MHC-1a-Restricted CD8⁺ T cell Responses Restrict SIV RNA expression During Antiretroviral Therapy

Benjamin Varco-Merth[1,2], Alejandra Marenco[1,2], William Goodwin[1,2], Omo Fadeyi[1,2], William Bosche[3], Scott Hansen[1,2], Jacob D. Estes[1,2], Brandon Keele[3], Jeffrey D. Lifson[3], Afam A. Okoye[1,2] and Louis J. Picker[1,2]

[1] Vaccine and Gene Therapy Institute, Oregon Health & Science University, Beaverton, OR, USA.
[2] Oregon National Primate Research Center, Oregon Health & Science University, Beaverton, OR, USA.
[3] AIDS and Cancer Virus Program, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, Maryland, USA.

411

Background

Major histocompatibility complex (MHC)-1a is a highly polymorphic gene region, the products of which are responsible for antigen presentation to CD8⁺ T cells. Certain MHC-1a allomorphs are associated with enhanced SIV control in the absence of antiretroviral therapy (ART), likely mediated by presentation of conserved, structurally important elements of the SIV proteome. However, the role of these so-called protective MHC-1a (pMHC)-restricted CD8⁺ T cell responses in establishment and persistence of the SIV reservoir remains unclear. Here, we contrasted SIV-infected rhesus macaques (RM) with (pMHC+) or without (pMHC-) known protective MHC-1a alleles (Mamu-A*01, -B*08, or -B*17) to assess the impact of protective alleles on cell-associated viral loads (CAVL) in blood and lymphoid tissues during treatment.

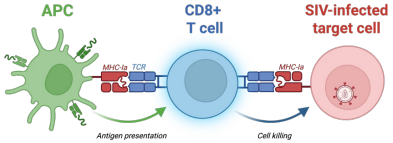


Figure 1. MHC-1 is expressed on both professional antigen presenting cells (APCs) and HIV-infected cells. Protective MHC-1 alleles are associated with enhanced control of HIV/SIV replication.

pMHC allomorphs enhance viral control during untreated SIV infection

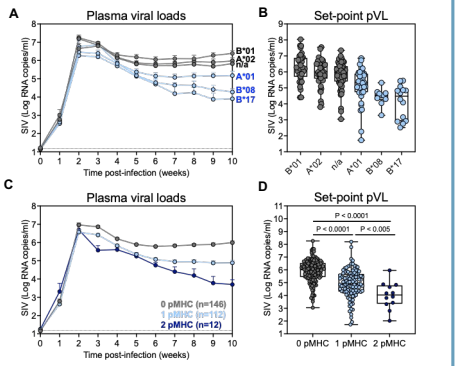


Figure 2. (A) Mean (+ SEM) SIVmac239 plasma viral loads (pVL) following infection in ART-naive RM with 0 ("n/a") or 1 common MHC-1 allele (Mamu-A*01, A*02, B*01, B*08 or B*17). (B) Set-point pVLs in ART-naive SIVmac239-infected RM with 0 or 1 common MHC-1 allele. (C) Mean (+ SEM) pVL following SIVmac239 infection in ART-naive RM with 0, 1 or 2 pMHC alleles. (D) Set-point pVL in ART-naive SIVmac239-infected RM with 0, 1 or 2 pMHC alleles.

Study Design

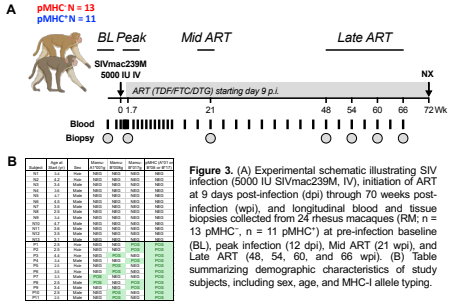


Figure 3. (A) Experimental schematic illustrating SIV infection (5000 IU SIVmac239M, IV), initiation of ART at 9 days post-infection (dpi) through 70 weeks post-infection (wpi), and longitudinal blood and tissue biopsies collected from 24 rhesus macaques (RM; n = 13 pMHC⁻, n = 11 pMHC⁺) at pre-infection baseline (BL), peak infection (12 dpi), Mid ART (21 wpi), and Late ART (48, 54, 60, and 66 wpi). (B) Table summarizing demographic characteristics of study subjects, including sex, age, and MHC-I allele typing.

