M. tuberculosis T cell epitope analysis reveals paucity of antigenic variation and identifies rare variable TB antigens
TB is the 2nd biggest killer disease

- 9.6 million sick
- 1.5 million killed
- 480,000 MDR TB

Global tuberculosis report 2015, WHO
MTBC phylogeographical distribution

- Lineage 2: East-Asian
- Lineage 3: East-African-Indian
- Lineage 4: Euro-American
- Lineage 7: Ethiopia
- Lineage 5: West-Africa
- Lineage 6: West-Africa
- Lineage 1: Indo-Oceanic
- Lineage 1: Indo-Oceanic

- Chimpanzee bacillus
- M. pinnipedii
- Ancient Peruvian Humans
- M. microti
- M. bovis
- M. caprae
- M. origys
African origin for MTBC

Human migrations resulted in the phylogeographical distribution we see today
Why study evolution of MTBC

T cell receptor

MHC class I
Tuberculosis immunity

Usual viral, bacterial, and protozoan pathogens:

Antibody or T cell recognition:

Antigen/epitope diversity

M. tuberculosis complex:

T cell recognition:

Antigen/epitope conservation
Rationale

M. tuberculosis

Epitope conservation

Transmission linked to immune response

Pathogen benefits

Identify variable T cell epitopes
Hypothesis:

Genes exist within the *M. tuberculosis* genome that encodes undiscovered T cell epitopes with naturally-occurring sequence variants.
Objectives

1-Identify highly variable coding regions in the *M. tuberculosis* genome.

2-Predict human CD4 and CD8 T cell epitopes with sequence variants in these variable coding regions.

3-Verify that epitopes stimulate immune responses in samples from subjects with newly-diagnosed TB.

4-Determine the impact of naturally-occurring sequence variation on the recognition of these predicted epitopes.
Identify highly variable coding regions in the *M. tuberculosis* genome

5% of the most variable genes
dN/dS>1

88 genes
Identify highly variable coding regions in the *M. tuberculosis* genome

7 genes
- Cell wall and cell processes x3
- Intermediary metabolism x2
- Lipid metabolism x1
- Information pathways x1
Predicted human CD4 and CD8 T cell epitopes with sequence variants in these highly variable coding regions.

How mutations in predicted epitopes affect predicted binding compared to random peptides?

CD4 binding affinity of 5%

CD8 binding affinity of 18%

150

207

56 nSNPs

~5 times more likely to affect (χ Squared P < 0.005)

No difference
Verify that epitopes stimulate immune responses in samples from subjects with newly-diagnosed TB.

14 candidate epitopes:
- 14 ancestral sequences
- 16 variant sequences

1. Rv2719c
   - CE1
   - LAAITLWLGTAOF (All lineage 1)
   - VAVOFGQMI (All lineage 6)

2. RimJ
   - CE11
   - EWTVRHTVAAVPACV (2 strains lineage 1)
   - GRMLPYVEL (Sub-lineage 4)

3. Rv0010c
   - CE4
   - QQTAWAPRTSGIAGC (1 strain lineage 1)
   - HSNIKRIEDFRRYG (Sub-lineage 1)

4. Rv0012
   - CE7
   - HTPPCENGE (1 strain lineage 4)
   - GTEIRSDAPRLDLV

5. Tb7.3
   - CE14
   - QAGDUAVI (Sub-lineage 6)

6. Rv0990c
   - CE8
   - AESLINPLSLRSISA (1 strain lineage 6)
Verify that epitopes stimulate immune responses in samples from subjects with newly-diagnosed TB.

**Candidate epitope**

**Anti INF-g**

**INF-g**

**Indicator**

**ELISA**

82 patients in The Gambia

Newly diagnosed TB
Sputum smear-positive
HIV-seronegative
Verify that epitopes stimulate immune responses in samples from subjects with newly-diagnosed TB.

Certain subjects showed similar IFN-g than positive control, indicating that the candidate epitope peptides were immunogenic.
Comparison of IFN-g responses after 2 and 6 months of TB treatment, compared with responses before treatment.

Verify that epitopes stimulate immune responses in samples from subjects with newly-diagnosed TB.

Evidence that responses to the peptides were attributable to TB.

The magnitude of the responses decreased significantly with successful treatment and resolution of TB.
Determine the impact of naturally-occurring sequence variation on the recognition of these predicted epitopes.
Determine the impact of naturally-occurring sequence variation on the recognition of these predicted epitopes

Amino acid substitutions altered immune responses induced in 10 of the 14 candidate epitopes
Conclusions

We discovered a novel set of antigens that contain T cell epitopes with naturally-occurring sequence variants.

We found that the majority of the naturally-occurring sequence variations in these antigens resulted in changes in human immune recognition.

These results reveal a group of *M. tuberculosis* antigens with novel properties that suggest that they may be valuable TB vaccine antigens.
Thanks to …

Prof. Joel D. Ernst
Dr. Richard Copin
NY School of Medicine

Dr. Bouke de Jong
Institute of Tropical Medicine, Antwerp

Dr. Jane Sutherland
Dr. Florian Gehre
Dr. Martin Antonio
MRC Gambia