Characterization of tissue resident myeloid cells in the liver and lung of SIV-infected rhesus macaques

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Introduction

Viral dissemination occurs early after infection targeting CD4 T cells and monocytes/macrophages. Monocytes derived from bone marrow and tissue resident macrophages (TRMs) derived from yolk sac, are short-lived and long-lived cells, respectively. HIV infects non-lymphoid tissues, such as liver and lung in which TRMs may represent viral reservoirs (VRs). Whereas we demonstrated that early antiretroviral therapy (ART) efficiently prevents infection of monocytes in the blood, spleen and intestine of SIV-treated rhesus macaques (RMs), little is known so far about the role of TRMs, and whether these cells may represent VRs in SIV-infected RMs.

Herein, we assessed the phenotypes of tissue resident macrophages by flow cytometry and their potential role as a viral reservoir in SIV-infected rhesus macaques.

Methodology

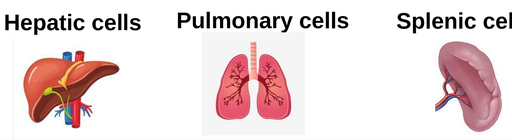
Rhesus macaques (RMs)

NATURAL INFECTION (NI)

SIVmac251 (10 AID_{50})

Euthanasia : **10 monkeys chronically infected**

Hepatic cells **Pulmonary cells** **Splenic cells**



Cell mechanical isolation → **Specific antibodies Staining by flow cytometry**

BD FACS Celesta

Cell DNA and RNA extraction → **Viral DNA and RNA quantification by qPCR**

Quant Studio 6

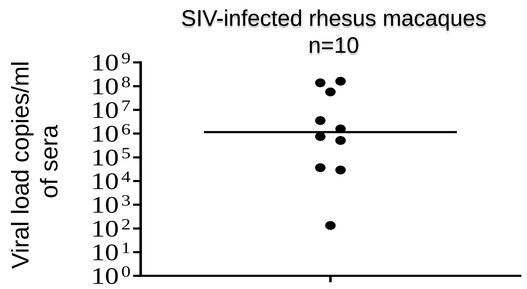
Rhesus macaques were infected with SIVmac251. Animals were sacrificed at different time point post-infection (n=10).

Cells from liver, lung and spleen were mechanically recovered. The phenotype of TRMs was analyzed by flow cytometry using specific antibodies including anti-CD14, anti-CD16, as well markers of TRMs such as CD44, CD59, CD35, CD117, CD206, MERKT, and LYVE.

The levels of viral DNA and RNA were quantified by qPCR.

