CD4+ Tissue-Resident Memory T Cells are an Important Reservoir for HIV Persistence

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Background

- Currently, peripheral blood is sampled to assess the efficacy of HIV eradication approaches.
- Memory CD4+ T cells are a key target for HIV infection and the major cellular reservoir for HIV in peripheral blood.
- CD4+ tissue-resident memory T cells (T_{RM}) are a potential cellular HIV reservoir that cannot be assessed by sampling peripheral blood.
- Key characteristics of T_{RM} include that they:[1-7]
  - Are present in lymphoid, non-lymphoid, and mucosal tissues
  - Do not recirculate
  - Are virtually absent from peripheral blood
  - Provide optimal immunity to site-specific pathogens
  - Express CD45RO and CD69
- Despite their potential importance to HIV persistence, the effect of HIV infection on T_{RM} has not been evaluated.
- Currently, *in vivo* studies of T_{RM} cannot be performed in humans because:
  - T_{RM} cannot be accessed by sampling human peripheral blood.
  - Studies of T_{RM} in humans are limited to analyses of biopsied and cadaveric tissue.[1]
- Therefore, we 1) performed a polychromatic flow cytometric analysis of peripheral blood and cells isolated from tissues of humanized BLT mice to characterize their human T_{RM} compartment, 2) determined the effect of HIV infection on CD4+ T_{RM}, and 3) evaluated the contribution of CD4+ T_{RM} to HIV persistence.

Materials and Methods

Generation of bone marrow-liver-thymus (BLT) humanized mice. BLT mice were generated by transplanting human fetal liver-derived CD34+ hematopoietic stem cells intravenously into irradiated NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ (NSG; The Jackson Laboratory) mice implanted with autologous human fetal liver and thymus tissue (Advanced Bioscience Resources) under the kidney capsule. Subsequently, the levels of human cells in the peripheral blood of BLT mice were monitored every 3-4 weeks with flow cytometry.[8-9]

Flow cytometric analysis of human tissue-resident memory T cells in BLT humanized mice. Peripheral blood and tissues were collected from BLT mice at necropsy. Mononuclear cells were isolated from the lymph nodes, spleen, bone marrow, liver, and lung as previously described.[8-9] Peripheral blood and tissue mononuclear cells were blocked with mouse IgG, mouse IgG2a$_k$, and rat IgG2a$_k$, then stained with antibodies specific for the following human markers: CD45, CD3, CD4, CD8, CD45RO, CD69, CCR7, HLA-DR, and CD25. Samples were analyzed with a BD LSRFortessa instrument and data analysis performed with BD FACSDiva software (version 6.1). Gating Strategy: Human tissue-resident memory T cells (T_{RM}) were gated as live (FSC/SSC)→CD45+ → CD3+ → CD4+ or CD8+ → CD45RO+ → CD69+.[1] T_{RM} that lacked expression of HLA-DR and CD25 were defined as resting. Human circulating memory T cells (T_{CM}) were gated as live (FSC/SSC)→CD45+ → CD3+ → CD4+ or CD8+ → CD45RO+ → CD69^{neg}.[1] Human T_{RM} and T_{CM} effector and central memory T cell populations were defined by CCR7 expression (effector memory: CCR7^{neg} and central memory: CCR7+).[1] T_{RM} were sorted from mononuclear cells isolated from tissues harvested from HIV-infected BLT mice using the following gating strategy: Live (FSC/SSC)→CD45+→CD3+→CD45RO+→CD69+.

HIV infection of BLT mice. Stocks of HIV (HIV-1$_{JR\text{-}CSF}$, HIV-1$_{THRO}$, HIV-1$_{CH040}$) were prepared and titered as previously described. BLT mice were exposed vaginally or orally to HIV-1 as previously described.[8-9] HIV-RNA was monitored longitudinally in plasma with real-time PCR as previously described.[8-9] HIV-infected BLT mice were necropsied 2-16 weeks post-exposure (mean: 7.75 weeks) and peripheral blood and tissues collected for flow cytometric analysis of human T_{RM}. HIV-DNA and HIV-RNA levels in T_{RM} were determined with real-time PCR analysis as previously described.[8-9] As a control, all samples were tested for the presence of human gamma globulin DNA or human TATA-box binding protein RNA by real-time PCR.

