Double-negative T cells are functionally distinct and maintained in SIV infected sooty mangabeys

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ABSTRACT

BACKGROUND: Recent studies have shown that CD4/CD8 double-negative (DN) T cells possess CD4 T-like functional properties and have been implicated in the lack of AIDS in natural hosts of SIV, including sooty mangabeys (SM). However, there are limited data in non-natural hosts of SIV like rhesus macaques (RM). This study aimed to compare DN T cells in SM and RM and to analyze the impact of chronic SIV infection on DN T cell function in both species.

METHODOLOGY: Peripheral blood DN T cells were evaluated in a cohort of SIV-negative and SIV-infected SM and RM. Functional analysis was performed using polychromatic flow cytometry and 28-plex luminex assay of TCR-stimulated (anti-CD3/anti-CD28 crosslinking) unfractionated and FACS-sorted purified T cell subsets respectively. Additionally, transcriptional profiling of 91 genes associated with T cell differentiation and function were evaluated in *ex vivo* and TCR-stimulated pure T cell subsets using a high-throughput microfluidics PCR platform (Fluidigm BioMark).

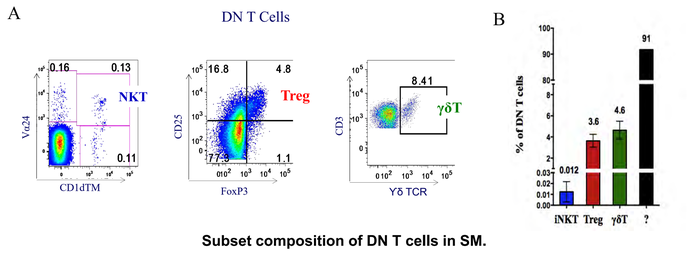
RESULTS: SIV-negative SM and RM DN T cells upregulated CD40L and produced Th1 (IFN-γ, IL-2), Th2 (IL-13) and Th17 (IL-17) cytokines in response to superantigen or TCR stimulation. However, SM DN T cells produced significantly more cytokines at the single-cell level, showed greater polyfunctionality, and were functionally distinct from RM DN T cells with regards to production of multiple cytokines and chemokines, including IL-2, IL-4, IL-5, IL-13, IL-17, G-CSF, GM-CSF, MCP-1, and MIF. SM DN T cells also had significant population of Tregs and higher frequency of CCR6-positive cells. *Ex vivo* gene expression profiling of purified T cell subsets showed transcriptional similarity of SM DN T cells to CD4+ T cells and strikingly significant differences from RM DN T cells. Surprisingly, RM DN T cells showed closest similarity to CD8+ and not CD4+ T cells. TCR-stimulated SM DN T cells showed 2- to 580-fold upregulation of 31 genes including effector molecules and activation markers in contrast to up to 9-fold upregulation of only15 genes in RM DN T cells. The genes showing greatest upregulation (>10-fold) in SM DN T cells included *il22, il17a, ifng, tnf, il2, il4, il5* and *il21*, confirming their enhanced Th17, Th1 and Th2 functionality. Following SIV infection, profound DN T cell hypofunctionality was seen in SIV-infected RM but not SIV-infected SM.

CONCLUSIONS: Besides being significantly higher in frequency, SM DN T cells are functionally distinct from RM and may differ in their ontogeny. The diverse functional properties of SM DN T cells that are not affected by SIV infection likely play a role in regulating immune activation and preventing disease progression to AIDS in these natural hosts of SIV infection.

OBJECTIVES

1. To characterize and compare the phenotype and function of DNT cells in sooty mangabeys (SM) and rhesus macaques (RM), representing AIDS-resistant and AIDS-susceptible nonhuman primate host species.
2. To investigate the impact of chronic SIV infection on DNT cell frequency and function in nonpathogenic and pathogenic hosts

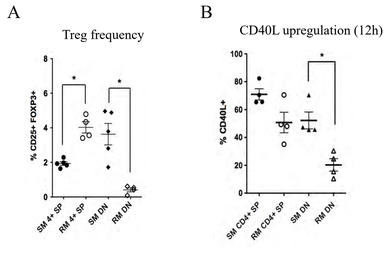
SM DN T cells comprise a minor proportion of NKT, Tregs and γδT cells



Subset composition of DN T cells in SM.

