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 viral reservoirs in SIV-infected rhesus macaques.

Methodology

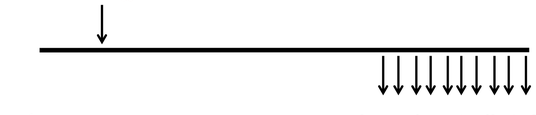
Rhesus macaques (RMs)

NATURAL INFECTION (NI)

SIVmac251 (10 AID_{50})

Euthanasia : 15 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

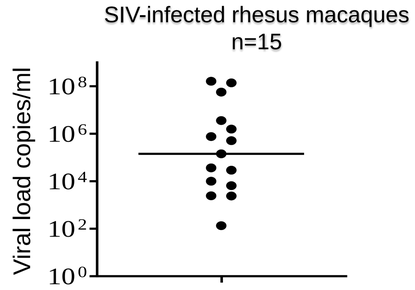
Rhesus macaques were infected with SIVmac251. Animals were sacrificed at different time point post-infection (n=15).

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, TIM-4, CD117, CD206, CD200R, CD64 and LYVE.

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

Results

FIGURE 1. VIRAL LOAD (VL) OF RHESUS MACAQUES



Viral loads were quantified by qRT-PCR. Each dot represents one individual.

FIGURE 2. MARKERS OF TISSUE RESIDENT MACROPHAGES

TRM markers	Functions	References
CD44 LYVE	Hyaluronic acid receptor (cell growth and migration)	Poon et al. J Immunol, 2015 Lim et al. Immunity, 2018
CD64	Fc receptor (clearance of immune complexes)	Gautier et al. Nat Immunol, 2012
CD206	Mannose receptor (cell activation)	Bain et al. Mucosal immunology, 2013
CD117	Tyrosine kinase and cytokines receptor (cell survival/proliferation/migration)	Ikarashi et al. J leukoc Biol, 2013
TIM-4	Phosphatidylserine receptor (phagocytosis)	Wong et al. Proc Natl Acad Sci, 2010
CD200R	Inhibitory receptor (anti-inflammatory)	Bain et al. Immunobiology, 2012

The expression of TRM markers were assessed in HLA-DR$^+$CD3$^-$CD20$^-$ cells expressing CD11b, as shown in the gating strategy below :

