Wnts-mediated astrocyte/CD8+ T cell interactions impacting HIV neuropathogenesis

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Abstract

Background: Depletion of CD8+ T cells accelerates neuroAIDS, although there is no clear evidence that CD8+ T cells control HIV/SIV in the CNS. A subset of CD8+ T cells, $CD4^{dim}CD8^{bright}$ T cells (DP T cells), is enriched in anti-HIV responses. This phenotype is generated through induction of Wnt/b-catenin signaling in CD8+ T cells. Wnts, small secreted glycoproteins, initiate Wnt/b-catenin signaling leading to gene regulation. Given that astrocytes are a rich source of Wnts, we evaluated whether astrocytes drive DP expression in vitro and in vivo.

Methods: Wnts mRNA and protein from human progenitor-derived astrocytes (PDAs) were determined by qRT-PCR and western blot, respectively. Day three-Astrocyte Conditioned Media (ACM) was added to αCD3/CD28 activated PBMCs and percent DP T cells were assessed at day six by flow. Wnts were depleted from ACMs by protein A/G magnetic beads coated with anti-Wnt 1, Wnt2b, Wnt 3, Wnt 5b, Wnt 10b, or IgG1 isotype control. NOD/SCID/IL-2rcγ -/- (NSG) mice were reconstituted with $2x10^7$ PBMCs (NSG-HuPBMCs) and infected by IP with 10^4 $TCID_{50}$ HIV_{BAL} at week 1. Three weeks post-infection, brain lymphocytes were isolated and expression of CD8, CD4, and HIVp24 determined by flow. Lastly, violet-labeled CD8+ T cells were injected into NSG brain, re-isolated at 1 week, and evaluated for CD4, CD45RA, CD27, CD28, and CCR7 expression by flow.

Results: PDAs secrete Wnts 1, 2b, 3, 5b, and 10b. ACM induced DP expression by 2-fold in a Wnt-dependent manner. CD8 Single Positive (SP) and DP T cells are found in the brain of HIV+ NSG-HuPBMCs mice. DP cells harbor HIV, but interestingly, at three weeks post-infection, greater than 90% of CD4 SP T cells were depleted, while 25% of DP cells survived. Brain injection of Violet-labeled CD8+ T cells induced DP expression by 10-fold, where >80% of DP and CD8 SP T cells expressed surface markers indicative of a Terminal Effector Memory (TEMRA) phenotype ($CCR7^-CD27^-CD28^-CD45RA^+$).

Conclusions: Astrocytes drive DP expression through Wnts. DP T cells are present in the CNS, are TEMRA, and HIV infected. Unlike CD4 SP T cells, DP T cells survive longer and may constitute a novel HIV CNS reservoir. These studies highlight a dynamic interaction between astrocytes and CD8 T cells, which can skew their differentiation and lead to a phenotype that, on one hand, can control HIV in the CNS but, on the other, heightens inflammatory responses in the CNS, contributing to HAND pathology.

Background

HIV invades the CNS shortly after infection. While studies in SIV and HIV indicate that CD8+ T cells are involved in controlling HIV replication in the brain the consequences of this, whether neuroprotective or pathogenic, are unclear. The brain is a complex site for immune responses, It is imperative to reduce pathogen burden but not at the expense of healthy brain tissue. A CD8+ T cell response that is too aggressive in clearing an infection in the brain can result in neuropathology. We and others have identified a population of CD8 T cells which dimly express CD4 on the surface (DP T cells). These cells constitute approximately 60% of all HIV-specific T cell responses as evaluated by tetramer analysis and are expanded in the blood of Long Term Non-Progressor HIV+ patients. CD4 expression on CD8+ T cells is mediated by enhanced β-catenin signaling. β-catenin is a transcriptional co-activator that regulates gene transcription resulting in many cellular processes including development, proliferation, survival, regeneration, wound healing and stress responses. The β-catenin pathway is stabilized by Wnt ligand binding to Frizzled and LRP5/6 receptors. Wnts are a family of 19 small secreted glycoproteins that are evolutionarily conserved. These studies will address how the Wnt rich microenvironment of the brain influences the anti-HIV responses made by CD8+ T cells.

