Characterizing SIV-infected plasmacytoid dendritic cell aggregates in rhesus macaque lymph nodes

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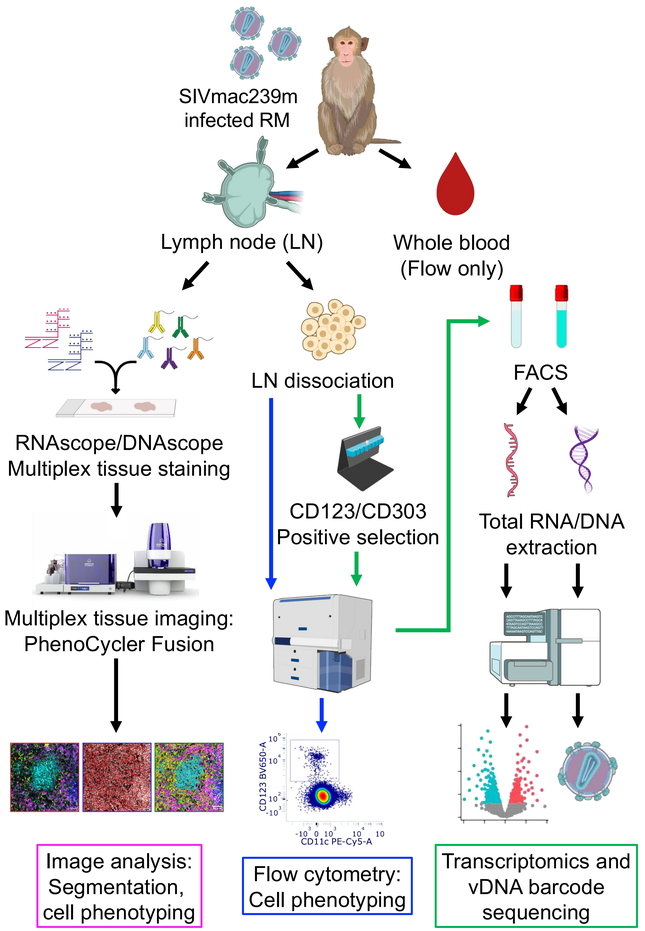
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BACKGROUND

Lymphoid tissues are major sites of HIV/SIV viral replication and reservoir persistence. The full spectrum of cells that harbor persistent virus and can contribute to viral rebound is not well understood, which hinders therapeutic targeting of these cell populations. We used multiplex fluorescent imaging to extensively characterize infected cells in lymphoid tissues of rhesus macaques (RM). We identified a pDC-like population of cells (cl-pDCs) harboring SIV genomes that formed unique structures in lymph nodes. We hypothesize these pDC-like cells may constitute an important subset of the persistent viral reservoir and respond to SIV infection in lymphoid tissues.

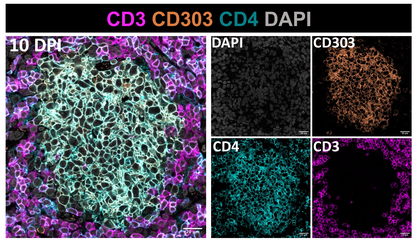
METHODS

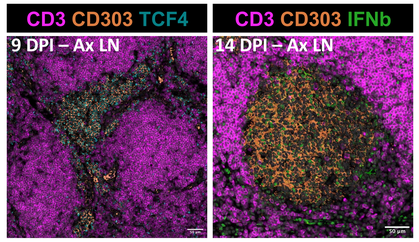


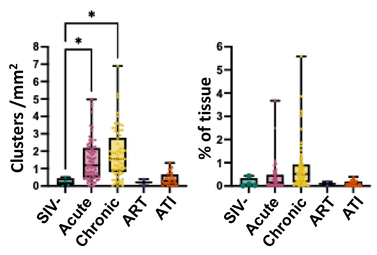
We identified a pDC-like population of cells, coined cl-pDCs, that harbor intact SIV viral genomes and form dense cellular aggregates and unique microenvironments in lymphoid tissues.

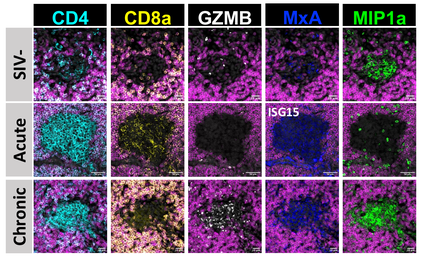
RESULTS

Identification and characterization of pDC-like cell clusters in RM LNs





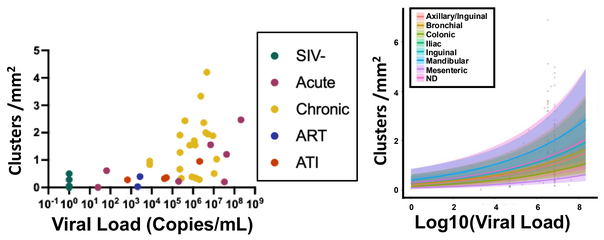




+	+/-		-						
CD303	SIV vRNA	IFNb	AIRE	CD2	CD45 pan	CTLA4	IBA1	Vimentin	Tbet
CD4	SIV vDNA	ISG15	BCL6	CD20	CD45RA	CXCL12	ICOS	MPO	
Neuropilin1	Apoptosis	Ki67	Caveolin	CD206	CD5	Cytokeratin	IFNg	PD1	
TCF4	CD107a	CD107a	CCL21	CD207	CD56	DC-SIGN	IgM	Podoplanin	
CD38	CD8a	NaK ATPase	***CD123***	CD21	CD68	Fibroblast	IL10	RUNX3	
MxA	GZMB	MIP1a	CD163	CD27	CD7	GATA3	IL18	T-bet	
SAMHD1	HLA-DR	PCNA	CD169	CD31	CD8b	Granulysin	IL2	TCRd	
	IDO		CD1c	CD3e	Collagen IV	GZMK	Tryp/Chym	TCF1	

