Development of a viral immuno-capture assay to assess the cellular origin and genetic diversity of HIV-1 viruses

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BACKGROUND

The viral reservoir retain sufficient levels of genetic variability to achieve rebound, even after years of efficacious viral control by cART. This genetic variability is key for the virus to recover from the extreme population size loss due to cART, facilitating the rapid rebound consistently observed in people living with HIV (PLWH) after treatment interruption/failure. Genetic diversity is also key in residual viremia on cART as it drives viral fitness, resistance to cART and/or host immune system. While T-CD4+ memory cells have been generally proposed to be the main HIV-1 reservoir, an increasing number of studies suggest that myeloid cells such as macrophages and mast cells constitute potential reservoirs during cART. **Our hypothesis is that, in plus of memory CD4+ cells, myeloid cells shape the reservoirs and by exhibiting different metabolism, replication dynamics and drug sensitivity characteristics, could favor HIV-1 intra-host genetic diversity, recombination, viral production from reservoirs.**

METHODS (1)

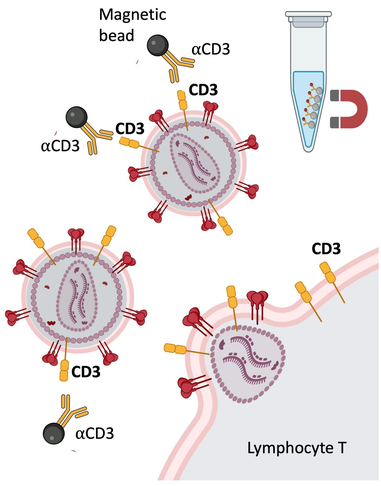


Figure 1: Viral Immuno-capture principle. Viruses budding from different cell types are isolated after being captured by an antibody directed against proteins specifically express by the cell type of interest incorporated in the virus. Here, T cells viruses are captured with antiCD3 antibodies bound to magnetic beads. The same approach is done on monocytes/macrophages with anti-CD14+ and with anti-CD203c+ for mast cells.

Viral Immuno-capture (v-IC) technique relies on the fact that nascent virions derive their membranes from the host cell membrane and acquire proteins specific to that host cell (See figure 1). Cell specific protein incorporation is assessed by LC-MS on purified virions produced by the cells type of interest (see figure 3). With the help of magnetic-bound antibodies directed against incorporated host cell proteins and a magnet, we can isolate and analyze viral genomes produced by specific cell types (See figure 2).

Blood CD14+ and colon resident CD203c+ myeloid cells produce more recombinant and genetically diverse HIV-1 virions than lymphocytes

METHODS (2)

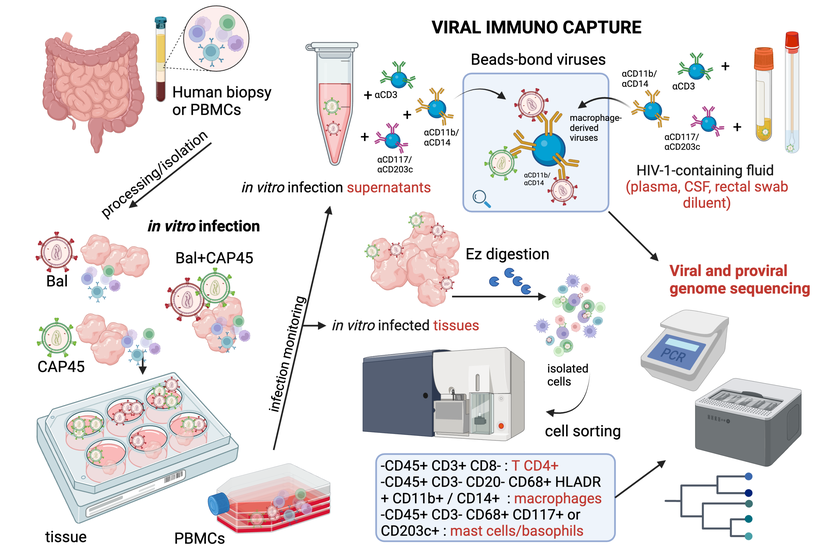


Figure 2: methodology. To study recombination and viral genetic diversity, naïve tissue gut biopsies or freshly isolated PBMCs are infected *in vitro* either with HIV-1 Bal (lab strain, subtype B), HIV-1 CAP45 (Transmitter Funder, subtype C), and with both, in absence and presence of ART. Viruses emerging from the whole lymphocyte population, monocytes/macrophages and mast cells are captured with beads coupled to anti-human –CD3, -CD14, -CD203c, extracted for RNA and sequenced. Every viral and proviral genome are quantified by q-PCR with Taqman technology. Made with Biorender.

