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CHARACTERIZATION OF FDC-BOUND VIRAL PRODUCTS DURING HIV/SIV INFECTION

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BACKGROUND

During chronic HIV/SIV infection, the B-cell follicle is a major site of virus replication, and large numbers of infectious viral particles accumulate on follicular dendritic cells (FDCs) in the form of immune complexes (ICs). Despite the importance of the B-cell follicle in HIV/SIV persistence, the establishment and composition of FDC-bound HIV/SIV ICs remains poorly characterized. In this study we analyzed FDC-bound viral products during HIV/SIV infection.

METHODS

Formalin fixed paraffin embedded (FFPE) lymph nodes (LNs) from acutely infected (7 dpi) and chronically infected (Non SAIDS, 69-118dpi) rhesus macaques were evaluated. Rhesus macaque tissue sections were stained with antibodies to CD20 (B-cells) and smooth muscle myosin heavy chain (FDC networks) and combined with in situ hybridization for SIV RNA (RNAScope ACD). Imagescope (Leica) was used for quantification. FDC-bound SIV RNA was quantified by the area of positive pixel on FDC networks with RNA+ cells excluded compared to the total area of the tissue. Data were analyzed using Mann-Whitney tests and Spearmen coefficients (r). Frozen human LNs and tonsils from chronically infected untreated and seronegative controls were also evaluated. Human tissues were stained with antibodies to IgD (rings FDCs) and Nef and analyzed qualitatively with Imagescope (Leica). All research involving human subjects conformed to the principles set forth in the Declaration of Helsinki and was approved by the University of Arizona Institutional Review Board. Animals were housed and cared for in accordance with AAALAC standards, and all animal procedures were performed according to protocols approved by the Institutional Animal Care and Use Committees of the Wisconsin National Primate Research Center and the University of Arizona..

RESULTS

Table 1: Clinical Characteristics of Rhesus Macaques

Animal	Viral Load at Necropsy	Days Post Infection	Axillary LN	Inguinal LN
R02002	3700000	7	x	
R04032	1300000	7	x	
R08002	493000	7	x	
R08008	2090000	7	x	
rh1998	1730000	7	x	
rh2279	3470000	7		x
rh2521	972	95	x	
rh2525	5630000	88	x	
rh2578	66800	69		x
rh2579	5380000	69	x	
rh2583	1180000	69		x
rh2584	109000	111	x	
Rh2284	2450000	84	x	
Rh2306	1380000	84	x	

Figure 1: Representative images demonstrating that SIV RNA (red) is detectable on the FDC network (green) of chronically infected animals (A), but not acutely infected animals (B).

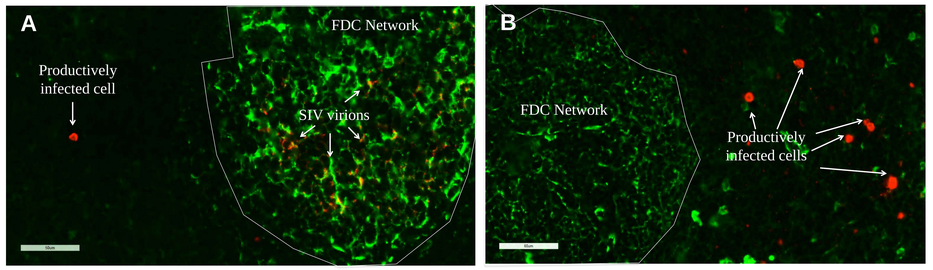


Figure 2: Representative image demonstrating how B cell follicles, germinal centers, and virus of FDCs were masked to allow for quantification. (A) FDC network (White) and SIV RNA (Gold) are shown and masked with a purple line. FDC bound SIV RNA was quantified within the mask, with productively infected cells excluded from the mask. (B) CD20 (Green) is shown with a yellow line masking the B cell follicle. (C) DAPI (Blue) is shown with a green line masking the outline of the tissue.

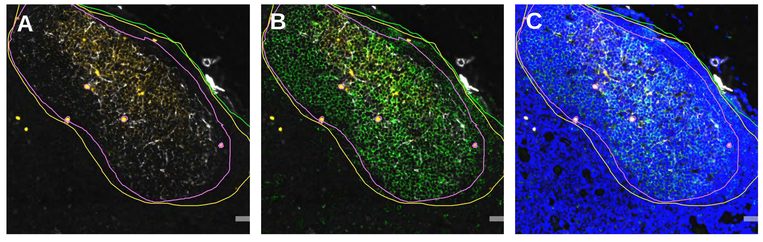


Figure 3: There is no significant difference between acutely and chronically infected animals in terms of relative size of B cell follicles (A) or FDC networks (B) although the latter tended to be larger in chronically infected animals. Bars indicate median values.

