Activation and Proliferation of PD-1+ Kidney Double-Negative T Cells Is Dependent on Nonclassical MHC Proteins and IL-2

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Background
- CD4-CDB-double-negative (DN) αβ T cells with innate-like properties represent a significant component of T cells in human and mouse kidneys.
- DN T cells constitute about 20-38% of αβ T cells in the normal kidney, whereas they exist as 1-5% of αβ T cells in the peripheral blood and lymphoid organs.
- They spontaneously proliferate in the steady state and protect against ischemic AKI.
- However, the mechanisms regulating DN T cell homeostasis and responses to external danger signals from "sterile" inflammation remain poorly understood.

Methods
- Knockout mouse models
- Flow Cytometry analysis
- Adoptive transfer of T and B cells
- In vitro co-culture system
- Ischemic AKI model
- Human kidney biopsies

Results

Figure 1: Gating strategy for identification of DN- T cell subsets. Lymphocytes were isolated from kidneys of WT mice; stained and acquired by LSR II. CD45+ cells were gated; followed gating of TCRαβ+ cells, which were then analyzed for the presence of CD4, CD8, and DN T cell subsets. CD1d tetramer+ cells were excluded as indicated in the figures.

Figure 2: β2m-dependent MHC1b, but not MHC II or MHC1a, molecules significantly reduce relative and absolute number of kidney DN T-cells.

Figure 3: Lack of β2m, but not MHC II, impairs activation, proliferation, and increased apoptosis of kidney DN T cells in the steady state.

Figure 4: Adoptive transfer of WT CD8 T-cells into β2m KO mice significantly increases absolute numbers and activates endogenous DN T-cells. Restoration of kidney DN T cells by adoptively transferred CD8 T-cells was not due to downregulation of CD8 coreceptors by donor T cells.

Figure 5: Injection of β2m KO mice with CD4 T cells, caused activation and expansion of kidney DN T cells, whereas injection of B cells caused activation without expansion.

Figure 6: Provision of IL-2 by CD8 T-cells is necessary for expansion of DN T-cells in vitro.

Figure 7: Identification of the NK1.1+ and PD-1+ DN T-cell subsets in mouse kidneys.

Figure 8: PD-1+ DN T-cell is the major responders to ischemic AKI by β2m-independent mechanisms.

Figure 9: Detection of the NK1.1+ and PD-1+ DN T-cell subsets in human kidneys.

Summary
- Lack of β2m, but not classical I or II MHC molecules impaired activation and proliferation of kidney DN T-cells.
- Kidney DN T-cells are largely dependent on conventional T-cells to drive their homeostasis by provision of IL-2.
- Lack of β2m selectively affected NK1.1 but not PD-1+ subset of kidney DN T-cells.
- PD-1+ DN T-cell is the major responders to ischemic AKI.