In Vitro Generation of Antigen-inexperienced Memory CD8+ T Cells by IL-15

Background

Memory-phenotype CD8+ T cells exist even within hosts that have not been exposed to foreign antigen (e.g., in human cord blood).[1] Classically, memory CD8+ T cells (T_{MEM}) result from antigen-specific interactions, but immunological memory can also be achieved through antigen-inexperienced memory-phenotype T cells — virtual memory CD8+ T cells (T_{VM}). T_{VM} arise in the periphery from naive CD8+ T cells and are driven by homeostatic cytokines.[1,2] They partially resemble classical T_{MEM} with their ability to rapidly mount an immune response upon antigen stimulation.[1,2,3] Putative human T_{VM} express NK and T_{MEM} markers (EOMES, NKG2A, KIR3DL1) and are phenotypically defined as [CD45RA+CD27-EOMES+NKG2A+panKIR+][9]. It has been shown that T_{VM} are largely depend on IL-15 and IL-4 for differentiation, expansion, and survival.[1,4,5] Ablation of IL-15 production or IL-15 signaling completely inhibits T_{VM} differentiation and survival.[5] BATF-3 dependent dendritic cells (DC) have been shown to be the predominant producers of IL-15 in the steady state, and BATF-3 deficient mice had a severely impaired T_{VM} population. T_{VM} differentiation in IL-15 deficient mice can be rescued by injecting IL-15/IL-15RA complexes.[6] Ablation of IL-4 is also known to compromise the T_{VM} population, indicating their dependence on IL-4[6,7,8] .However, this cytokine dependency has mainly been shown in mice. Whether IL-15 and/or IL-4 directly contributes to T_{VM} differentiation in humans is to be determined.

Hypothesis

Treatment of bulk CD8+ naive T cells with IL-15 and/or IL-4 *in vitro* will convert them into [CD45RA+CD27-EOMES+panKIR+] antigen-inexperienced memory CD8+ T cells (T_{VM}).

Rationale

It has been shown that CD45RA+CD27-panKIR+ T_{VM} cells accumulate in patients with human immunodeficiency virus (HIV). They are positively correlated with IL-15 and IFN-y serum concentrations.[9] However, there is still no clear evidence for cytokine-induced activation of T_{VM} in human infectious diseases. T_{VM} are also inversely correlated with latent HIV viral load and have been demonstrated to play a protective role in influenza, helminth infections, and other chronic infections.[10,11,12]

A putative T_{VM} equivalent population in humans has been defined. Given that human T_{VM} have a similar responsiveness to antigen stimulation and phenotype to mouse T_{VM}, and that they are positively correlated with IL-15 serum levels in patients with chronic infections, it is plausible that IL-15 mediates the expansion/differentiation of human T_{VM} cells as well.[1,2,9]

Objectives

1. Establish proportion of Tvm in isolated Tn / bulk CD8+
2. Generate Tvm from bulk CD8+ T cells
3. Generate Tvm from isolated Tn
4. Assess the function of Tvm following 48hrs CD3/28 stimulation

Methods

Establish proportion of Tvm in PBMCs

1. Using flow cytometry, sort PBMCs with CD3,CD8,Livedead,CD45RA,CD27,CCR7, EOMES, KIR3DL1,KIR2D,NKG2A. Determine the Tvm population percentage, gated on CD3+CD8+CD45RA+CCR7-CD27-EOMES+KIR3DL1+ and/or KIR2D+ and NKG2A+[5]

Generate Tvm from PBMC culture

1. Thaw PBMCs in a 6-well plate overnight (or 24hrs)
2. Coculture PBMCs in round-bottom well with:
a. control (no cytokines)
b. IL-15 (20ng/mL)
c. IL-4 (20ng/mL)
d. IL-15 + IL-4 ((20ng/mL each)
3. Using flow cytometry, sort groups a-d with CD3,CD8,Livedead,CD45RA,CD27,CCR7, EOMES, KIR3DL1,KIR2D,NKG2A. Determine the Tvm population percentage, gated on CD3+CD8+CD45RA+CCR7-CD27-EOMES+KIR3DL1+ and/or KIR2D+ and NKG2A+[5]

Generate Tvm from bulk CD8+ T cells

1. Thaw PBMCs in a 6-well plate overnight (or 24hrs)
2. Isolate CD8+ T cells using CD8 sorting kit.
3. Coculture bulk CD8 T cells with:
a. control (no cytokines)
b. IL-15 (20ng/mL)
c. IL-4 (20ng/mL)
d. IL-15 + IL-4 ((20ng/mL each)
4. Sort cells by flow cytometry according to the panel in (3) above, w/out CD3

Generate Tvm from isolated Tn

1. Thaw PBMCs in a 6-well plate overnight (or 24hrs)
2. Isolate CD8+CD45RA+CCR7+ Tn using Tn magnetic sorting kit.
3. Coculture Tn cells with:
a. control (no cytokines)
b. IL-15 (20ng/mL)
c. IL-4 (20ng/mL)
d. IL-15 + IL-4 ((20ng/mL each)
4. Sort cells by flow cytometry according to the panel in (3) above, w/out CD3

**Putative human Tvm markers are CD8+CD3+CD45RA+CD45RO-CD27-CCR7-EOMES+KIR3DL1+ and/or KIR2D and NKG2A+.[5,9] KIR3DL1 and KIR2D collectively comprise the panKIR marker, which may be the ideal marker for Tvm Identification, rather than EOMES.[17]

Assess the function of Tvm following 48hrs CD3/28 stimulation

1. Isolate CD8+CD45RA+CCR7+ Tn using Tn magnetic sorting kit.

2. Coculture CD45RA+CCR7+ bulk Tn with:
a. IL-15+IL-4+IL-2
b. IL-15+IL-2
c. IL-4+IL-2
d. Control (IL-2)
*10ng/mL each cytokine for 7 days [4,16]

3. For groups a-d, stimulate with CD3+CD28 for 48hrs and split into another unstim population:
Treatment, Stim (ts)
as. IL-15+IL-4+IL-2; CD3/28
bs. IL-15+IL-2; CD3/28
cs. IL-4+IL-2; CD3/28
Treatment, Unstim (tu)
au. IL-15+IL-4+IL-2
bu. IL-15+IL-2
cu. IL-4+IL-2
Control, Stim (cs)
ds. IL-2; CD3/28
Control, Unstim (cu)
du. IL-2

4. Using flow cytometry, sort above groups with CD8,Livedead,CD45RA,CD27,CCR7,EOMES,panKIR, NKG2A,granzyme B,perforin,(granulysin).[9] Compare granzyme B and perforin expression between ts and cs.

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