Abstract Number: 380 Liver Macrophages and HIV-1 Persistence

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Abstract

Background: Cellular reservoirs of HIV-1 infection that persist despite combination antiretroviral therapy (cART) are impediments to a cure. The extent to which tissue resident macrophages (TRM) sustain infectious HIV-1 in patients on cART is unknown. We hypothesized that liver macrophages (LM), also known as Kupffer cells, comprising 80-90% of all TRM are a reservoir of HIV-1.

Methods: To test this hypothesis we used both in vitro and in vivo studies. Purified LM from 3 human donors were infected in vitro with an R5-tropic GFP reporter HIV-1 virus and supernatants were tested periodically for the presence of viral RNA. To assess their role in vivo, LM were purified from liver explants taken from HIV-1 infected persons with uncontrolled (n=1) and cART-suppressed viremia (n=2); viral outgrowth assays were performed on LM isolated from HIV-1 infected persons with cART-suppressed viremia to determine if purified LM contain infectious HIV-1. LM purity was confirmed using sensitive qPCR assays, and when required LM were incubated with a high-affinity T cell immunotoxin.

Results: LM were infected in vitro and were found to support infectious virus production for >170 days; LM supernatants were sufficient to propagate HIV-1 in reporter cells. Infectious virus was also recovered during and after in vitro exposure to cART for 24 days. Poly(A) HIV-1 RNA was detectable in LM supernatant from the individual with uncontrolled viremia (N7) 18 days after explantation, indicating virus release. Viral outgrowth assays demonstrated LM supernatants from HIV-1 infected persons (LT01 and LT02) with cART-suppressed viremia could transmit infection to reporter cells: LT01 and LT02 LM were maintained ex vivo for 36 and 95 days, respectively, before stimulation. After stimulation of LM, reporter cells were inoculated with LM supernatants for 36 (LT01) and 11 (LT02) days. Reporter cells were lysed and found to have proviral DNA (LT01: 2.4 cp/10^6 cells; LT02: 4.9 cp/10^6 cells). Reporter cells treated with LM supernatants from LT02 also released HIV-1 RNA at a level of 55 cp/100 μL on day 11 after inoculation. Notably, LT02 had a pre-cART plasma HIV-1 RNA level of 74 cp/mL .

Conclusions: These data provide strong evidence that LM, the largest TRM population, release infectious HIV-1 after a prolonged duration; therefore, LM may represent an important reservoir of HIV infection and potential impediment to cure.

Introduction

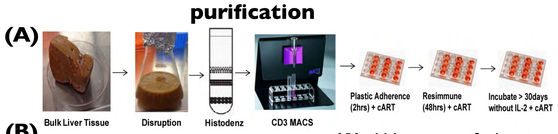
Clinical evidence has revealed that HIV-1 cure is possible. However, long-lived cellular reservoirs of HIV-1 which exist in all patients are a major challenge thwarting early eradication of HIV. The best-characterized cellular reservoir is the resting memory CD4+ T cell. Tissue macrophages are abundant, live for years, and are susceptible to HIV-1 infection; however, their role as a long-lived HIV-1 cellular reservoir has not been adequately addressed in humans. We studied human liver macrophages (Kupffer cells), comprising up to 90% of all tissue macrophages as a potential HIV-1 cellular reservoir.

Materials and Methods

Ex vivo: To assess their role *in vivo*, LM were purified both from liver explants taken from HIV-1 infected persons with uncontrolled (n=1) and cART-suppressed viremia (n=2). Viral outgrowth assays were performed on LM isolated from HIV-1 infected persons with cART-suppressed viremia to determine if purified LM contained infectious HIV-1. LM purity was confirmed using sensitive qPCR assays and, when required, LM were incubated with a high-affinity T cell immunotoxin, Resimmune™.

In vitro: For *in vitro* HIV-1 infections primary human Kupffer cells (KC) purchased from Life Technologies were infected with GFP-tagged R5-tropic HIV-1 virus, pSF162R3 Nef+. *In vitro* infected cells were used to study viral kinetics of HIV-1 infection over time by testing culture supernatants periodically for the presence of viral RNA. During the course of the infection (after 115 days post infection) antiretrovirals was also used to observe the effect of treatment on *in vitro* infections of these cells using previously described concentrations.

Fig 1:Purification schema with evidence of purification



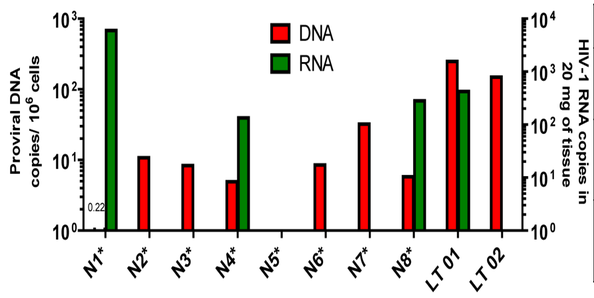
(A) LM are purified using multi-step protocol that progressively depletes T cells: **(B)** Using a sensitive qPCR assay for CD3ε that can detect a single T cell, the protocol described in **(A)** results in no detectable T cells (Post-Resimmune). cART is incubated at all steps of the protocol to prevent inadvertent *ex vivo* LM infection by dying or phagocytosed T cells.