pMHC⁺ RM restrict viral replication in blood more quickly than pMHC⁻ RM

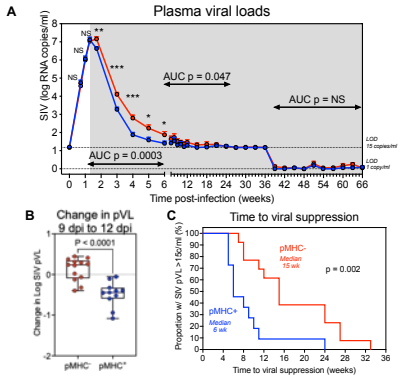


Figure 4. (A) Mean (+ SEM) SIVmac239 plasma viral loads (pVL) through 66 wpi. (B) Change in SIV pVL from 9 dpi to 12 dpi. (C) Time to viral suppression defined as first of two consecutive time points below limit of detection (LOD).

Cell-associated SIV RNA is lower in pMHC⁺ RM

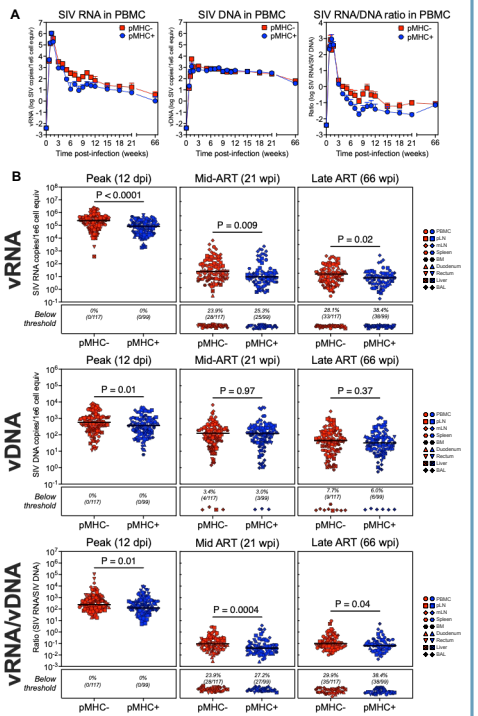


Figure 5. (A) Mean (+ SEM) of cell-associated SIV RNA (left), DNA (middle) and RNA/DNA ratio (right) in PBMCs. (B) Cell-associated SIV RNA (top), DNA (middle) and RNA/DNA ratio (bottom) at Peak (12 dpi, left), Mid-ART (21 wpi, middle) and Late ART (66 wpi, right) time points.

Protective MHC-1 allele-mediated immune responses restrict SIV replication in plasma and tissues during ART

CD8⁺ memory T cells in pMHC⁺ RM are activated early in lymph nodes

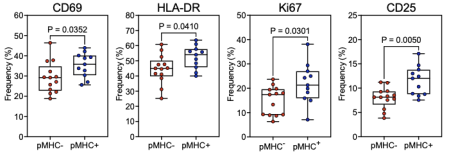


Figure 6. Comparison of CD8⁺ memory T cells expressing CD69⁺, HLA-DR⁺, Ki67+ or CD25⁺ in lymph nodes at peak (12 dpi) SIV infection.