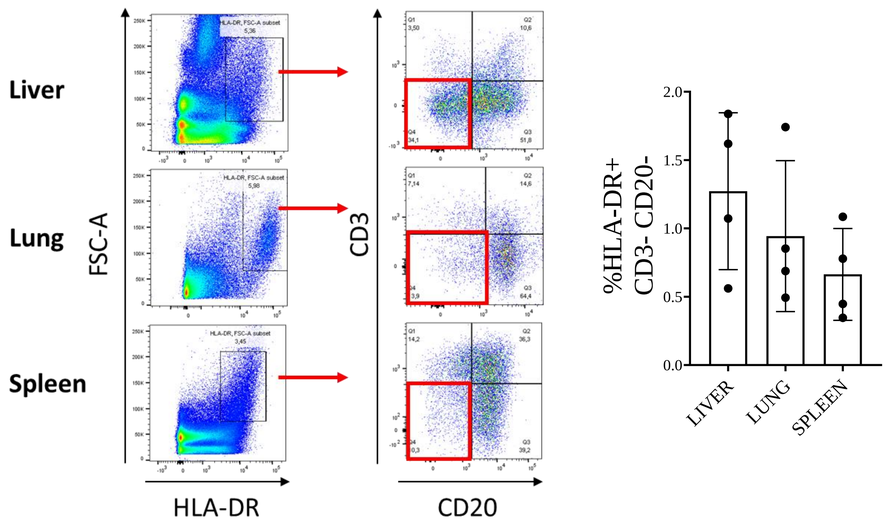
Results

FIGURE 1. VIRAL LOAD OF SIV-INFECTED RHESUS MACAQUES



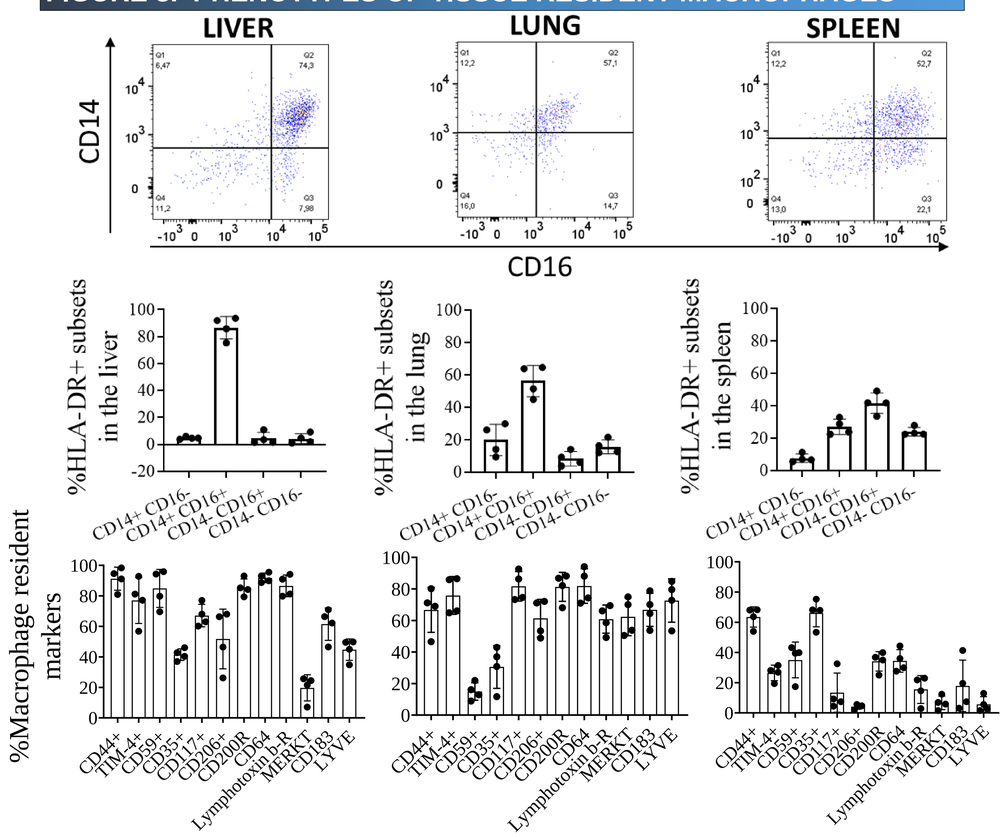
Viremia of SIV-infected RMs reached until 10^8 copies/ml.

FIGURE 2 FLOW CYTOMETRIC ANALYSIS OF MYELOID CELLS IN SIV-INFECTED RHESUS MACAQUES



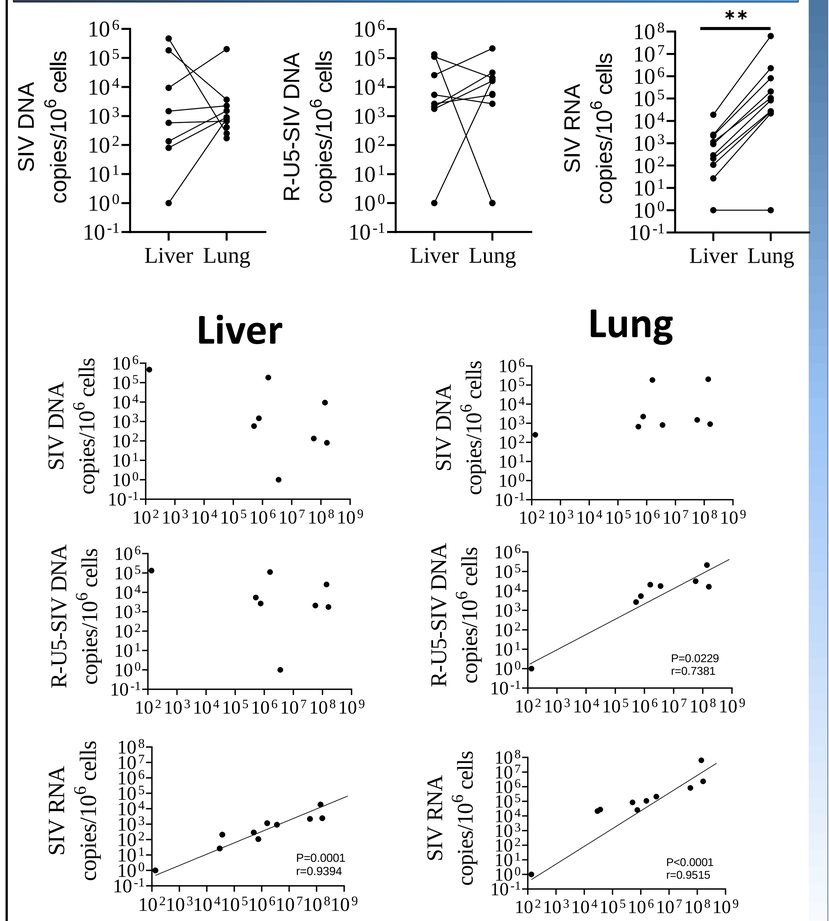
Overall, the proportion of myeloid cells HLA-DR+ CD3- CD20- is higher in the liver than in the spleen and lung.

FIGURE 3. PHENOTYPES OF TISSUE RESIDENT MACROPHAGES



Our data revealed that TRMs phenotypes differ depending on tissues analyzed. Myeloid cells from the lungs and livers of SIV-infected RMs expressed mostly CD117, CD206 and LYVE markers. By performing a mechanical procedure, instead to use a cocktail of proteases, we preserved CD14 shedding that allowed to identify infiltrate cells. Thus, we also detected infiltrate monocytes (CD14+) that do express TRM markers in the infected tissues.

FIGURE 4. LIVER AND LUNG INFECTION IN SIV-INFECTED RMs



Our data also demonstrated that liver and lung of SIV-infected RMs both contain comparable levels of viral DNA and retrotranscripts SIV DNA (R-U5). Interestingly, R-U5 SIV DNA is positively correlated with viremia only in the lung.

Moreover, we observed that viral RNA levels are significantly higher in the lung compared to the liver. For both, SIV RNA are also positively correlated with the viremia.

Conclusions

Thus, we characterized the phenotypes of long-lived tissue resident macrophages in the lung and liver in comparison to the spleen of rhesus macaques.

Because we showed that non-lymphoid tissues as liver and lung can be infected by SIV, it remains to determine the contribution of TRMs from liver and lung in maintaining viral reservoirs under antiretroviral therapy.

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