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Results

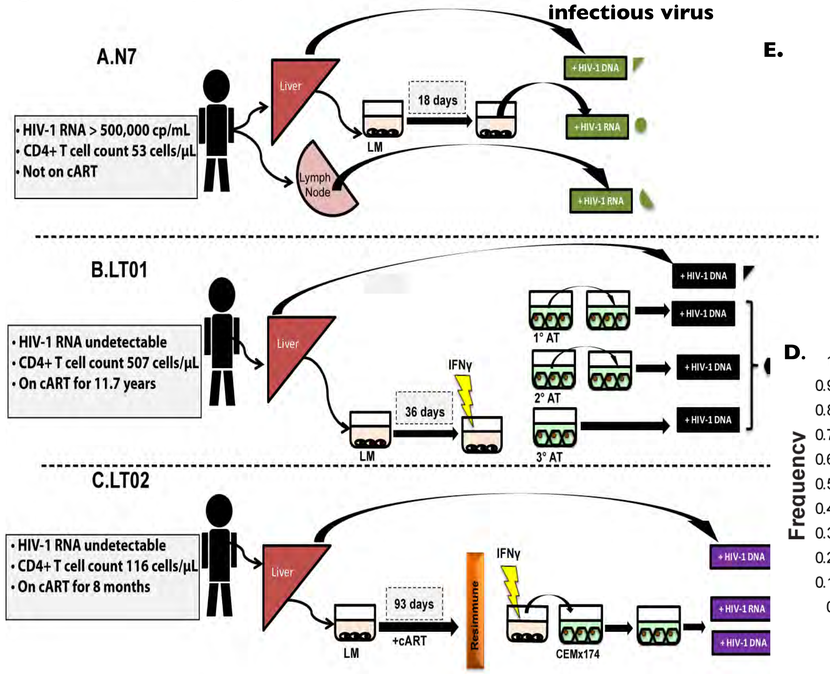
Fig 2: HIV-1 DNA is detectable in liver tissue from HIV-1 infected persons



	N1*	N2*	N3*	N4*	N5*	N6*	N7*	N8*	LT01^	LT02^
CD4+ T cell count (cells/μL)	-	4	-	9	379	860	53	67	607	116
HIV-1 RNA (cp/mL)	-	-	-	-	UD	UD	>500,000	136,896	UD	UD
cART	TDF/ddI/ NFV	None	None	None	-	TDF/FTC/ EFV	None	None	TDF/FTC/ Ral	ABC/3TC/ DTG

Whole liver tissue was obtained from HIV-1 infected individuals at time of death (*) from NDRI and during liver transplantation (^). After whole cell lysis, samples were tested for HIV-1 proviral DNA and RNA. Cell numbers were estimated using ERV3 DNA quantification from the same samples. The table indicates the latest available CD4+ T cell count, cART status, and plasma HIV-1 RNA before the recovery of liver tissues. UD – undetectable using a standard clinical assay. *; HIV-1 RNA, cART status, HCV and HBV status are reported by NDRI. Data that was unavailable is indicated by "-". HIV-1 cART, HCV and HBV status were available through clinical testing for ^. N2, N5, N8, LT01 and LT02 were HCV+ while N2 and N3 were HBV+.

Fig 3: Liver Macrophages isolated from HIV-1 infected persons with uncontrolled and with controlled viremia harbor infectious virus



