Memory CD4 T Cells from The Liver Are Infected During SIV Infection in Rhesus Macaques

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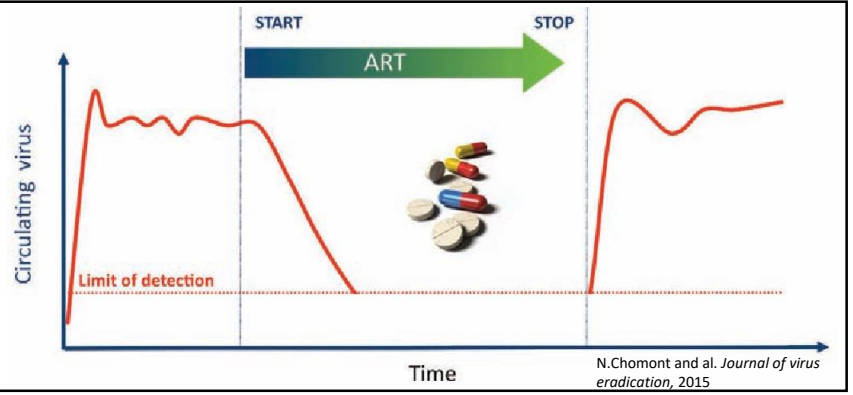
No conflicts of interest to declare

Background :

Despite the introduction of highly active antiretroviral therapy, HIV continues to be a major global public health issue as a chronic disease. The liver has been shown to be an HIV-infected organ causing liver disease and co-morbidity in people living with HIV. We have established a model of Rhesus Macaque (RM) infected with SIV, taking the opportunity to further analyze the nature of infected cells in the liver. Herein, we specifically assessed the role of CD4+ T cells.



N.Chomont and al. *Journal of virus eradication*, 2015

Methodology :


SIVmac251


Rhesus Macaque

SIV
(20AID50)

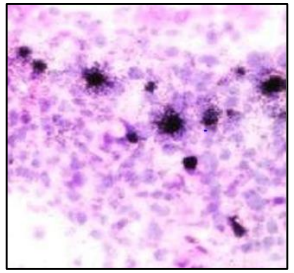
Day of euthanasia — 90 — 120 — 320

12 monkeys chronically infected

Rhesus Macaques were infected with the SIVmac251 (20 AID50) and sacrificed at different time points post-infection.


Liver


Blood


In situ hybridization

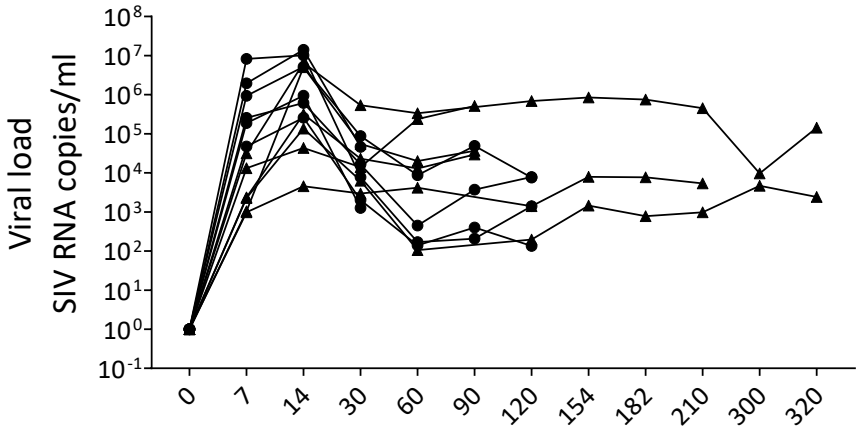

qPCR

Flow cytometry


Cell sorting

Cells from the liver were mechanically recovered without proteases to maintain the integrity of surface molecule expression. We also recovered peripheral blood in comparison. *In situ* hybridization was used to detect vRNA in hepatic tissue. Flow cytometry was assessed to analyze cell phenotypes by using specific antibodies. CD4 T cells were sorted by flow cytometry. qPCR was performed for viral load quantification and cell-associated viral DNA quantification.

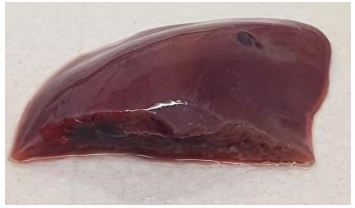
Figure 1. Plasma viral load (VL) of SIV-infected RMs



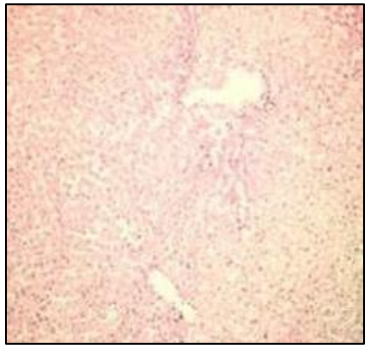
RMs developed VLs reaching 10^3 to 10^6 copies/ml.

