Lobule zone-dependent accumulation of Vδ1 and Vδ2 γδ-T cells in livers of SIV+ infant macaques

Brooke I. Johnson[1], Kimberly A. Meyer[1], Jeremy V. Smedley[2], Donald L. Sodora[1,3], and Nina Derby[1]

Center for Global Infectious Disease Research, Seattle Children's Research Institute, Seattle, Washington, USA[1], New Iberia Research Center, University of Louisiana, Lafayette, Louisiana, USA[2], Department of Pediatrics, University of Washington, Seattle, Washington, USA[3]

Introduction

γδ-T cells are enriched in liver and barrier tissues compared to peripheral blood where they aid in the response to gut-derived antigens and maintain tissue homeostasis[1]. They are depleted from the blood during HIV[2] and SIV[3] infections, yet what happens to them and the importance of the effect are not well characterized. We hypothesized that γδ-T cells may be involved in liver dysfunction during HIV infection. As physiological metabolic functions are spatially divided in liver lobules[4], we applied a spatially resolved approach – GeoMx digital spatial profiling (DSP) – alongside standard histological and immunofluorescence microscopy techniques to characterize SIV-driven changes in transcriptional profile and cell subsets within the periportal and centrilobular functional zones of the liver lobule in infant macaques.

Methods

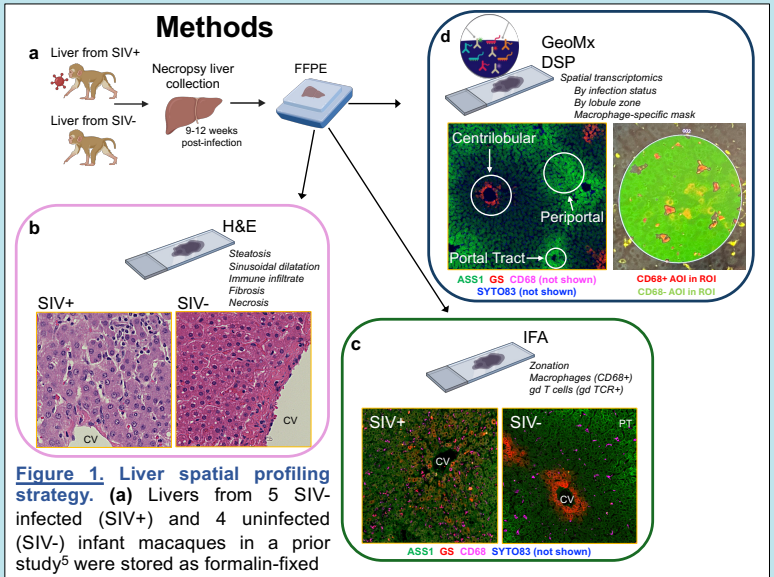


Figure 1. Liver spatial profiling strategy. **(a)** Livers from 5 SIV-infected (SIV+) and 4 uninfected (SIV-) infant macaques in a prior study[5] were stored as formalin-fixed paraffin-embedded (FFPE) blocks. Slides were prepared for **(b)** H&E staining, **(c)** immunofluorescence (IFA), and **(d)** GeoMx DSP with the human whole transcriptome atlas. ASS1=arginosuccinate synthetase 1 (expressed in periportal hepatocytes)[6], GS=glutamine synthetase (expressed in hepatocytes around central veins)[6], CD68=macrophage marker, and Syto83=DNA stain. CV=central vein. PT=portal tract. 80 regions of interest (ROIs) were placed based on ASS1/GS, representing 3 periportal, 3 centrilobular, and 2 PT ROIs per macaque.

Results

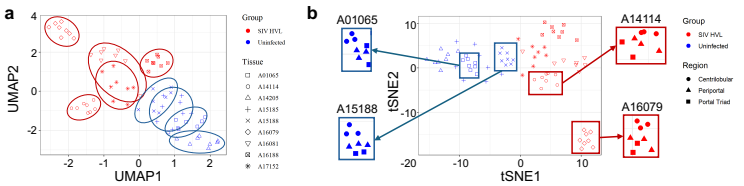


Figure 2. Global impact of SIV infection on hepatic gene expression. Transcriptional data from each ROI were analyzed using dimentionality reduction. **(a)** UMAP showing the overall transcriptional difference between SIV+ (red) and SIV- (blue) livers. **(b)** tSNE showing that transcriptional differences segregate by zone within each macaque liver.

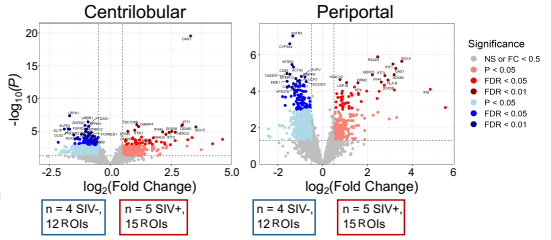


Figure 3. Transcriptional changes in centrilobular and periportal zones of SIV+ infant macaque liver. Data were analyzed in a Linear Mixed Model with Benjamini-Hochberg correction. Volcano plots indicate that SIV+ and SIV- livers possess 151 centrilobular zone differentially expressed genes (DEGs) and 139 periportal zone DEGs (based on FDR<0.05).

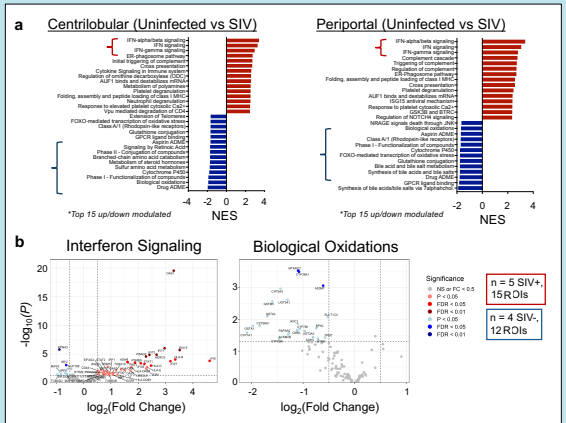


Figure 4. IFN responses and oxidative stress in the livers of SIV+ infant macaques. **(a)** Gene set enrichment analysis (GSEA) shows that IFN responses are the most upregulated and oxidative metabolism and bile the most downregulated by SIV (red vs. blue brackets). NES=normalized enrichment score. **(b)** Volcano plots identify affected genes in the IFN signaling and Biological Oxidations pathways in centrilobular zone ROIs.

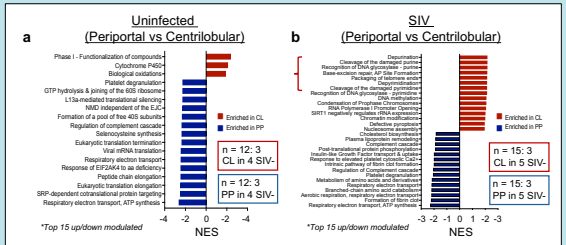


Figure 5. SIV-driven centrilobular DNA damage response. **(a)** GSEA indicates zonation of hepatic function in uninfected infant macaque liver in alignment with the literature[4]. **(b)** DNA damage response is enriched in the centrilobular zone of SIV+ infant macaque liver (red bracket).

ID	Sex	Age SIV+	Viral Load (copy/ml)	Kg	Steatosis	Inflammation	Fibrosis, Necrosis, Congesion
A15188	M	na	na	1.8	na	Patchy mild portal, lobular	na
A15065	M	na	na	2.2	60-70%	Patchy mild portal, CV	na
A14205	M	na	na	1.9	10%	na	na
A15185	M	na	na	2.1	na	Mild portal, lobular	na
A17152	F	13w	4.04×10^8	1.3	na	Severe lymphocytic & neutrophilic, CV	na
A14114	M	18w	2.15×10^8	1.9	na	Severe portal, focal lobular and CV	CV congestion
A16081	F	15w	3.91×10^8	1.3	na	Patchy portal, moderate CV	CV fibrosis & necrosis
A16079	F	12w	2.43×10^8	1.2	na	Moderate portal & lobular lymphocytic	CV necrosis
A16188	M	15w	2.55×10^8	1.2	30%	Patchy portal & lobular	CV necrosis

Figure 6. Histological evidence of central vein DNA damage. H&E-stained slides were scored blind by a pathologist at Seattle Children's Hospital, revealing SIV-associated inflammation, and centrilobular fibrosis and necrosis.

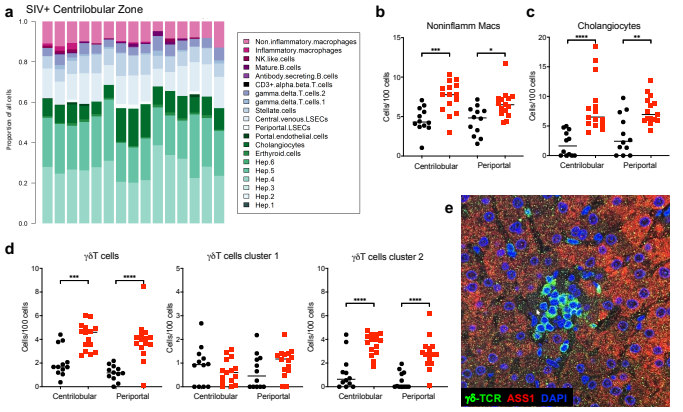
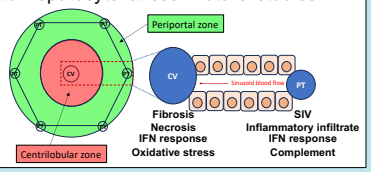


Figure 7. Cluster 2 γδ-T cell accumulation in SIV+ infant macaque livers. **(a)** SpatialDecon algorithm[7] for mixed cell deconvolution using published adult human liver scRNAseq[8] as a reference was applied to identify cell subsets affected by SIV infection across lobule zones. Subsets in centrilobular zone ROIs are shown. Non-inflammatory macrophages **(b)**, cholangiocytes **(c)**, and γδ-T cells **(d)** were enriched in centrilobular and periportal zones of SIV+ macaques. γδ-T cell enrichment was driven by cluster 2 γδ-T cells, which transcriptionally resemble phosphoantigen-reactive Vδ2-γδ-T cells[8]. **(e)** Cells expressing γδ-TCR were also identified in SIV+ infant macaque liver by IFA.

Conclusions and Future Directions

Spatially resolved techniques reveal that SIV infection alters physiological hepatocyte function as well as immunity in the infant liver. Oxidative stress, IFN signaling, hepatocyte damage, and accumulation of macrophages and γδ-T cells are hallmarks of the infection. We hypothesize that Vδ2-γδ-T cells may accumulate in the liver in response to hepatocyte stress. Future studies will aim to understand the impact of HIV/SIV on hepatic γδ-T cell function and of altered γδ-T cell function on liver physiology. Our long-term goal is to identify strategies to improve liver function in infants and adults living with HIV.



Acknowledgements and References

This study was supported by an SCRI Spatial CoLab Pilot Grant (ND, DLS) and R01 AI34830 (DLS). The authors acknowledge Dr. Gail Deutsch, MD for help with liver pathology.

1. Ibadapo-Obe, 2023, JHEP Reports. 2. Plhl, 2024, J Infect Dis. 3. Walker, 2021, Frontiers Immunol. 4. Ben-Moshe, 2019, Nat Rev Gastroenterol Hepatol. 5. Wood, 2021, Plos Pathogens. 6. Derby, 2024, Viruses. 7. Danaher, 2022, Nat Commun. 8. MacParland, 2018, Nat. Commun.