaged has, like most consumer economies, accumulated a vast debt. This is a debt of theory, and some of us are soon going to have an exciting time paying it back—with interest, I hope” (Brenner, 1997).

• Models instantiate theories
• Simulations
• We need to replace the black box with mechanistic models

Reconstruction of cellular signalling networks and analysis of their properties.
Bovine tuberculosis in UK

**BOVINE TB IS OUT OF CONTROL**

WE TAKE ACTION ON TB IN COWS. **9.0 MILLION** CATTLE WERE TESTED IN 2014 IN BRITAIN. **269,358** CATTLE HAVE BEEN CULLED IN GREAT BRITAIN DUE TO TB BETWEEN 2006 AND APRIL 2015.

TB IS SPREADING ACROSS THE COUNTRY. THERE WERE **4,692** NEW OUTBREAKS IN BRITAIN DURING 2014.

BADGERS HAVE TB
- **UP TO 1 IN 3** BADGERS IN ENGLAND AND WALES HAVE TB. TB HAS TO BE CONTROLLED IN WILDLIFE.

- **£500 MILLION** ESTIMATED COST OF TB CONTROL IN ENGLAND AND WALES OVER THE NEXT DECADE WITHOUT TAKING FURTHER ACTION.
- **£662 PER BADGER** WHAT IT COST THE WALES ASSEMBLY TO VACCINATE EACH BADGER IN 2010.
- **£1 BILLION** WHAT IT WOULD COST TO CONTROL TB IN ENGLAND AND WALES OVER THE NEXT DECADE.

BADGER VACCINATION WILL NOT CURE A BANDER THAT IS ALREADY INFECTED.

- **94%** REDUCTION IN TB ENCE ADE AQIOUS IN THE EARLY 1990s.

**HEALTHY CATTLE, HEALTHY BADGERS = HEALTHY COUNTRYSIDE**

#TBfree

www.tfreeengland.co.uk
Test and cull

• In the UK, there is a compulsory bovine TB screening and slaughter programme for cattle.
  – In Great Britain (GB) the standard screening test for bTB is the single intradermal comparative cervical tuberculin (SICCT) test which compares immune responses to intradermal injections of bovine and avian tuberculin, purified protein derivatives (PPD) from M. bovis (bovine) and M. avium (avian) respectively.

• Despite the fact that the BCG vaccination has proven effective in protecting cattle against the disease, vaccination is currently not used because the BCG interferes with the skin-test currently used to detect the infection (giving a positive result whether an animal has been vaccinated against, or is infected with, TB.)
Bovine TB in India

- The problem in India is further confounded by the special status of the cow in Hinduism that prevents any test and cull strategy from being implemented.
  - BCG is currently not allowed because it interferes with the PPD skin test.
Bovine tuberculosis and vaccination UK

• Development of cattle and wildlife bTB vaccine is part of Welsh Government and Defra’s Eradication strategies:
Vaccination against bovine TB: the only potential candidate:

- **bacille Calmette-Guérin (BCG)** – a proven technology
  - Live, attenuated *M. bovis*
  - Attenuated in cattle since 1912
  - Used in humans since 1921
  - Safe in a wide range of species
  - Cheap
  - Recommended by WHO for humans
  - Variable efficacy in cattle, at population and individual animal levels
  - Compromises specificity of tubercuin-based tests like SICCT – needs DIVA test to differentiate infected and vaccinated animals
BUT, BCG – Variable efficacy

Meta-analysis of the literature reveals:

Human Trials

<table>
<thead>
<tr>
<th>Publication</th>
<th>Population</th>
<th>Country</th>
<th>Protection %</th>
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<tbody>
<tr>
<td>Hubrig 1959</td>
<td>East Germany</td>
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<tr>
<td>Ellwood 1972</td>
<td>Malawi</td>
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<tr>
<td>Cheneau 1975</td>
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<td>Zuckerman 1975</td>
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<td>Buddle 1995a,b</td>
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<td>Vordermeier 2002</td>
<td>U.K.</td>
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Cattle Trials

<table>
<thead>
<tr>
<th>Publication</th>
<th>Protection %</th>
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</thead>
<tbody>
<tr>
<td>Vordermeier 2002</td>
<td>0 25 50 75</td>
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</table>
DIVA test:

• BCG is not fully protective in all animals.

• Still require surveillance tests for *M. bovis* infection.

• Tuberculin-based reagents compromised in respect to specificity (although SICCT reactivity wanes quickly between 6-9 months post-vaccination)

• Need novel DIVA (Differentiation of Infected and Vaccinated Animals) tests that react with infected animals but not vaccinated animals
## Current DIVA antigens

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Comments</th>
<th>DIVA blood test</th>
<th>DIVA skin test</th>
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<tbody>
<tr>
<td>ESAT-6</td>
<td>Gene deleted in BCG</td>
<td>YES</td>
<td>YES Protein</td>
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<tr>
<td></td>
<td></td>
<td>Overlapping peptides</td>
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<tr>
<td>CFP-10</td>
<td>Gene deleted in BCG</td>
<td>YES</td>
<td>YES Protein</td>
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<tr>
<td></td>
<td></td>
<td>Overlapping peptides</td>
<td></td>
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<tr>
<td>Rv3615c</td>
<td>Gene present in BCG Lower gene expression</td>
<td>YES</td>
<td>YES Protein</td>
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<tr>
<td></td>
<td>Gene product not secreted</td>
<td>Overlapping peptides</td>
<td></td>
</tr>
<tr>
<td>Rv3020c</td>
<td>Gene present in BCG</td>
<td>NO</td>
<td>YES Protein</td>
</tr>
</tbody>
</table>