- Identified a pDC-like CD3-CD4+CD303+ cell forming unique clusters in LNs
- Clustered pDCs (cl-pDCs) lack expression of CD123 → not a classic pDC
- cl-pDCs can be found during all stages of SIV infection and express inflammatory and cytotoxic proteins during infection

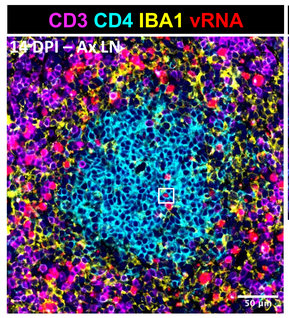
Abundance of cl-pDCs correlates with systemic infection

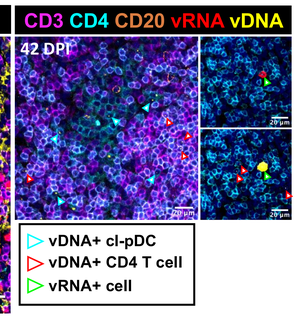


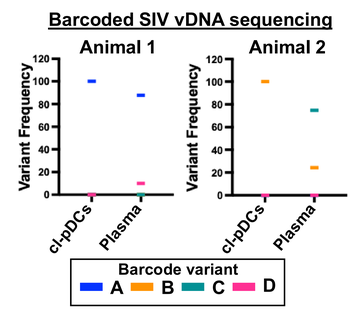
- More cl-pDCs in peripheral draining LNs (mandibular, inguinal, axillary)
- The number of clusters increases with systemic infection and inflammation

This project has been funded in whole or in part with federal funds from the National Cancer Institute, National Institutes of Health, under Contract No. 75N91019D00024. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

cl-pDCs can produce viral RNA and harbor intact viral DNA genomes

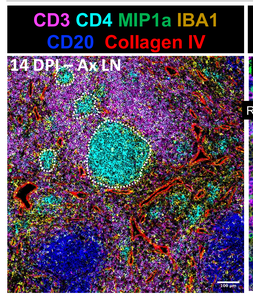


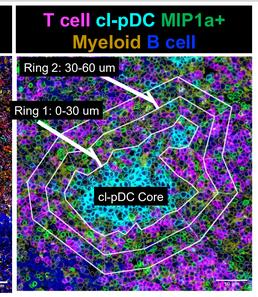


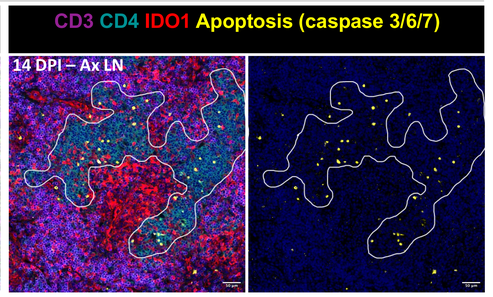


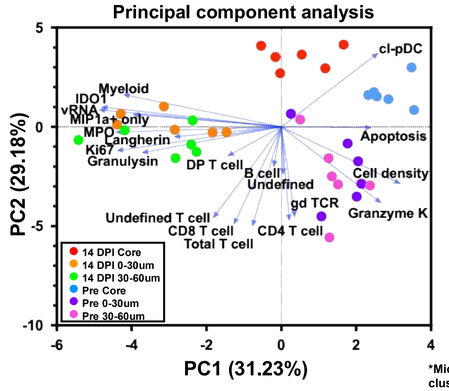
- vRNA and vDNA were detected in cl-pDCs by RNAscope and DNAscope
- Intact, full-length vDNA sequences were detected in sorted cl-pDCs using barcoded SIVmac239m
- The detected viral variant in cl-pDCs matched variants found in blood

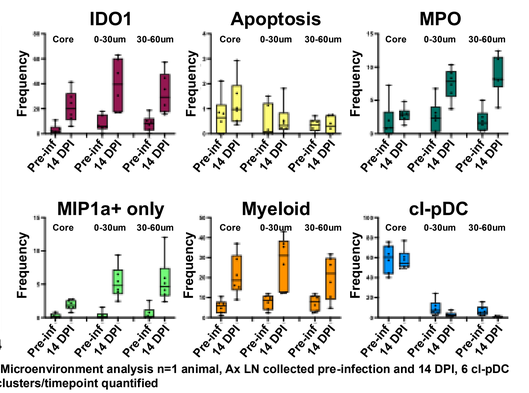
Ongoing work: Understand cl-pDC function and tissue microenvironments











*Microenvironment analysis n=1 animal, Ax LN collected pre-infection and 14 DPI, 6 cl-pDC clusters/timepoint quantified

- The frequency of IDO1-producing cells, myeloid cells, and neutrophils surrounding cl-pDC clusters increases in acute infection
- High levels of apoptosis are localized to cells found within cl-pDC cores

Plain language summary

While CD4+ T cells are the are the major cell type infected with SIV/HIV, here, we identified a previously undescribed cell that can be infected with virus and forms unique structures in the lymph node.