Results

Human Tissue-Resident Memory T Cells are Present in the Tissues of BLT Mice but Virtually Absent From Peripheral Blood

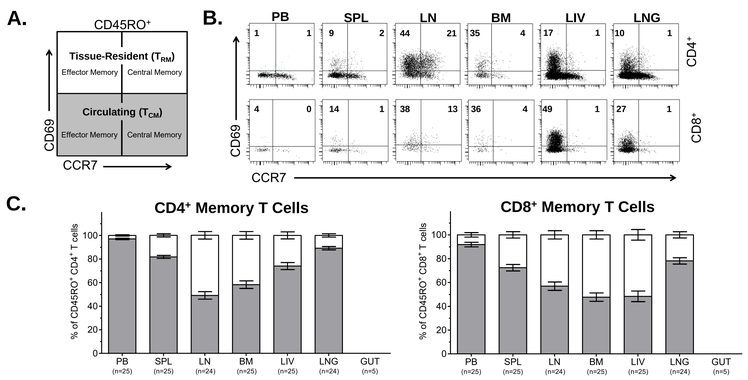


Figure 1. Human tissue-resident memory T cells are present in the tissues of BLT mice but virtually absent from peripheral blood. (A) Gating strategy for identifying human tissue-resident (T_{RM}) and circulating (T_{CM}) memory T cell populations: Live (FSC/SSC)→CD45+→CD3+→CD4+ or CD8+→CD45RO+. (B) Representative flow plots depicting the distribution of human T_{RM} and T_{CM} populations in the peripheral blood (PB) and tissues (SPL=spleen, LN=lymph nodes, BM=bone marrow, LIV=liver, LNG=lung) of BLT mice. (C) Mean percentage (± SEM) of human memory (CD45RO+) CD4+ and CD8+ T cells in the PB and tissues of BLT mice that are T_{RM} (white bars) and T_{CM} (gray bars).

Resting CD4+ Tissue-Resident Memory T Cells are Present in the Tissues of BLT Mice

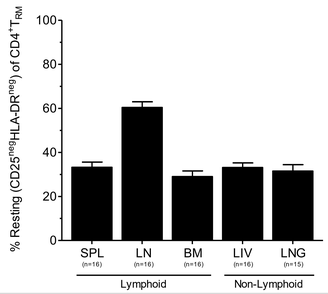


Figure 2. Resting CD4+ tissue-resident memory T cells are present in lymphoid and non-lymphoid tissues of BLT mice. Mean percentage (± SEM) of resting (CD25^{neg}HLA-DR^{neg}) CD4+ T_{RM} in the tissues (SPL=spleen, 33%; LN=lymph nodes, 61%; BM=bone marrow, 29%; LIV=liver, 33%; LNG=lung, 32%) of BLT mice.

CD4+ Memory T Cells are Significantly Depleted From the Tissues of HIV-Infected BLT Mice

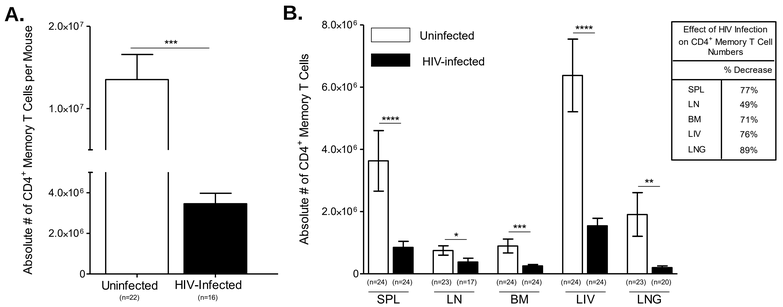


Figure 3. CD4+ memory T cells are significantly depleted from the tissues of HIV-infected BLT mice. Mean absolute number (± SEM) of CD4+ memory (CD45RO+) T cells in the tissues (SPL=spleen, LN=lymph nodes, BM=bone marrow, LIV=liver, and LNG=lung) of uninfected (white bars) and HIV-infected (black bars) BLT mice. Data represent the mean absolute number of CD4+ memory T cells (A) per mouse and (B) per tissue. We used a two-tailed Mann-Whitney test to compare the absolute of number of CD4+ memory T cells in uninfected and HIV-infected BLT mice (*p<0.05, **p<0.01, **p<0.001, and ****p<0.0001).