- **DIVA blood test**
  - response to ESAT-6/CFP-10 peptide cocktail and/or Rv3615c peptide cocktail
- **DIVA skin test**
  - response to 3 protein reagent (ESAT-6/CFP-10/Rv3615c)
  - response to 4 protein reagent (ESAT-6/CFP-10/Rv3615c/Rv3020c)
Current DIVA tests

• Detect Infected among Vaccinated Animals.
• Existing diagnostic test platforms for bovine TB.
  – Blood based IFN-\(\gamma\) release assay (IGRA).
  – Tuberculin Skin test (SICCT).
• DIVA IGRA (e.g. Bovigam test): High sensitivity but lower Specificity compared to SICCT, expensive and main cost contributor to vaccine strategy implementation
• DIVA skin test (Martin Vordermeir): Promises higher specificity than IGRA, but currently lower sensitivity. Nevertheless, may be more cost-effective than IGRA?
• Need to develop a compatible sensitive and specific BCG vaccine and DIVA skin test combination
Development of recombinant BCG vaccine and complementary diagnostics for TB control in cattle

Partners

- University of Surrey
  - McFadden, Stewart, Kierzek
- Animal Health Veterinary Laboratories Agency
  - Vordermeir, Villarreal-Ramos
- Health Protection Agency
  - Rawlins
- Tamil Nadu Veterinary and Animal Sciences University, Chennai
  - Dr. V. Maroudam
- Centre for Development of Advanced Computing, Pun
  - Dr. K. Sunitha Manjari
Current Approach

Attenuation of BCG

Antigens

Genes

M. bovis

Current DIVA tests

Current DIVA antigens non-protective non-essential
Novel rBCG Approach

attenuation of BCG genes

antigens

M. bovis

protective non-essential

Non-protective non-essential
Aims

A. Construction of a dcBCG strain deleted in antigens dispensable for protection

B. Development of exDIVA skin test based on current DIVA antigens plus additional antigens deleted from dcBCG
Aims

The key challenge of the project is to successfully identify exDIVA antigens that are:

- Dispensable for persistence in cows
- Immunogenic
- Dispensable for protection in cows
A. Construction of dcBCG strain

- BCG can be recovered from bovine lymph nodes several weeks after inoculation
  1. Make transposon mutant library in BCG
  2. Inoculate library into bovine lymph nodes
     - Mutants that are unable to persist in the cow will be lost from the library
     - Mutants that are able to persist in the cow will not be lost from the library
     - Identify genes that are dispensable for persistence in cows
  3. Delete dispensable genes encoding antigens from BCG
Phage based transposon mutagenesis of BCG Pasteur 1173P2

3.2 x 10^5 mutants on 7H11 with Kan

Library injected into prescapular lymph nodes and recovered after 6 weeks

<table>
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<th>INPUT mutants</th>
<th>OUTPUT mutants</th>
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<td>gene 7</td>
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<td>INPUT mutants</td>
<td>OUTPUT mutants</td>
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<td>-</td>
</tr>
<tr>
<td>gene 7</td>
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</tbody>
</table>

Genes dispensable for persistence in cows
Potential DIVA antigens able to be deleted from BCG to generate dcBCG?

Genes required for persistence in cows
Virulence genes?
Currently constructing dcBCG strains deleted in dispensable genes

- The key challenge of the project is to successful identify exDIVA antigens that are:
  - Dispensable for persistence in cows
  - Immunogenic in skin test
  - Dispensable for protection in cows
New DIVA antigens

- The next stage is to identify which dispensable genes are antigenic in a skin test
The tuberculin purified protein derivative (PPD) is a widely used diagnostic antigen for tuberculosis. However it is poorly defined. Most mycobacterial proteins are extensively denatured by the procedure employed in its preparation, which explains previous difficulties in identifying constituents from PPD to characterize their behaviour in B- and T-cell reactions.

We performed a proteomics-based characterization of PPD from several different sources by LC-MS/MS, which combines the solute separation power of HPLC, with the detection power of a mass spectrometer.

The technique is able to identify proteins from complex mixtures of peptide fragments.
Proteomics of PPD

- Proteins were initially separated by one-dimensional SDS-PAGE which showed a smear of mostly low molecular weight protein fragments.
- The gel was then cut into slices and the proteins subjected to in-gel trypsin digestion before LC-MS/MS.
- A total of 171 unique proteins were identified by LC-MS/MS analysis based on at least one identified peptide per protein with a MASCOT confidence level above 95%.
Proteomics of PPD

- The identified proteins were classified according to the M. tuberculosis annotation.
- The predominant class are predicted cytoplasmic proteins, accounting for 77.9%. Membrane or secreted proteins with different signal peptides (Tat, Sec and Non-Classical secretion) accounted for only 22.4% of the proteins present.
- The most common protein class among the avium PPD was also cytoplasmic proteins.
- Twenty-one proteins were classified as secreted. Of this, Rv0685, Rv1174c, Rv1411c, Rv1860, Rv1886c, Rv1980c are also found in bovine preparations.
- Analysis was qualitative, not quantitative
But which antigens are responsible for immune response?

- Over the last decade AHVLA has assessed the immunogenicity in infected cattle of 500 proteins by the interferon gamma release assay (IGRA).
- The observed hierarchy of responses ranged from proteins not recognized at all (0% responder frequencies) to antigens being recognized by more than 90% of animals tested.
Purification and testing of defined antigenic cocktails as DIVA skin test reagents

- Dispensable antigens will be cloned and expressed in E. coli and insect cells and purified.
- Antigens will be tested in:
  - Measurement of DIVA antigenicity (IGRA-based blood test) of immunogenic proteins in naturally infected and control cows.
  - Measurement of skin test reactivity of prototype exDIVA tests in cows.
Development of recombinant BCG vaccine and complementary diagnostics for TB control in cattle