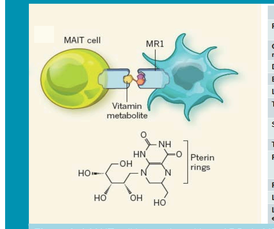
# MAIT Cells are Highly Enriched in Bronchoalveolar Lavage Fluid of Patients with TB


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## BACKGROUND

Mucosal Associated Invariant T (MAIT) cells are non-conventional innate-like CD8+ lymphocytes that have a semi-invariant ab T cell receptor (featuring TRAV1-2). MAIT cells respond to a range of bacterial, mycobacterial and fungal pathogens. They are restricted by the evolutionarily conserved MR-1 (MHC Class 1 Related Protein 1) which presents vitamin B metabolites of microbial origin.




| | Conventional T cells | MAIT cells |
|---|---|---|
| **Restricting MHC protein** | HLA-A, HLA-B and HLA-C (humans), and H-2 (mice) | MR1 |
| **Chromosomal location of MHC gene region** | Chromosome 6 (humans) and chromosome 17 (mice) | Chromosome 1 |
| **Diversity (allelic and polymorphic)** | High | Low |
| **Evolutionary conservation** | Low | High |
| **Ligands** | Peptides | Pterin analogues |
| **TCR repertoire** | Diverse αβ TCRs | Vα7.2–Jα33 (humans) and Vα13–Jα33 (mice) |
| **Selecting cell in the thymus** | Non-haematopoietic epithelial cell | Haematopoietic cell (possibly immature T cell) |
| **Thymic effector capacity** | No | Yes |
| **Requirements for T cell activation** | Ligand–MHC molecule | Pterin analogue–MR1, but no TLR or NLR |
| **Response kinetics** | Clonal expansion required | Early in infection |
| **Localization** | Lymphoid tissue and blood | Lung and liver |
| **Long-lived immunity to specific exogenous antigen** | Yes | Unknown |

**Figure 1**. A MAIT cell interacting with an APC via MR-1 and a table comparing conventional T cells and MAITs. Figure from Chua and Hansen, Nature, Nov 2012. Table from Gold and Lewinsohn, Nature Reviews Microbiology, Jan 2013.

*M.tb*-reactive MAIT cells are found in the peripheral blood of people with no history of *M.tb* exposure, but are absent from the circulation of people with active TB. *Thus we hypothesized that in pulmonary TB, MAIT cells would be enriched in the pulmonary mucosa and have a Th1 functional profile.*

## METHODS

Bronchoalveolar lavage (BAL) fluid and peripheral blood were collected from a cohort of patients undergoing clinical bronchoscopy in Durban, South Africa.

- **Pulmonary TB** cases were confirmed microbiologically or histologically with either a positive *M.tb*. BAL culture/PCR or Ziehl-Neelsen positive necrotizing granulomas from pulmonary parenchyma.
- **Controls** were defined as having no evidence of inflammatory or infectious lung disease and negative bacterial, fungal and mycobacterial BAL cultures.

BAL cells (and matched PBMC when available) were:

- Characterized by flow cytometry to assess the frequency of MAITs (TRAV1-2+) among CD8+ T cells.
- Stimulated with CD2/CD3/CD28 beads and assessed for TNFa and IL-17 production by intracellular cytokine staining.

## RESULTS

| Characteristic | Control | TB | p-value |
|---|---|---|---|
| Number of subjects | 11 | 8 | |
| Age, median (IQR) | 51 (45-73.5) | 50 (24-67.3) | 0.15 |
| Female, n (%) | 6 (54.5) | 6 (75.0) | |
| HIV-positive, n (%) | 3 (27.3) | 3 (37.5) | |
| On ARV, n (% of HIV-positives) | 3 (27.3) | 3 (37.5) | |
| CD4 count cells/mm3 (range, HIV-positive only) | 177-463 | 343-395 | 0.70 |
| %TRAV1-2+ of BAL CD8+, median (IQR) | 5.0 (4.3-5.8) | 22.9 (16.4-33.9) | 0.0005 |
| %TRAV1-2+ of PBMC CD8+, median (IQR) | 6.9 (5.5-9.1) | 5.8 (5.6-6.2) | 0.53 |

**Table 1.** Comparison of demographic and clinical characteristics and and TRAV1-2+ cell enrichments in TB cases and controls. Mann Whitney U test was used to statistically compare groups.

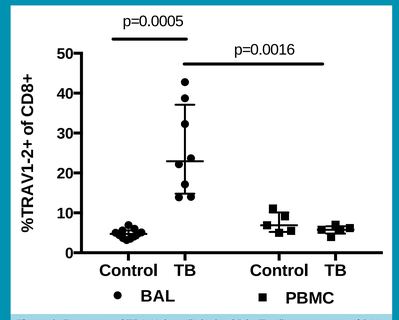



**Figure 2.** Frequency of TRAV1-2+ cells in the CD8+ T cell compartment of BAL and PBMC in TB cases and controls. Mann Whitney U test was used to statistically compare groups.