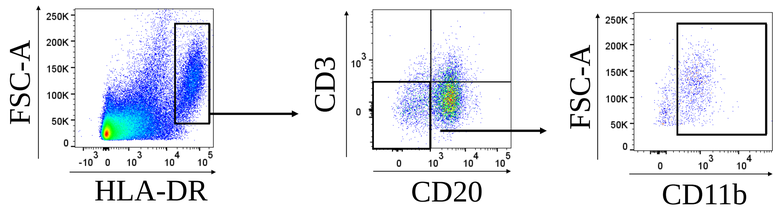
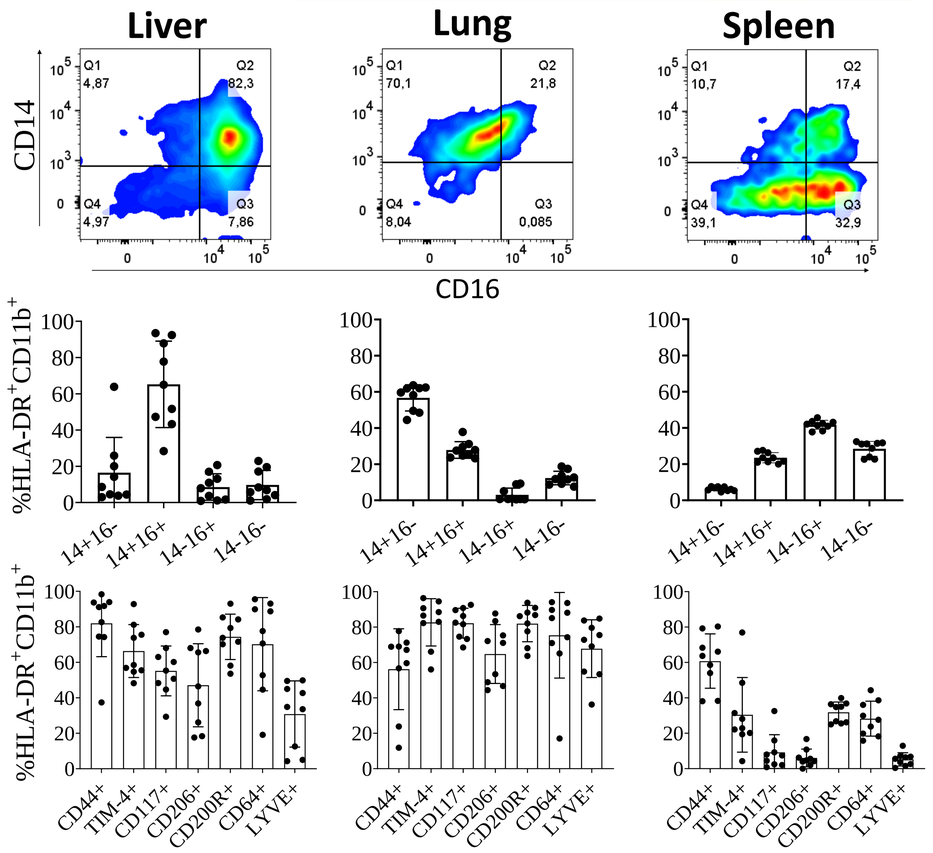
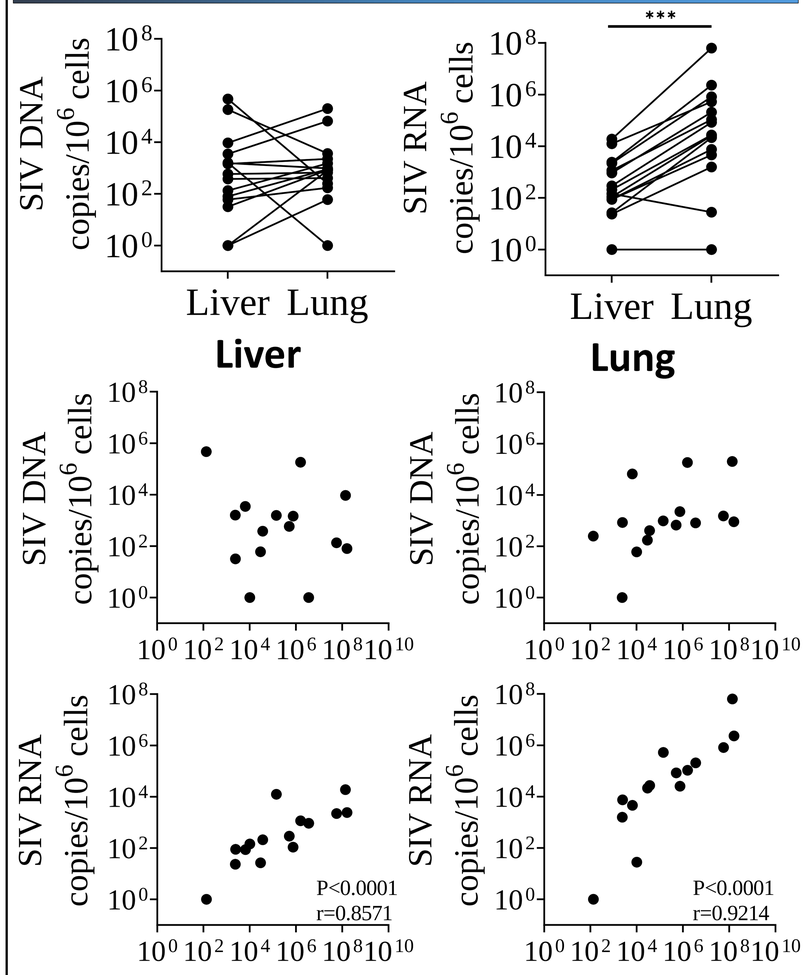


FIGURE 3. PHENOTYPES OF TISSUE RESIDENT MACROPHAGES



Analysis show that TRMs phenotypes are mostly observed in the lungs and livers compared to the spleens of SIV-infected RMs. By performing a mechanical procedure, instead to use a cocktail of proteases, we preserved CD14 shedding that allowed to identify infiltrate cells.

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



Concomitantly with the viremia observed during natural infection, our data revealed that liver and lung of SIV-infected RMs both contain comparable levels of viral DNA.

Moreover, our data showed that viral RNA levels are significantly higher in the lung compared to the liver. Interestingly, for both, SIV RNA were strongly 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.

Because we demonstrate that liver and lung can be infected by SIV and may represent a source of viral seeding, it remains to determine the contribution of resident macrophages from liver and lung in maintaining viral reservoirs under antiretroviral therapy.

Acknowledgment

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