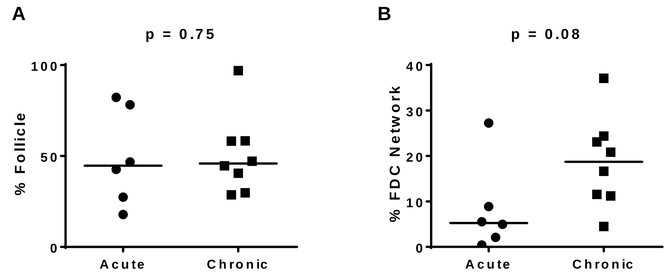


Figure 4: In chronic SIV infection, plasma viral load is not significantly correlated with the size of the FDC networks (A), but is strongly correlated with the size of the FDC-bound SIV reservoir (B).

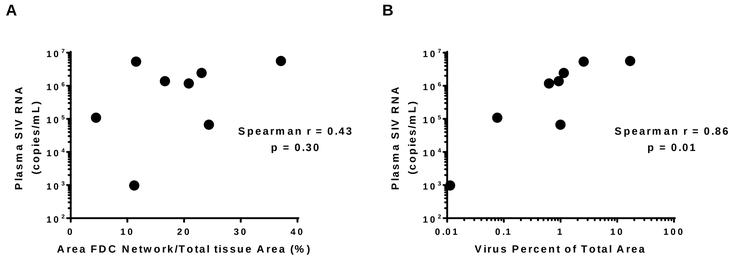
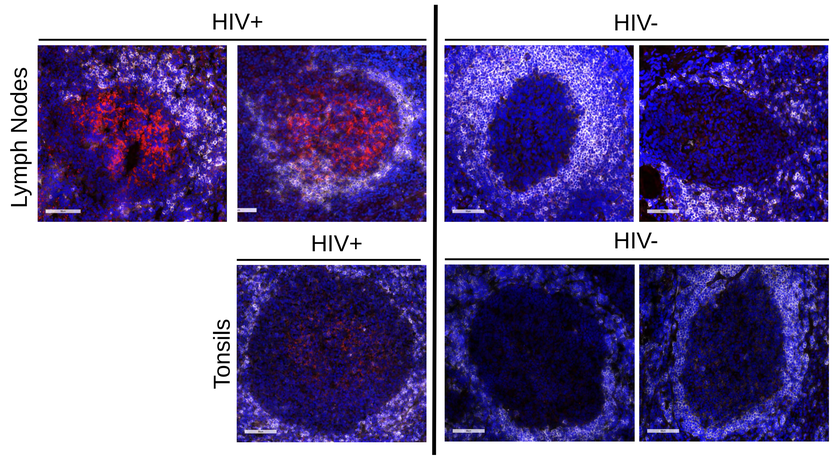


Figure 5: Robust Nef staining is visible on the FDC networks in tissues from HIV infected participants, but is absent in uninfected individuals. Red=Nef, FDC Networks/germinal centers= IgD (White) negative areas surrounded by IgD positive areas.



SUMMARY

- No FDC bound SIV reservoir is detectable at day 7 during acute infection, but it is readily detectable in chronically infected animals
- The relative size of B cells follicles does not change between acute and chronic infection, although FDC networks tend to be larger in chronic disease.
- In chronic SIV infection, plasma viral load strongly correlates with the size of the FDC-bound SIV reservoir.
- Nef accumulates on FDC networks during chronic HIV infection.

CONCLUSIONS

- No FDC-bound SIV RNA is established during the first 7 days of acute SIV infection despite high viral loads, consistent with the notion that antibodies are necessary for the establishment of this reservoir.
- Lack of correlation between the size of the FDC network and plasma viral load suggests that the FDC network itself is not a determinant of viral load.
- The strong correlation between FDC bound SIV reservoir and plasma viral loads suggests that they are in equilibrium with each other.
- Because Nef is a known immune-modulator, the accumulation of Nef on FDC networks may contribute to dysfunction of Tfh and other follicular cell subsets.

Acknowledgments

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