Development of Viral Biomarkers in SHIV Infected Non-Human Primates

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We detected SIV-RNA signal by ISH in LNs and GALT of animals with low to undetectable plasma viremia above the background of tissue from uninfected individuals. SIV-p27 IHC signal was detected in LNs of some animals with high viremia, but less efficiently in other tissue compartments and aviremic animals. LNs from animals with plasma viremia >10^3 c/ml presented co-compartmentalization of SIV-RNA and-p27 with FDCs. SIV-p27 measured by Simoa was not readily detected in blood cells but could be detected in rectal and jejunal biopsies of animals with higher plasma viral loads.

BACKGROUND

As HIV-1 infection has transformed from a fatal crisis into a chronic, life-long disease managed by anti-retroviral therapy (ART), attention has shifted towards curative therapies. A regimen which may allow for viral control off ART will likely require a combination of agents to address HIV reservoirs and induce immune control. These complex interactions are difficult to study in vitro and thus require access to in vivo models. Infection of Rhesus macaques with SIV or chimeric HIV allows study of viral pathogenesis, biomarkers, and emerging cure interventions. Here we describe efforts to apply in situ hybridization (ISH) and immunohistochemistry (IHC) imaging techniques, and ultrasensitive immunoassays (Simoa) to detect host and viral proteins and RNA in samples derived from infected non-human primates (NHPs).

METHODS

We collected biological samples from lymph nodes (LNs), gut associated lymphoid tissue (GALT), and blood from a cohort of NHPs including uninfected and SHIV162p3-infected viremic and aviremic animals. We measured plasma viral loads by quantitative PCR (assay limit of detection, LOD<10^2 copies/ml) and used Simoa ultra-sensitive immunoassay technology to detect SIV-p27 in blood and tissues. Other tissue specimens were fixed, paraffin embedded, and subjected to microtomy for the detection of SIV-p27, viral RNA and follicular dendritic cells (FDCs) by ISH or IHC with commercially available reagents followed by computational image analysis.

RESULTS

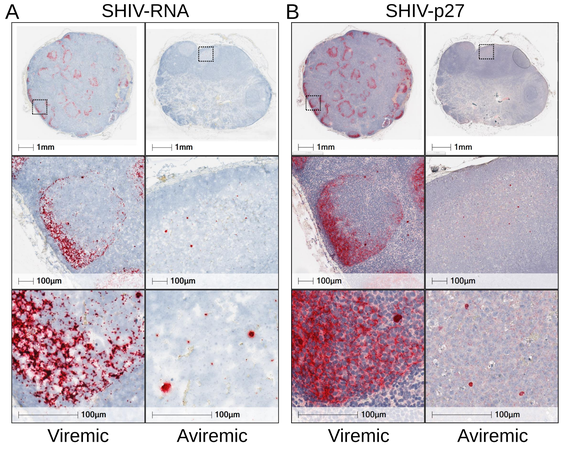


Fig. 1 Detection of SHIV-RNA and -p27 markers in SHIV162P3 infected rhesus macaques. Axillary lymph nodes were surgically removed, fixed in 4% paraformaldehyde for 48 hours, and paraffin embedded followed by microtomy. Representative magnification images of 5 µm thick sections stained for A) SHIV-RNA (RNAScope® 2.5 LS Probe- SIVmac239, 312818, ACDBio) and B) SHIV-p27 protein (Anti-SIV p27 Gag, 4324, ABL Inc.). Regions of interest are indicated by dash-lined boxes. Rows from top to bottom show low, 10x, 40x magnifications.

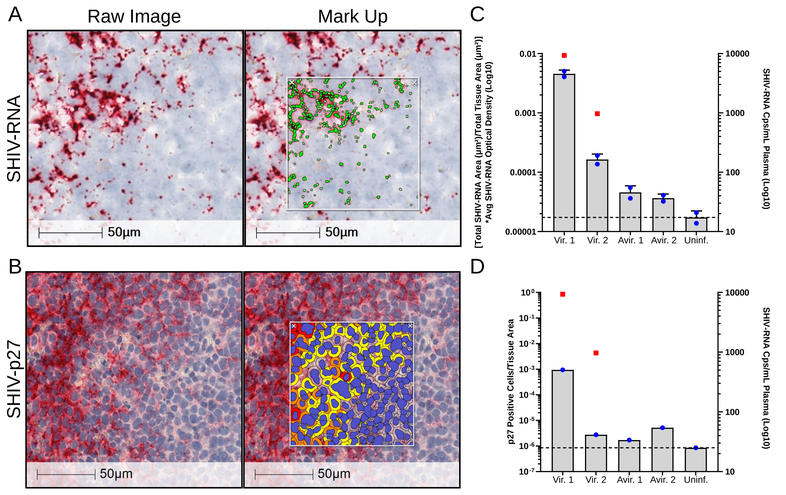


Fig. 2 Digital quantification of SHIV-RNA and -p27 signals in histological staining. Chromogenic signal captured in the images shown in Figure 1 computationally analyzed with HALO (Indica Labs). A) Representative regions of interest stained for SHIV-RNA and B) -p27 (left panels), and relative computational detection (mark up, right panels). C) SHIV-RNA area and D) SHIV-p27 positive cells detected in adjacent sections (blue dots), and relative average (gray bars). Red squares indicate animals' plasma viral loads (LOD<10^2 copies/ml). Sections of an inguinal lymph node surgically obtained from an uninfected (uninf.) animal were used to set background (dashed lines). Vir., viremic; Avir., aviremic.

