# FUNCTIONAL, ANTIGEN-SPECIFIC STEM CELL-LIKE MEMORY ($T_{SCM}$) CD4+ T CELLS ARE INDUCED BY HUMAN *M. TUBERCULOSIS* INFECTION

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**Background**

Induction of anti-mycobacterial T cell responses by vaccination or natural infection is not sufficient to consistently confer protection against TB disease. A better understanding of the *Mycobacterium tuberculosis* (M.tb)-specific memory T cell repertoire is needed. Compared to central ($T_{CM}$) and effector ($T_{EFF}$) memory T cells, stem cell-like memory T cells ($T_{SCM}$) have superior self-renewing capacity, longevity and proliferative potential, functions associated with maintenance of long lasting immunity. Our knowledge of $T_{SCM}$ function derives primarily from studies of virus-specific CD8+ $T_{SCM}$. Little is known about CD4+ $T_{SCM}$ function in response to bacterial infections. We aimed to determine if M.tb infection generates antigen-specific CD4+ $T_{SCM}$ and if so, characterise their functional ontology.

**Methods**

We studied immune responses to natural M.tb infection in PBMCs from three cross-sectional QuantiFERON (QFT)+ adult cohorts and a longitudinal adolescent cohort of recent QFT converters. M.tb-specific CD4 T cells were detected by flow cytometry using MHC-class II tetramers bearing Ag85, CFP-10 or ESAT-6 peptides, or by intracellular cytokine staining after whole blood stimulation with Ag85B, CFP-10, ESAT-6 or BCG. Transcriptional analyses of M.tb-specific tetramer+ CD4+ (Tet+) $T_{SCM}$ (CD45RA+CCR7+CD27+), sorted by FACS, were performed by microfluidic quantitative real-time PCR. The transcriptomic profile of Tet+ $T_{SCM}$ was confirmed on independent cohorts by measuring expression of chemokine receptors, cytotoxic molecules and cytokines using flow cytometry.

**Results**

Mtb-specific Tet+ $T_{SCM}$ were induced by primary M.tb-infection and maintained thereafter. CD95- Tet+ $T_{SCM}$ displayed a very distinct transcriptomic profile from bulk CD4+ naïve T cells ($T_N$) and more similar to bulk $T_{SCM}$, M.tb-specific $T_{SCM}$ and $T_{EFF}$. CD95 expression, a typical marker of CD8+ $T_{SCM}$, by Tet+ $T_{SCM}$ was variable. By contrast, Tet+ $T_{SCM}$ were predominantly CXCR3+, an alternative $T_{SCM}$ marker. Tet+ $T_{SCM}$ expressed significantly higher protein levels of CCR5, CCR6, CXCR3, Granzyme A, Granzyme K and Granulysin than bulk $T_N$ and CD95+ $T_{SCM}$. These M.tb-specific $T_{SCM}$ were also functional, producing IL-2, IFN-γ and TNF-α upon antigen stimulation.

**Conclusions**

Our comprehensive analyses show that M.tb CD4+ $T_{SCM}$ are induced during primary M.tb-infection and are distinct from bulk $T_N$ and $T_{SCM}$. CXCR3 was a more robust marker of M.tb-specific CD4+ $T_{SCM}$ than CD95. M.tb-specific CD4+ $T_{SCM}$ displayed chemokine receptor profiles consistent with memory Th1/17 cells and, surprisingly, expressed effector functions including cytotoxic markers and Th1 cytokines.

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