Figure 2. *In situ* hybridization of hepatic tissue

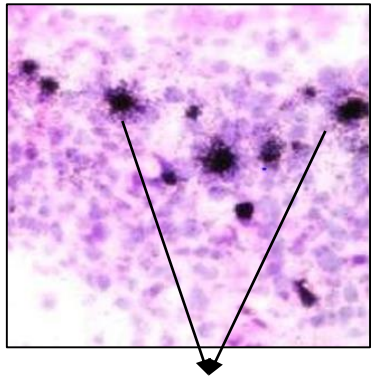
Liver section



Naive



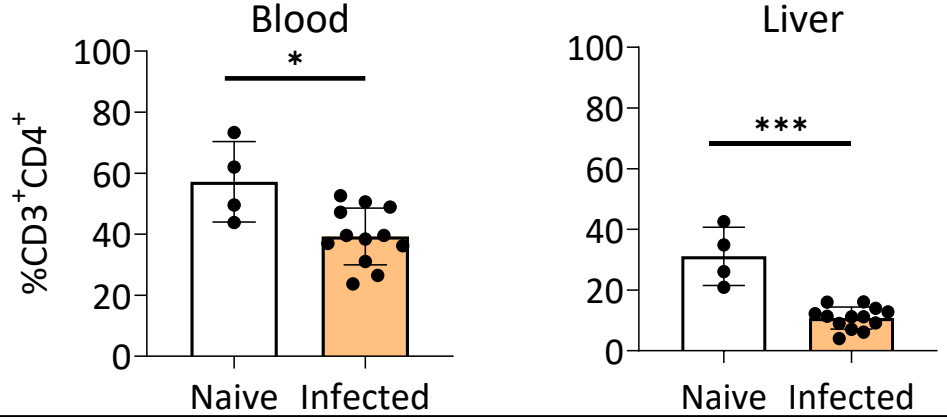
SIV



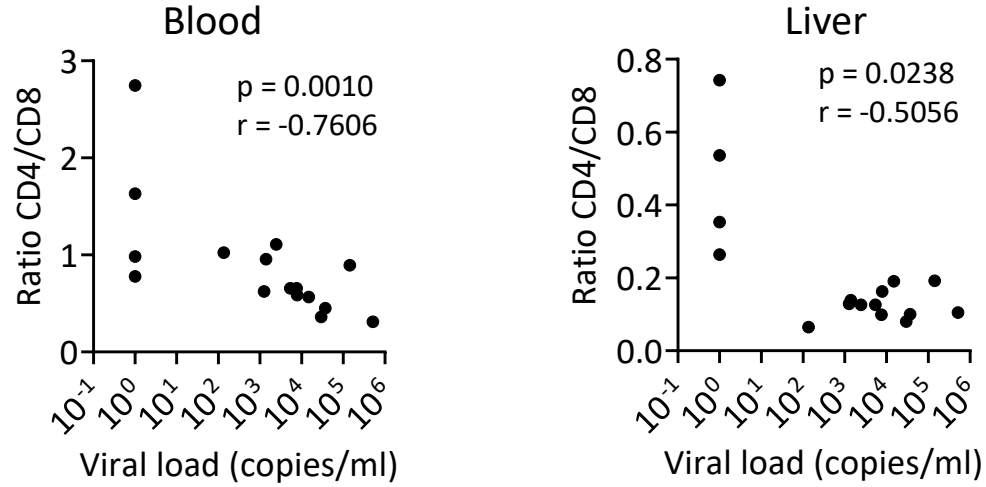
Spots of replicating virus

To validate our model, we assessed in hepatic tissue viral replication by in situ hybridization. Thus, in infected individuals, spots of viral replication (in black) demonstrate the presence of productive SIV-infected cells in the liver of SIV-infected RMs.

