Balance between Protection and Pathogenic Response to Aerosol Challenge with *Mycobacterium tuberculosis* (Mtb) in Mice Vaccinated with TriFu64, a Fusion Consisting of Three Mtb Antigens

Sadaf Sulman 1,2, Benjamin O. Savidge 1,3, Kawther Alqaseer 1,3,4, Mrinal K. Das 1,3, Neda Nezam Abadi 1,5, John E. Pearl 1,3, Obolbek Turapov 1,3, Galina V. Mukamolova 1,3, M. Waheed Akhtar 2 and Andrea May Cooper 1,3

1Department Respiratory Sciences, University of Leicester, Leicester LE1 7RH, UK.
2School of Biological Sciences, University of the Punjab, Lahore 54590, Pakistan.
3Leicester Tuberculosis Research Group—LTBRG, University of Leicester, Leicester LE1 7RH, UK
4Department of Basic Science, Faculty of Nursing, University of Kufa, P.O. Box 21, Kufa, Najaf Governorate, Najaf 540011, Iraq.
5APC Microbiome Ireland, University College Cork, T12 YT20 Cork, Ireland
No conflict of interest
• Tuberculosis vaccines capable of reducing disease worldwide have proven difficult to develop.
• BCG is effective in limiting childhood disease, but adult TB is still a major public health issue.
• Development of new vaccines requires:
  • Identification of antigens that are both spatially and temporally available throughout infection,
  • Immune responses to which reduce bacterial burden without increasing pathologic outcomes.
• Subunit vaccines containing antigen require adjuvants to drive appropriate long-lived responses.
We generated a multi-epitope triple-antigen fusion (TriFu64), consisting of three *Mtb* antigens:

- **Virulence associated EsxN (Rv1793),**
  - ESAT-6 like early-stage antigen expressed by *Mtb* shortly after infection,
  - Induces a strong T-cell immune response

- **PPE42 (Rv2608),**
  - Drives strong humoral and variable T cell responses and has been shown to reduce bacterial burden in a mouse model when used as a vaccine

- **Latency associated Rv2628**
  - One of the eight DosR-regulated genes induced in response to stress
  - Cytokine producing T-cells specific for this antigen are seen after prolonged infection
Construction of the fusion proteins
Material and Methods

• The recombinant *Mtb* antigens and fusion protein were generated by molecular cloning and purified.

• TriFu64 and other antigens were delivered at a dose of 0.5 $\mu$g/mouse in either Imject Alum or MPL/TDM/DDA.

• The vaccinated mice were aerosol challenged with *Mtb* H37Rv strain with a dose of 100 colony forming units (CFU).

• Lymphocytes isolated from spleen of vaccinated mice were re-stimulated with antigens (5 $\mu$g/ml). The supernatant was analysed for TNF$\alpha$ and IL-17 production through ELISA test.

• RNA was extracted from total lung tissue of *Mtb* challenged mice and was cDNA amplified followed by quantitative Real Time PCR (qRT-PCR).

• Lung histology was examined.
Protective Immune Responses

Reduction in bacterial burden in vaccinated mice challenged with *Mtb* via the aerosol route.
Induction of differential antigen-specific cellular responses by MPL/TDM/DDA adjuvanted antigens.
Vaccination with TriFu64 results in weight loss during the response to aerosol infection.
RT-PCR and lung histology

(C) Gene transcription $2^{\Delta \Delta Ct}$

(D) Ratio of $2^{\Delta \Delta Ct}$ specific genes

(E) Saline MPL/TDM | TriFu64 MPL/TDM | ESAT-6$_{1-20}$ MPL/TDM

RT-PCR and lung histology
Conclusion

• Combination of different *Mtb* antigens with different functional, spatial and temporal availability
  • Induce inflammatory cytokines (IL-17 and TNF-α)
  • Impact bacterial growth
  • Associated with weight loss

• Defining function of specific T-cells during vaccination and challenge to ascertain their role in protection

• Correlates of protection should also involve consideration of correlates of pathogenic potential (Sadaf *et al.*, 2021)
Supervisor
M. Waheed Akhtar
School of Biological
Sciences

Supervisors
Andrea M. Cooper
John Pearl
Galina Mukamolova
Obolbek Turapov

Funded by: