SPECT/CT IMAGING OF CD4-POOL SUGGESTS A NOVEL CORRELATE OF BENIGN DISEASE PROGRESSION

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Background: Global changes in the amount of lymphoid tissue have not been studied in the settings of acute, chronic progressive (CP) or long-term non-progressive (LTNP) SIV infection.

A better understanding of changes in the total body immune system, especially in those organs capable of expanding or contracting in size, e.g. lymph nodes and spleen, may advance our knowledge of HIV/SIV pathogenesis.

We have previously shown the feasibility of *in vivo* CD4-pool imaging in rhesus macaques infected with SIV/SHIV, or following total body irradiation and hematopoietic stem cell transplantation[1,2].

Methods: Healthy uninfected (n=10) or SIV (mac251 or mac239)-infected (n=10) rhesus macaques were studied with *in vivo* CD4 pool SPECT imaging using ^{99m}Tc- F(ab´)$_2$-CD4R1, and volumetric imaging with Computed-Tomography (CT).

Only animals with no evidence of immunogenicity to the radiotracer were included in both cross-sectional and longitudinal analysis.

In the cross-sectional analyses, the first scan after 5 months of untreated infection was used. In the longitudinal analyses, 3 animals were imaged longitudinally at baseline, acute, and chronic phase.

CD4 pool in the clusters of axillary lymph-nodes (AxLns) and spleen was quantified as maximum Standardized-Uptake-Value (SUVmax) of the anti-CD4 probe.

The volume of AxLn (LN-size-Max, and LN-size-total) and spleen were determined from the co-registered CT images. LN-size-Max was estimated by manually placing a Volume-of-Interest (VOI) on the single lymph node (LN) with maximum uptake of the anti-CD4 probe. LN-size-total was estimated by manually placing a VOI on all the AxLn's on both right and left side combined.

Five of the 10 SIV-infected animals showed features of long-term non-progressors with CD4 T-cell count >600 cells/µl during the entire period of untreated infection (median nadir:743 cells/µl; median SIV-RNA set-point:3,760 copies/ml. 4/5 had a protective haplotype (Mamu A*01, B*08 or B*17)).

The remaining five animals showed features of chronic progressors with CD4 T counts <500 cells/µl during the entire period of untreated infection (median nadir:177 cells/µl; median SIV-RNA of 140,000 copies/ml, 1/5 had a protective haplotype).

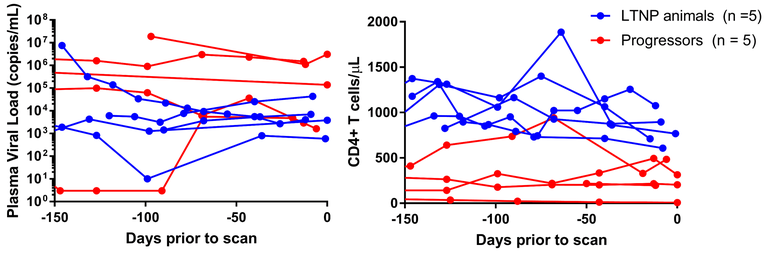


Figure 1. Plasma viral load (SIV-RNA copies/mL) and CD4+ T cell counts (cells/µl) in 10 SIV-infected animals (5 long-term non-progressors and 5 chronic progressors) up to 5 months prior to scan date.

Results: The SUVmax of the AxLns and the spleen was positively correlated with the peripheral blood CD4 T-cell count (r, AxLn = 0.51 (P=0.01); Spleen = 0.74 (P<0.001)). In addition, a positive correlation was observed between the SUVmax and the LN-size-Max (r=0.42, P=0.03).

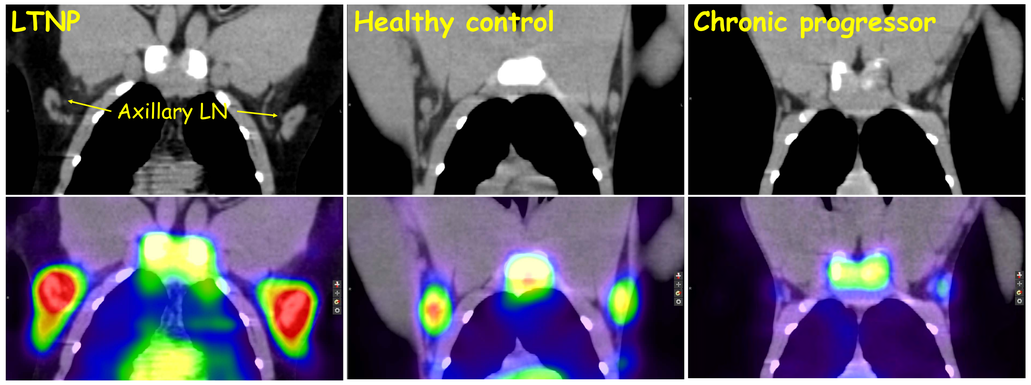


Figure 2. Top panels show Computed Tomography (CT) image in coronal view and the bottom panels show Nuclear Medicine anti-CD4 SPECT image superimposed with CT image in coronal view.

- AxLns were larger in the LTNP group compared to the healthy controls (P<0.01) or chronic progressive groups (P<0.05).
- Spleen volume (normalized on body weight, mL/kg) in the LTNP group was higher than the healthy controls (P=0.066) but not statistically significantly different from the chronic progressors (P=0.75).

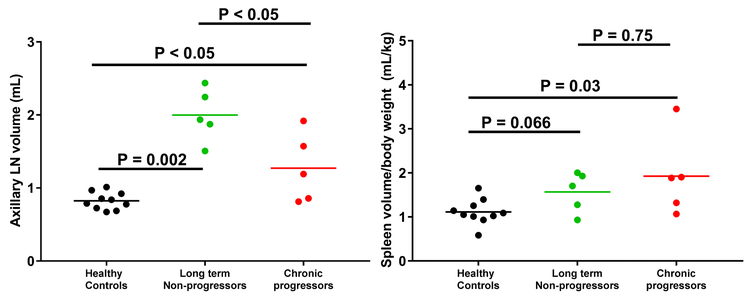


Figure 3. Panels show normalized splenic volume (mL/kg) and Axillary LN volume (mL) in 10 healthy controls, 5 long-term non-progressors, and 5 chronic progressors.

Within the 10 SIV-infected (and untreated) animals, we observed:

- a **negative** association between Axillary LN-size-total and plasma viral load (r = -0.56, P<0.05).
- a **positive** association between normalized splenic volume and plasma viral load (r = 0.54, P=0.05).

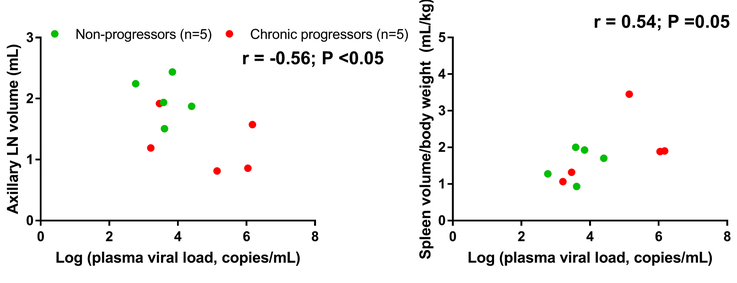


Figure 4. Panels show normalized splenic volume and Axillary LN volume vs. plasma viral load (copies/mL).

In the longitudinal study, splenic CD4-pools decreased in all 3 animals in acute phase, concomitant with paradoxical increases in the AxLn CD4 pool as well as increases in the volume of AxLns. In the chronic phase of infection, the size of AxLns was reduced in 2 animals (with chronic progressive features) but not in the third animal (with LTNP features, B017+).

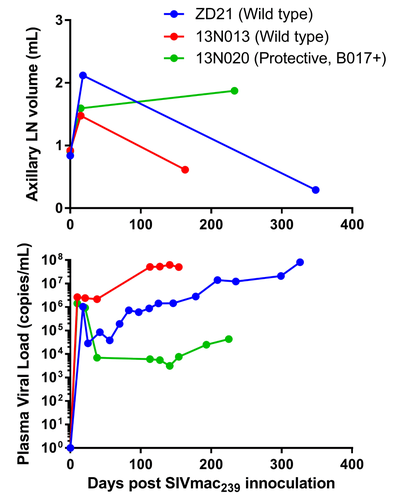


Figure 5. Changes in Axillary LN volume (estimated from CT image) during acute (~2 weeks post-inoculation) and chronic (>5 months post-inoculation) infection in three animals imaged longitudinally.

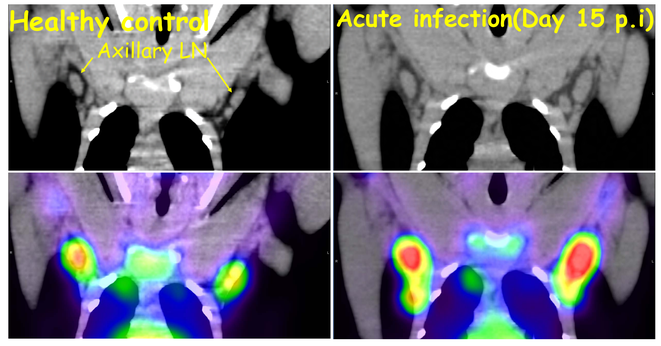


Figure 6. Top panels show Computed Tomography (CT) image in coronal view and the bottom panels show Nuclear Medicine anti-CD4 SPECT image superimposed with CT image in coronal view. The volume of the lymph nodes and the CD4 pool of the lymph nodes increased in Acute infection (~2 weeks post-inoculation)

Conclusions: SPECT/CT imaging during acute and chronic SIV infection highlighted an unexpected association between the size of lymph nodes and the CD4 pool, that may suggest a novel correlate of non-progressive SIV infection.

The molecular mechanisms governing the stromal fibroblastic reticular cells (FRC) network stretching, to allow lymph node expansion\contraction, are being revealed recently (*Acton et al. Nature 2014, and Astarita et al. Nature Immunology 2014*).

Whether irreversible or partially reversible lymph node elasticity induced by SIV replication in tissues is in part responsible for the seemingly stochastic lymph nodes CD4 pool reconstitution observed in previous studies[3,4,5] or differences in disease progression, deserves further investigation[6].

References:

1. Di Mascio et al, Blood 2009
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4. Di Mascio et al, 2013, International AIDS society, Kaula Lampur, Malaysia
5. Di Mascio et al, 2012, International AIDS conference, Washington DC, United States
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