(A) Representative dot plots of SM DN T cells stained with antibodies specific for NKT cells, Tregs and γδ T cells. (B) Frequencies of NKT cell, Treg and γδ T cell in circulating DN T cells of sooty mangabeys.

SM DN T cells comprise a higher proportion of Tregs and upregulate CD40L to a higher extent than RM DN T cells

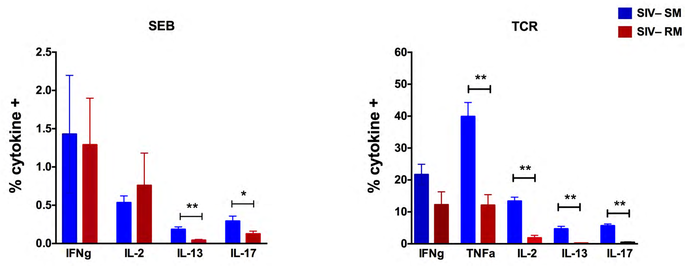


Ex vivo frequency of Tregs and upregulation of CD40L in CD4 T and DN T cells of SM and RM.

(A) Circulating Treg frequency based on CD25 and FOXP3 staining in SM and RM PBMC.

(B) CD40L upregulation in SM and RM CD4 T and DN T cell subsets following 12h in vitro stimulation with PMA/Ca Ionomycin. Bars denote mean values. * P<0.05 Mann Whitney test.

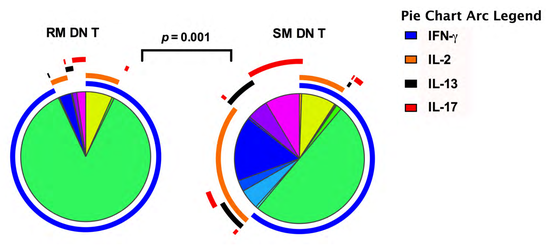
Significantly higher levels of IL-17 and IL-13 production by SM DN T cells in comparison to RM DN T cells



SM DN T cells display a more potent response to TCR stimulation than RM DN T cells

Intracellular Cytokine Staining in RM and SM DN T cell subsets for IFN-γ, IL-2, IL-13, IL-17, and TNF-α following 12h SEB (A) or TCR (B) stimulation of PBMC. Bars denote mean values with SEM. * P<0.05, ** P<0.01, Mann Whitney test.

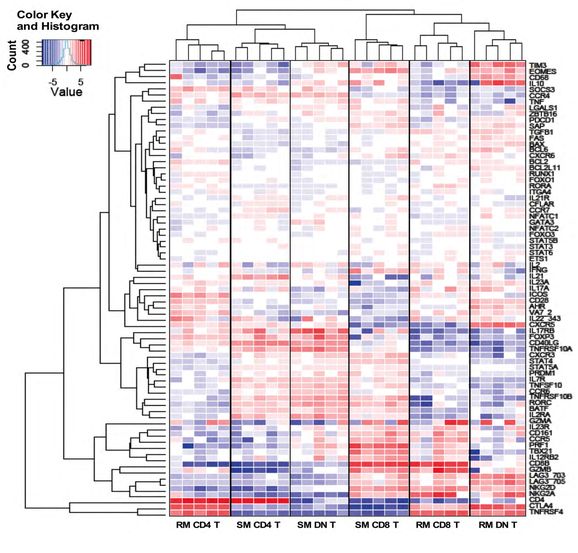
SM DN T cells display higher polyfunctionality in comparison to RM DN T cells



SM DN T cells are more polyfunctional than RM DN T cells

SPICE-generated pie chart representations of the functional DN T cell profiles. Pies represent the capacity of DN T cells in SIV-egative SM and RM (n=5 per group) to secrete one or any combination of the four cytokines IFN-γ, IL-13, IL-17, and IL-2.