Figure 3. Liver infection is associated with CD4 T cell depletion

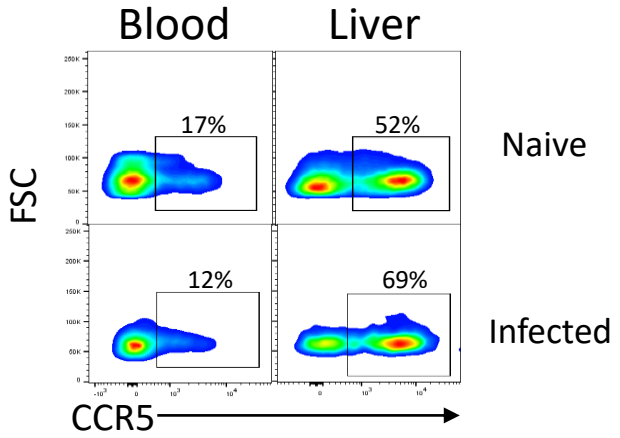


Given that the liver is infected during the course of SIV infection, we assessed the level of CD4 T cells in hepatic tissue in comparison to the blood. As expected, the frequency of CD4 T cells from the liver was lower in SIV infected RMs (12%) in comparison to healthy RMs. Statistical analyses were performed using Mann Whitney test. *, $p<0.05$; ***, $p<0.001$.

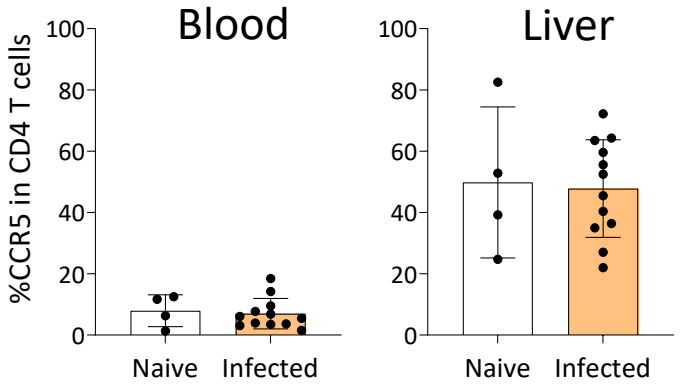


We then assessed the CD4/CD8 ratio to evaluate the impact of SIV infection. Thus, we observed a positive correlation between viral load and CD4/CD8 ratio both in the blood and liver. Spearman analyses was used for correlations.

Figure 4. CCR5 expression in CD4 T cells

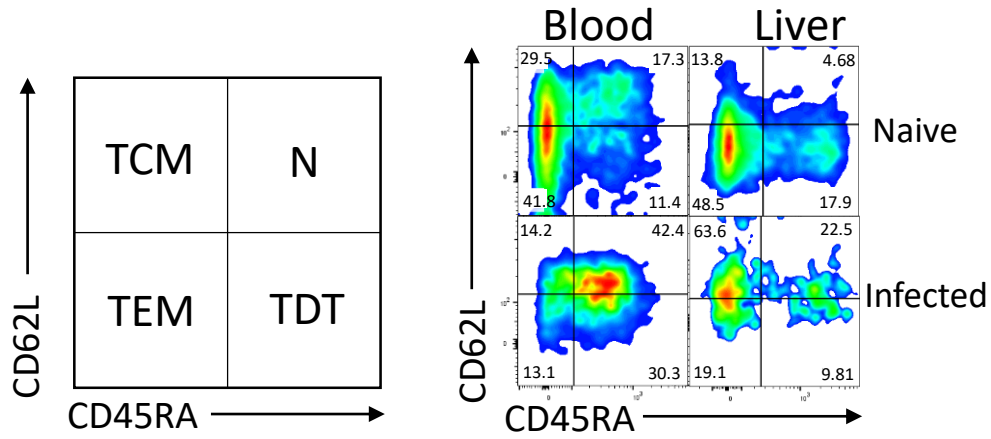


Because CCR5 is the main chemokine receptor used by SIV to infect CD4 T cells, we assessed CCR5 expression on CD4 T cells. Above, representative dot plots depicting the expression of CCR5 in a naive and SIV-infected monkey.