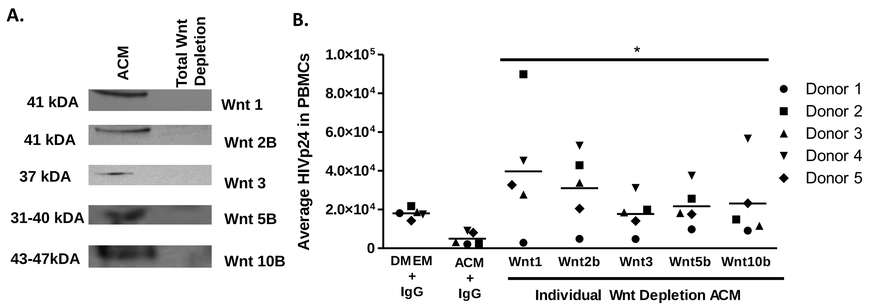


Figure 1. Astrocytes secret Wnt ligands which inhibit HIV infection of PBMCs

A) Efficiency of Wnt depletion from Astrocyte Conditioned Media (ACM): 1 $x10^6$ PDAs were cultured for 6 days, 50µl of ACM was assessed by western blot for Wnt 1, 2b, 3, 5b, and 10b. ACM was depleted for Wnt ligands by immunoprecipitation and efficiency of depletion assessed by Western blot. **B) Wnts secreted from astrocytes inhibit HIV replication in human PBMCs:** αCD3/αCD28 activated PBMCs were infected with of HIV_{BAL} (2ng/ $1x10^6$ cells) then cultured in DMEM, ACM, or individual Wnt depleted ACM for 6 days. HIVp24 was measured by ELISA. Data is representative of four donors. Asterisks denote $p<0.05$ determined by student's-T test.

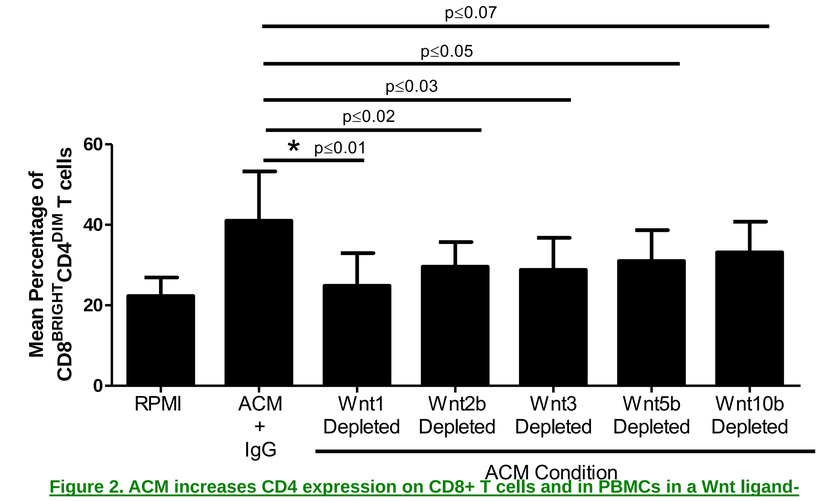


Figure 2. ACM increases CD4 expression on CD8+ T cells and in PBMCs in a Wnt ligand-dependent manner

aCD3/aCD28 activated PBMCs were left untreated or treated with ACM or ACM depleted of each individual Wnt ligand by immunoprecipitation then cultured with PBMCs for three days. Mean percentage of DP T cells was determined by FACS analysis. Asterisks denote $p<0.05$ determined by students-T test.