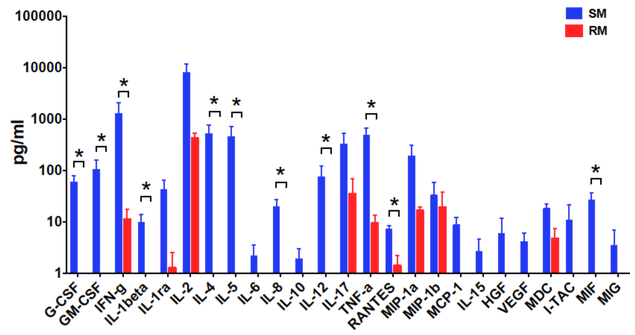
Constitutive gene expression of SM DN T cells is strikingly different from RM DN T cells



Transcriptional profile of ex vivo DN T cells in SM and RM in comparison to CD4 & CD8 T cell subsets

Gene expression heat map of FACS-sorted T cell subsets from 5 SM and 5 RM showing the unsupervised hierarchical clustering of cells on top. SM DN T cells cluster closest to SM CD4 T cells and then to RM CD4 T cells, but RM DN T cells cluster with CD8 T cells.

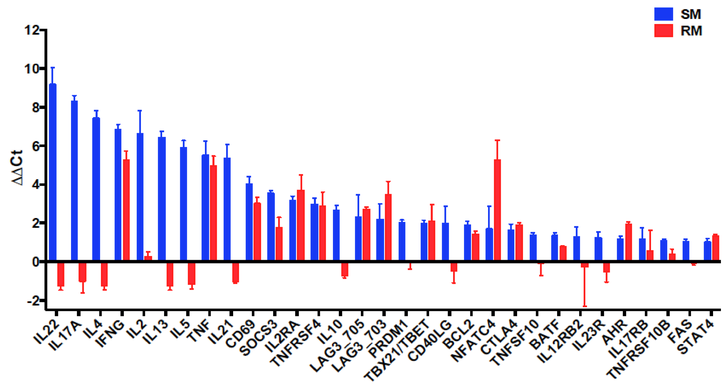
SM DN T cells are functionally distinct from RM and secrete a diverse array of cytokines and chemokines



Diverse cytokine secretion by SM DN T cells in response to mitogen stimulation

Cytokine secretion by FACS-sorted DN T cells from RM and SM following 12h stimulation with PMA/Ca-Ionomycin measured by luminex multiplex assay. Bars denote mean values with SEM. * P<0.05, Mann Whitney test.

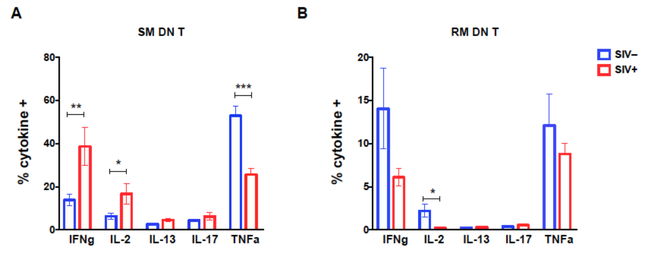
Significant transcriptional upregulation of Th17, Th2 and Th1 cytokines in TCR-stimulated DN T cells of SM



Upregulation of effector molecules by sorted DN T cells in response to TCR stimulation in SM and RM

Quantitative real time gene expression analysis of TCR stimulated purified DN T cells from SIV-negative SM and RM (n=4 per group) showing upregulation over unstimulated cells as ΔΔCt values. Data shows mean values and SEM bars.

Enhanced IFNg and IL-2 cytokine production of SM DN T cells and diminished responses of RM DN T cells in chronic SIV infection



Impact of chronic SIV infection on SM and RM DN T cells

Intracellular Cytokine Staining in SIV-infected SM (A) and RM DN T cell subsets (B) for IFN-γ, IL-2, IL-13, IL-17, and TNF-α following 12h TCR stimulation of PBMC. Bars denote mean values with SEM. * P<0.05, ** P<0.01, *** P<0.001, Mann Whitney test.

CONCLUSIONS

- DN T cells in both SM and RM can produce IL-2 and IL-17 and upregulate of CD40L similar to CD4+ T cells.
- DN T cells in SIV-negative SM differ from SIV-negative RM with regards to:
  - containing significantly higher proportions of Tregs
  - producing significantly more Th2 and Th17 cytokines
  - comprising greater polyfunctionality
- DN T cell function is enhanced for IL-2 and IFN-γ production in SIV-infected SM but is significantly impaired in SIV-infected RM.
- Besides being functionally distinct from RM, SM DN T cells may differ from RM DN T cells in their ontogeny.
- The diverse functional properties of SM DN T cells that are not affected by SIV infection, and an increase in IL-2 and IFN-γ function likely play a role in regulating immune activation and preventing disease progression to AIDS in these natural hosts of SIV infection .

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