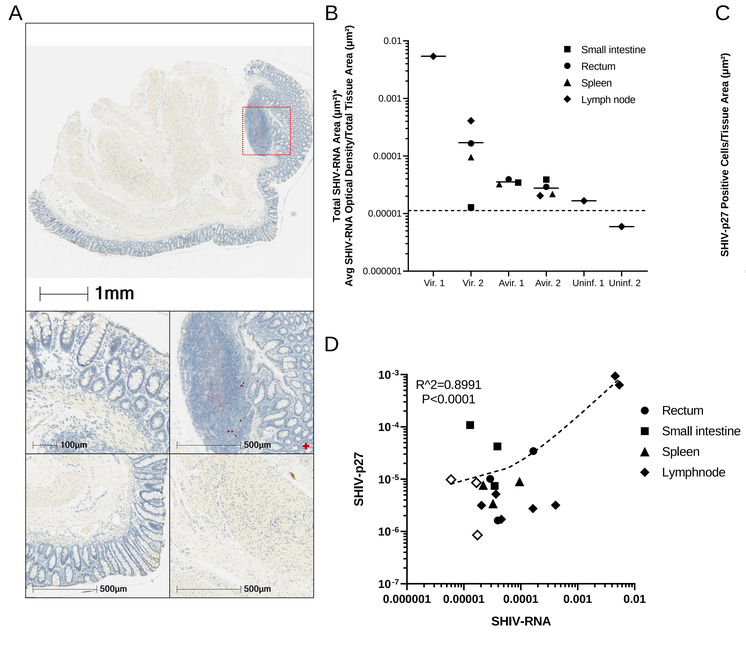


Fig. 3 Correlation of SHIV-RNA and –p27 markers in lymphoid tissue. A) Representative section of a rectal biopsy from a viremic animal (top panel) and regions of interest (bottom panels) showing intestinal glands, lamina propria, sub mucosal tissue, and lymphoid aggregates where SHIV-RNA was detected (red dash-lined box; red plus symbol). Quantification of B) viral RNA, C) p27 markers and D) relative correlation in lymphoid compartments from all animals. Horizontal lines, mean value; dashed lines, average background stain set with uninfected tissue (uninf.); Vir., viremic; Avir., aviremic.

	SHIV-RNA	SHIV-p27 gut	
Animal ID	**Plasma (c/ml)**	**Colorectal (pg/ml)**	**Jejunal (pg/ml)**
Viremic 1	6.11E+03	11.90 (6.13; 17.67)	0.55 (0.54; 0.56)
Viremic 2	2.14E+03	0.15 (0.00; 0.29)	0.14 (0.24; 0.04)
Aviremic 1	<1.00E+02	0.01 (0.00; 0.02)	0.02 (0.00; 0.04)
Aviremic 2	<1.00E+02	0.02 (0.00; 0.04)	0.03 (0.00; 0.06)
Uninfected	N/A	0.01 (0.00; 0.02)	0.02 (0.00; 0.04)

Table 1. Biochemical quantification of SHIV-p27 in gut biopsies. Rectal and jejunal specimens were lysed, and immunoprecipitated p27 quantified from eluates by digital enzyme-linked immunosorbent assay with single molecule array detection (Simoa, Quanterix HD-X). Animals' plasma viral loads were measured at tissue collection (LOD<10^2 c/ml).

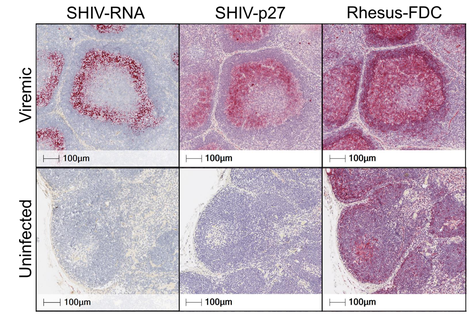


Fig. 4 Detection of follicular dendritic cells marker in lymph nodes. Adjacent sections of inguinal lymph nodes collected from a viremic (top panels) and an uninfected (bottom panels) individuals stained for SHIV-RNA, -p27 and rhesus FDC (F3803, Sigma).

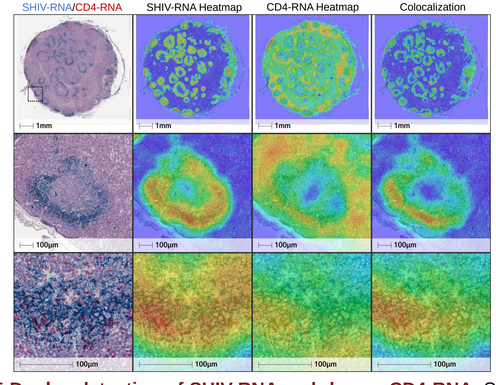


Fig. 5 Duplex detection of SHIV-RNA and rhesus CD4-RNA. SHIV-RNA and *M. mulatta* CD4-RNA (RNAScope 2.5 LS Probe-Mmu-CD4-C2, 481878-C2) stained with green and red chromogenic probes respectively (left panels). Individual RNA markers density heatmaps and relative colocalization (right panels).

CONCLUSIONS

Tissue SIV-RNA and -p27 ISH/IHC markers quantification correlated in samples across the compartments tested. Using ISH/IHC, Simoa, and qPCR assays, we are assembling a set of biomarkers to investigate viral RNA and protein in NHPs with variable levels of viremia. These assays can be multiplexed with host biomarkers to further characterize the microenvironments that sustain virus persistence and provide insights into the effectiveness of clearance of infected cells from tissues.