CD4+ Tissue-Resident Memory T Cells are Significantly Depleted From the Tissues of HIV-Infected BLT Mice

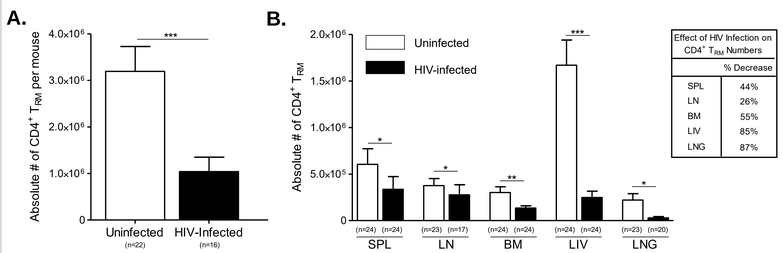


Figure 4. CD4+ tissue-resident memory T cells are significantly depleted from the tissues of HIV-infected BLT mice. Mean absolute number (± SEM) of CD4+ tissue-resident memory T cells (T_{RM}) in the tissues (SPL=spleen, LN=lymph nodes, BM=bone marrow, LIV=liver, and LNG=lung) of uninfected (white bars) and HIV-infected (black bars) BLT mice. Data represent the mean absolute number of CD4+ T_{RM} (A) per mouse and (B) per tissue. We used a two-tailed Mann-Whitney test to compare the absolute of number of CD4+ T_{RM} in uninfected and HIV-infected BLT mice (*p<0.05, **p<0.01, **p<0.001, and ****p<0.0001).

HIV-DNA and HIV-RNA are Present in T_{RM} of HIV-Infected BLT Mice

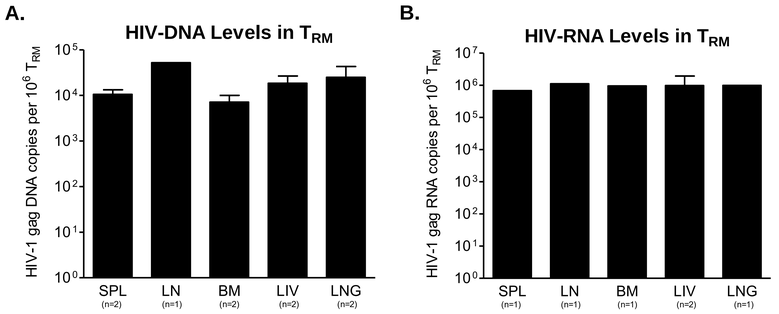


Figure 5. HIV-DNA and HIV-RNA are present in T_{RM} of HIV-infected BLT mice . (A) HIV-DNA and (B) HIV-RNA levels in T_{RM} sorted from the tissues (SPL=spleen, LN=lymph nodes, BM=bone marrow, LIV=liver, LNG=lung) of HIV-infected BLT mice (4-7 weeks post-exposure) as determined by real-time PCR. Gating strategy for T_{RM} sorting: Live (FSC/SSC)→CD45+→CD3+→CD45RO+→CD69+.

Conclusions

- Human CD4+ and CD8+ tissue-resident memory T cells are present in lymphoid and non-lymphoid tissues of BLT mice but are virtually absent from peripheral blood, like in humans.[1]
- HIV infects and depletes CD4+ memory T cells and CD4+ T_{RM} in tissues.
- CD4+ T_{RM} contain substantial levels of viral DNA and viral RNA.

Future Directions

- Assess if CD4+ T_{RM} levels rebound following ART
- Determine if CD4+ T_{RM} serve as a cellular reservoir for HIV during ART

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