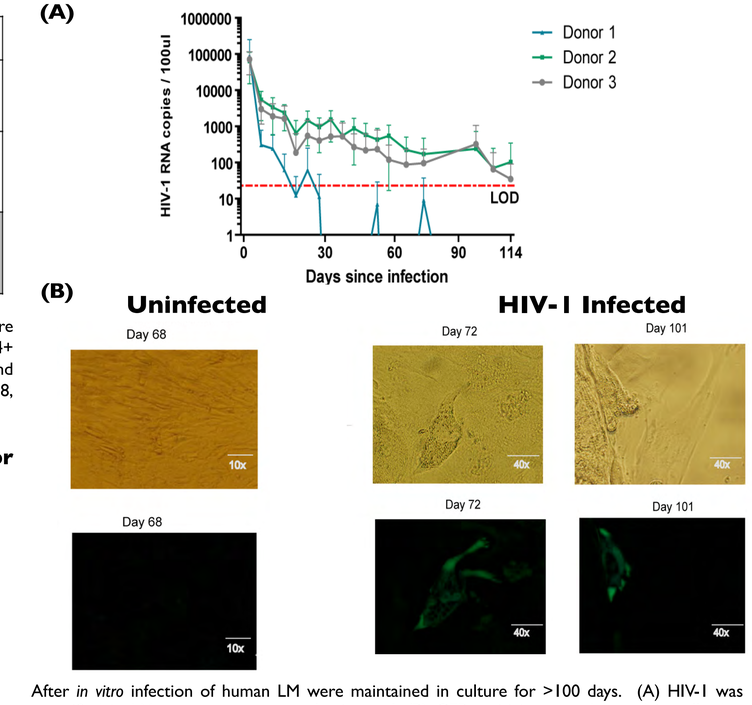
After isolation of primary human LM from HIV-1 infected subjects [**(A)** N7; **(B)** LT01; and **(C)** LT02)], cells were maintained *ex vivo* for the indicated duration. **(E)** T cell contamination was measured using sensitive qPCR for CD3:**(A)** Supernatants from N7 LM contained detectable HIV-1 RNA. **(B)** and **(C)** HIV-1 RNA was not immediately detectable in LM supernatants from LT01 and LT02 **(B)** LM from LT01 were stimulated with IFN γ and then treated with a combination of LPS and poly I:C; supernatants were transferred to activated T lymphoblasts 6 hours (1° AT), 72 hours (2° AT), and 22 days (3° AT) after activation. Supernatants from 1° AT and 2° AT reporter cells were transferred to fresh AT cells 19 days after inoculation. After 36 days, activated T lymphoblasts were lysed, and HIV-1 proviral DNA was detected **(C)** 1% T cell contamination was detected in LT02 LM after isolation therefore, cultures were maintained for 93 days in the absence of IL-2 to further enrich cultures for LM in the presence of cART. Thereafter, LM were treated with Resimmune™, over 48 hours. LM were subsequently treated with IFN γ and HIV-1 rTat. Supernatants were transferred to uninfected CEMx174. HIV-1 RNA was detectable in cell supernatants on day 11 and HIV-1 proviral DNA in lysed CEMx174. **(D)** qPCR amplicons were sequenced (126 bp) to confirm that all isolates were specific for HIV-1, and aligned to a reference sequence. LT01 sequences were limited to 36 bp. The identity in nucleotide sequence between study isolates was compared in the context of 100 unrelated subtype B HIV-1 nucleotide sequences from the LANL HIV-1 database spanning the same 126bp amplicon. Height of bars indicates the frequency with which each percent identity appears among all pairwise comparisons between sequences. Gray bars indicate pairwise comparisons between LANL database from unrelated donors. Values falling within the 95th percentile of all comparisons between unrelated donor sequences are shown. Cyan bars indicate inter-person differences while the red bar indicates intra-persons differences.

Conclusions

These data provide strong evidence that LM, the largest TRM population, release infectious HIV-1 after a prolonged duration; therefore, LM may represent an important reservoir of HIV infection and potential impediment to cure.

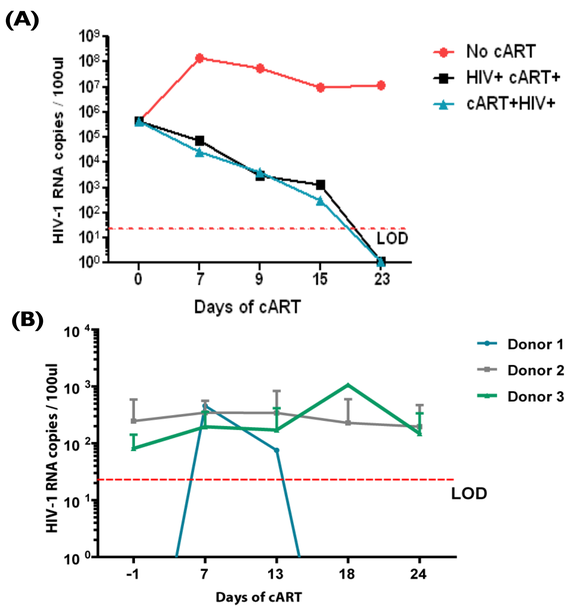
Results

Fig 4: Long-term viral kinetics of HIV-1 infected Liver Macrophages



After *in vitro* infection of human LM were maintained in culture for >100 days. (A) HIV-1 was quantified in LM supernatants at regular intervals. (B) GFP-positivity was observed in cells that were morphologically consistent with LM during the entire course of infection.

Fig 5: HIV-1 production of GFP positive Liver Macrophages in culture during treatment with cART



After 115 doi, cART (TDF, FTC, and Ral) was added to *in vitro* LM cultures (A) Over the course of 24 days during which infected LM were exposed to cART, viral RNA was stably released into supernatants (B) To confirm the efficacy of cART in HIV-1 suppression, activated CD4+ T cells were treated (A) before (blue) and after (black) infection with the Rf strain.