SIV-specific CD8⁺ T cells are maintained at lower frequencies in pMHC⁺ RM

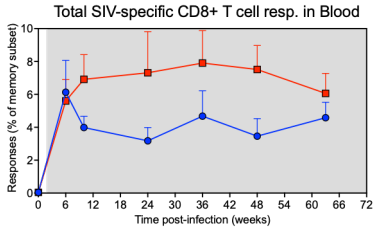


Figure 7. Comparison of total SIV-specific CD8⁺ T cell responses (mean + SEM) in BAL in pMHC⁺ vs pMHC⁻ RM. Response frequencies were measured by intracellular cytokine staining for TNFα and/or IFNγ following stimulation with pools of consecutive, overlapping 15-mer peptides spanning the SIV Gag, Nef, and Pol proteins

CD8⁺ memory T cells are less effector-differentiated in pMHC⁺ RM

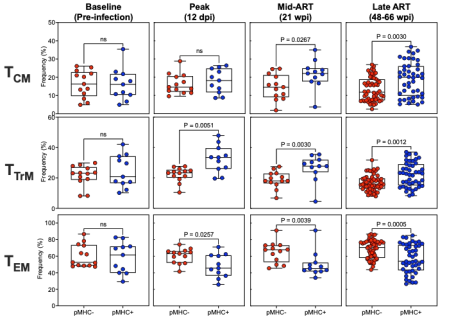


Figure 8. Comparison of the frequencies of CD8⁺ central memory (TCM), transitional memory (TTM) and effector memory (TEM) subsets in blood, as a proportion of total CD8⁺ memory T cells at Baseline, Peak (12 dpi), Mid-ART (21 wpi) and Late ART (48-66 wpi) time points.

Interferon-stimulated gene expression is elevated in pMHC⁻ RM during ART



Figure 9. Heatmap of single-cell RNA-seq analysis of interferon-stimulated gene expression in PBMCs demonstrating higher expression in pMHC⁻ RM (red) compared with pMHC⁺ RM (green) during acute infection (12 DPI), with elevated expression sustained during late ART (54–66 WPI).

pMHC impact post-ATI viral dynamics only after viral rebound peaks

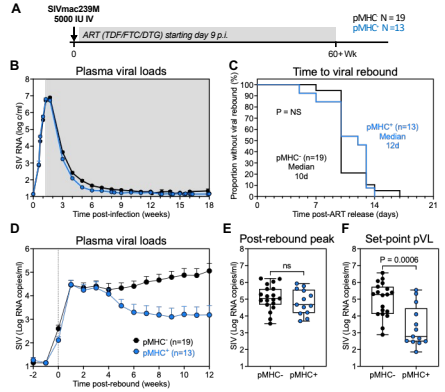


Figure 10. (A) Schematic of the experimental protocol showing SIVmac239 infection and ART initiation 9 days post-infection. (B) Mean (+ SEM) SIV plasma viral loads (pVLs). (C) Kaplan–Meier analysis of SIV rebound kinetics. (D) Mean (+ SEM) pVL profiles stratified by MHC group. (E) Post-rebound peak SIV pVLs. (F) Post-rebound set-point SIV pVLs.

Summary and conclusions

- Protective MHC-1a-restricted CD8⁺ T cell responses limited SIV replication in blood, beginning as early as 12 days post-infection
- pMHC⁺ RM had reduced SIV RNA in tissues but SIV DNA was not significantly lower
- SIV-specific CD8⁺ T cell responses in PBMC were lower frequency and less effector differentiated in pMHC⁺ RM and were associated with reduced inflammation.
- Post ATI, protective MHC-1a-restricted CD8⁺ T cell responses had no effect on time to rebound or peak viral replication, but reduced post-peak viral set points.

Plain language summary

The presence of optimized, virus-targeting CD8⁺ T cells improves suppression of the viral reservoir during ART.

Acknowledgements

This work was funded by grants from the Bill and Melinda Gates Foundation and the National Institute of Allergy and Infectious Diseases (UM1AI164560).
We would like to thank the veterinary staff at the ONPRC. SIVmac239M was provided by BK, ART was provided by Gilead Sciences. We would like to thank Ben Bimber, Maanasa Kaza and GW McElfresh for scRNAseq analysis.