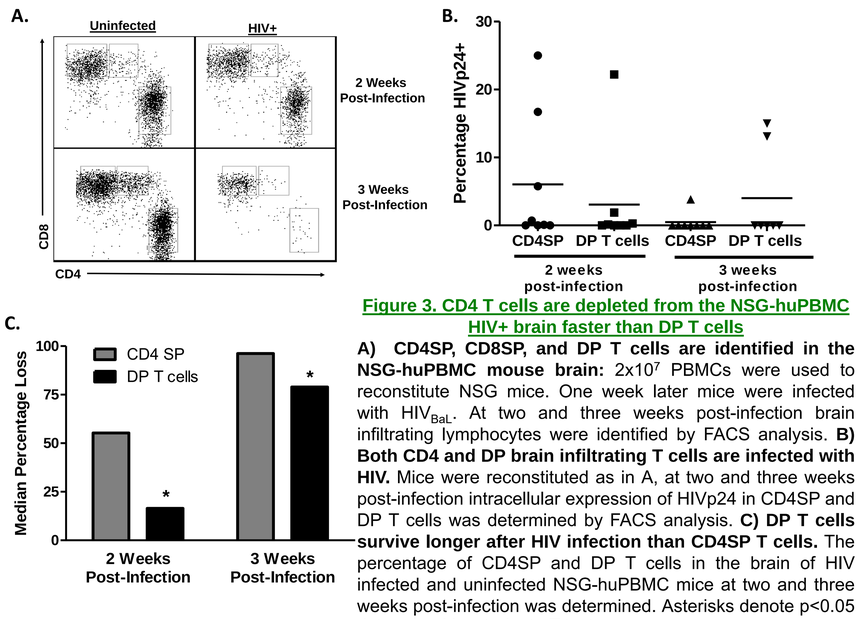


Figure 3. CD4 T cells are depleted from the NSG-huPBMC HIV+ brain faster than DP T cells

A) CD4SP, CD8SP, and DP T cells are identified in the NSG-huPBMC mouse brain: $2x10^7$ PBMCs were used to reconstitute NSG mice. One week later mice were infected with HIV_{BaL}. At two and three weeks post-infection brain infiltrating lymphocytes were identified by FACS analysis. **B) Both CD4 and DP brain infiltrating T cells are infected with HIV.** Mice were reconstituted as in A, at two and three weeks post-infection intracellular expression of HIVp24 in CD4SP and DP T cells was determined by FACS analysis. **C) DP T cells survive longer after HIV infection than CD4SP T cells.** The percentage of CD4SP and DP T cells in the brain of HIV infected and uninfected NSG-huPBMC mice at two and three weeks post-infection was determined. Asterisks denote $p<0.05$ determined by students-T test.

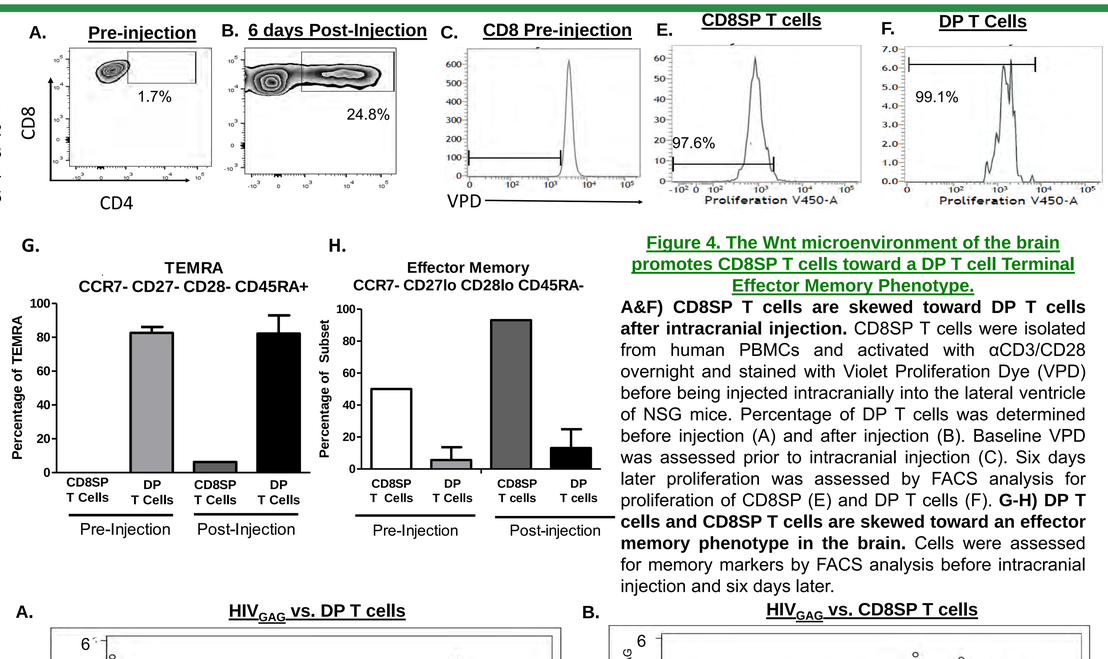


Figure 4. The Wnt microenvironment of the brain promotes CD8SP T cells toward a DP T cell Terminal Effector Memory Phenotype.