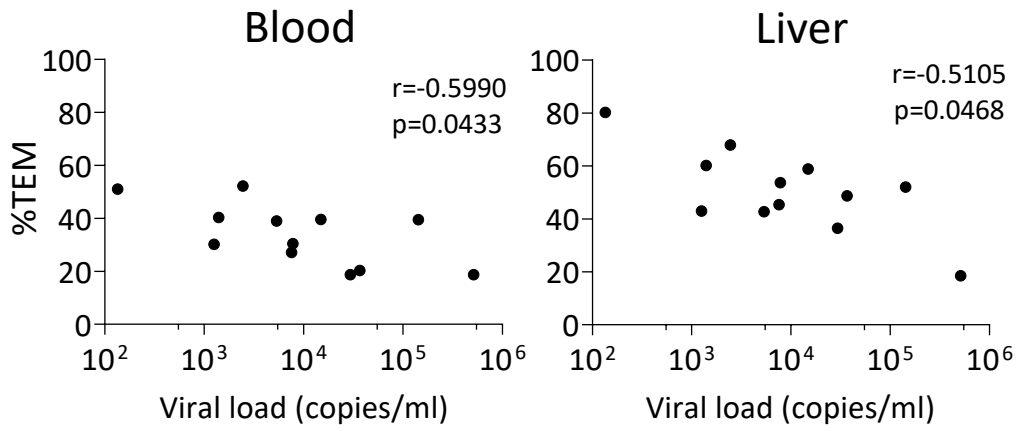


No significant difference was observed between the percentages of CD4 T cells expressing CCR5 from naive and infected RMs both in the blood and liver. Interestingly, we found that about 40% of CD4 T cells expressed CCR5 in the liver, whereas 8% of CCR5 is expressed in blood CD4 T cells.

Figure 5. CD4 T cell subsets in the blood and liver

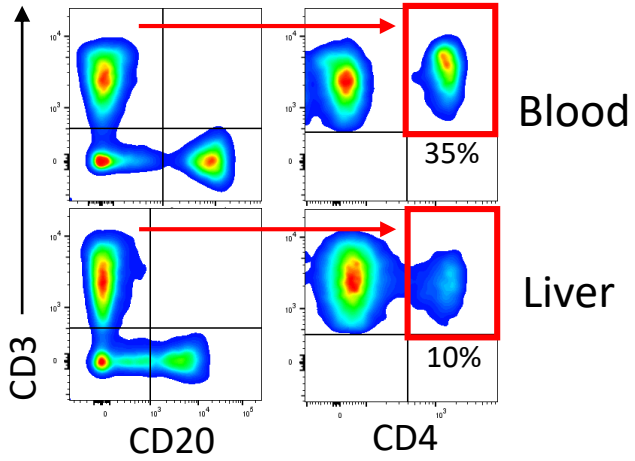


Gating strategy was used to analyse CD4 T cell subsets including naive (N: CD45RA+ CD62L+), central memory (TCM: CD45RA- CD62L+), effector memory (TEM: CD45RA- CD62L-) and terminally differentiated (TDT: CD45RA+ CD62L-) CD4 T cells. Compared to the blood, memory CD4 T cells represent the main subsets in SIV-infected individuals.

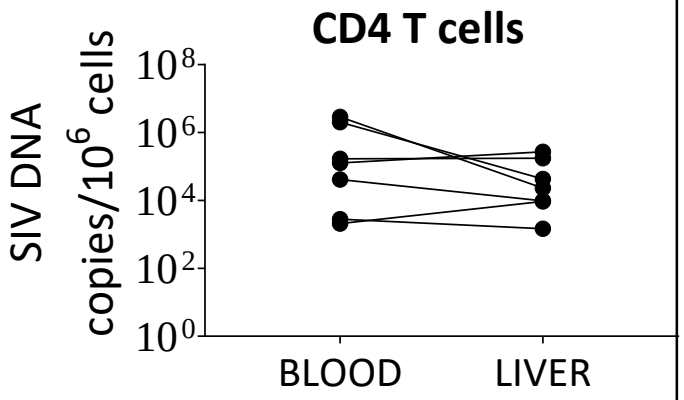


As expected, percentages of TEM were negatively correlated with plasma viral loads both in the blood and liver of SIV-infected RMs. These results demonstrated the impact of SIV infection on effector memory cell levels in each compartment. Spearman analysis was used for correlations.

Figure 6. CD4 T cell infection



We first sorted CD4 T cells from the blood and liver by flow cytometry. Cells were sorted from the CD3+CD20- population and then viral DNA was quantified by qPCR.



CD4 T cells from the liver contain vDNA. Similar levels of total vDNA in CD4 T cells from the liver and the blood (about 10^4 copies/10^6 cells).

CONCLUSION

Herein, we demonstrate that CD4 T cells from the liver are significantly depleted, correlating with plasma viral load. We also showed that CD4 T cells in the liver are mainly memory cells. Importantly, the phenotype of CD4 T cells in the liver excludes a possible blood contamination. Furthermore, despite higher levels of CCR5 expression in the liver than in the blood, the levels of total vDNA are similar.

PERSPECTIVES

Altogether, our results indicated that CD4 T cells from the liver of SIV-infected RMs may represent possible viral reservoir under ART. Thus, further analyses are in progress to assess the extent of viral infection of CD4 T cells in ART-treated monkeys.

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The monkeys

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