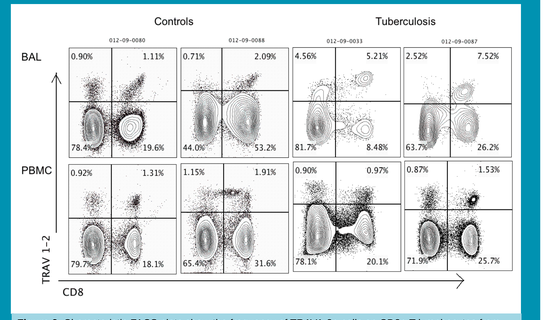



**Figure 3.** Characteristic FACS plots show the frequency of TRAV1-2+ cells on CD3+ T lymphocytes from paired BAL (top row) and PBMC (bottom row) of 2 individual TB cases and 2 controls. Cells were gated on FSC SSC characteristics, live on viability stain, CD14- and CD3+.

In pulmonary TB cases, TRAV1-2+ cells comprised a significantly higher percentage of lung-resident CD8+ T cells (median 22.9%, IQR 16.4-33.9%) than in controls (median 5.0%, IQR 4.3-5.8%, p=0.0005). In contrast, during pulmonary TB, TRAV1-2+ CD8+ T-cells were not enriched in the periphery demonstrating significant MAIT cell compartmentalization (p=0.0016, Figures 2 and 3)

Upon non-specific activation, lung resident and peripheral TRAV1-2+ CD8+ cells produced TNFa but not IL-17. There was a trend towards higher TNFa production by lung-resident compared to peripheral TRAV1-2+CD8+ cells. (Figure 4).

In one example of pulmonary TB, the lung-resident TRAV-1+ CD8+ MAIT cells produced very high levels of TNFa and did not produce IL-17 above background. The peripheral TRAV1-2+ CD8+ population produced far less TNFa (Figure 5).

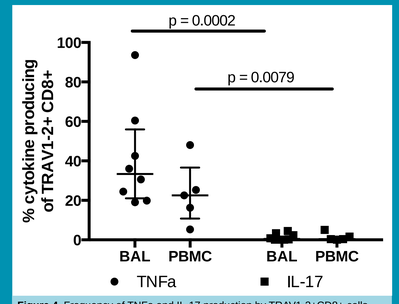



**Figure 4.** Frequency of TNFa and IL-17 production by TRAV1-2+CD8+ cells from BAL and periphery. There were insufficient numbers of cells to stratify into TB and control groups. In all instances background cytokine production was subtracted. Mann Whitney U test was used to statistically compare groups.

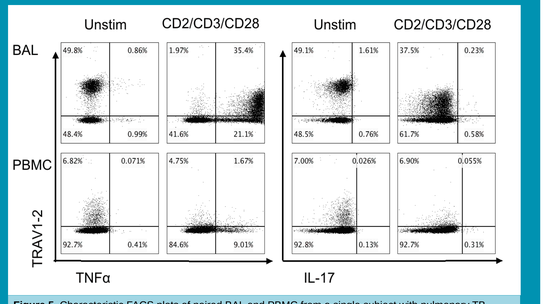



**Figure 5.** Characteristic FACS plots of paired BAL and PBMC from a single subject with pulmonary TB. Upon non-specific T cell activation with CD2/CD3/C28 beads (Miltenyi), the TRAV1-2+CD8+ cells from the BAL produce very high levels of TNFa, in contrast to the TRAV1-2+CD8+ in the peripheral blood. Very little IL-17 is made by TRAV1-2+CD8+ T cells in lung or periphery. Cells were gated on FSC SCC characteristics, live on viability stain, CD14-, CD3+ and CD8+.

## CONCLUSIONS

MAIT cells, an emerging class of innate lymphocytes, are highly enriched in the lungs during pulmonary TB. Pulmonary TRAV1-2+CD8+ cells produce TNFa but not IL-17.

Interestingly, though we and others have shown that HIV severely depletes MAITs in the peripheral blood, here we find MAITs to be enriched in the lungs of both HIV-positive and HIV-negative patients during active pulmonary tuberculosis. This suggests that MAITs may be redistributed to mucosal surfaces rather than destroyed by HIV via activation induced apoptosis as has been proposed. Additional research into the phenotype, function and compartmental distribution of MAITs in HIV infection is needed.

Further research on the role of MAIT cells in protective immunity against TB is urgently needed; given their restriction by the monomorphic MR-1, their potential in novel TB vaccine strategies should be explored.

## ACKNOWLEDGMENTS


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