A&F) CD8SP T cells are skewed toward DP T cells after intracranial injection. CD8SP T cells were isolated from human PBMCs and activated with αCD3/CD28 overnight and stained with Violet Proliferation Dye (VPD) before being injected intracranially into the lateral ventricle of NSG mice. Percentage of DP T cells was determined before injection (A) and after injection (B). Baseline VPD was assessed prior to intracranial injection (C). Six days later proliferation was assessed by FACS analysis for proliferation of CD8SP (E) and DP T cells (F). **G-H) DP T cells and CD8SP T cells are skewed toward an effector memory phenotype in the brain.** Cells were assessed for memory markers by FACS analysis before intracranial injection and six days later.

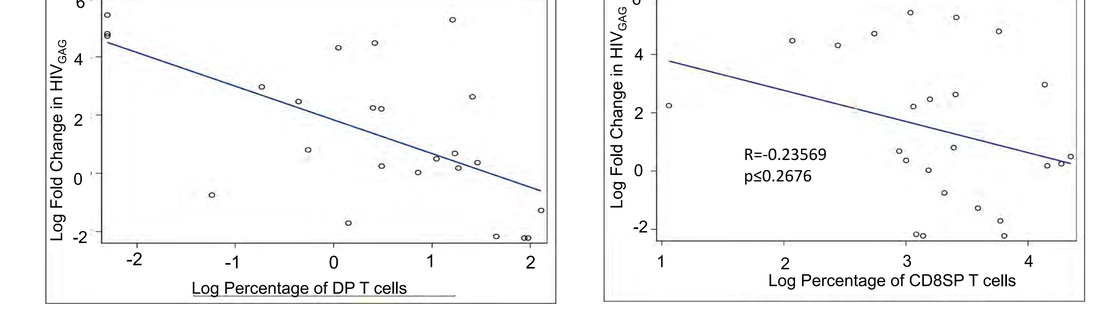


Figure 5. DP T cells, not CD8SP T cells, control HIV transcripts in the brain of NSG-huPBMC HIV+ mice

A&B) HIV_{GAG} transcripts inversely correlate with DP T cells, not CD8SP T cells in the brain of HIV infected NSG-HuPBMC mice. NSG-huPBMC mice were reconstituted with $2x10^7$ human PBMCs and infected with $TCID_{50}$ HIV_{BaL}. Two weeks later, frequency of brain infiltrating CD8SP and DP T cells was assessed from one hemisphere of the brain and HIV_{GAG} transcripts were amplified using qRT-PCR with a C_T value of 35 used as the upper limit of detection. All samples were normalized to GAPDH then further normalized to the sample with the highest detectable HIV_{Gag} transcript C_T value <35. Frequency of DP (A) or CD8SP (B) T cells was then compared to HIV_{GAG}.

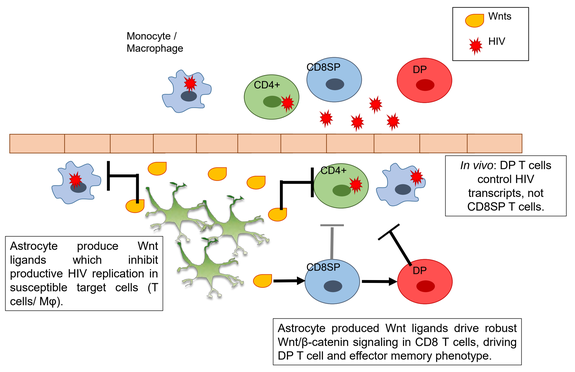


Figure 6. Model depicting interplay between Wnt ligands and infiltrating T cells in the HIV infected brain.

Conclusions

- Wnt ligands produced by astrocytes promote the DP T cell phenotype.
- Wnt ligands inhibit productive HIV infection in PBMCs which infiltrate the brain.
- CD4SP and DP T cells are susceptible to HIV infection in the brain of NSG-huPBMC HIV+ Mice; however DP T cells are better able to survive HIV infection compared to CDSP T cells.
- The Wnt rich microenvironment of the brain alters the phenotype of CD8SP T cells toward an effector memory population and DP T cells.
- DP T cells, not CD8SP T cells, inhibit HIV transcripts in the brain of NSG-huPBMC HIV infected mice.
- Collectively, these data highlight Wnt ligands as significant mediators facilitating communication between astrocytes and PBMCs in the context of HIV infection in the CNS

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