RESULTS (1)

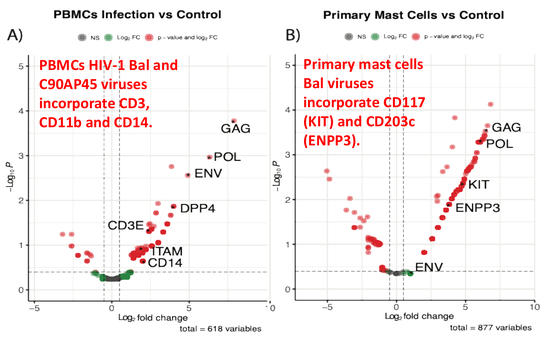


Figure 3: Incorporation of host cell proteins in HIV-1 viruses

Proteomic content of concentrated viruses from in vitro infected supernatant with one or two different subtypes (Bal, subtype B and CAP45, subtype C) are compared to non-infected PBMCs or mast cells supernatant from the same donor. The volcano plot shows the differences in protein quantity between non infected and infected supernatants and their significance.

RESULTS (2)

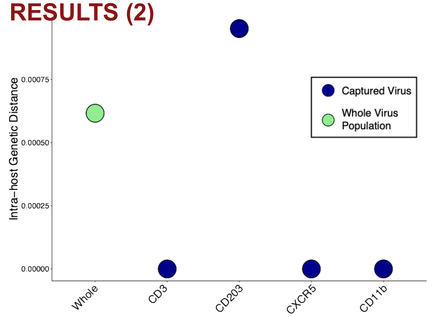


Figure 4: Viruses emerging from gut resident mast cells are significantly more genetically diverse. Gut explants have been infected *ex vivo* with HIV-1 Bal for 13 days, and viruses captured in supernatant with anti- CD3 for viruses emerging from whole lymphocyte population, -CD203c from mast cells, -CXCR5 from lymphocyte T helper and -CD11b from monocytes/macrophages.

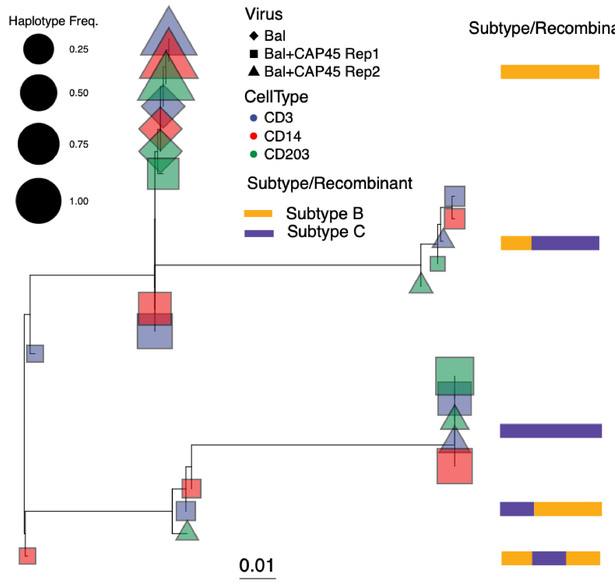


Figure 5: Dually infected PBMCs myeloid cells produce more recombinant viruses. PBMCs are infected for 6 days with Bal only (subtype B), CAP45 only (subtype C), or both. The phylogenetic tree depicts the viral populations immune-captured with human anti-CD3, -CD14 and -CD203c coated beads. Viral genome sequencing (*gag*) reveals that myeloid cells (CD14+, CD203c+) generate more recombinant viruses than lymphoid cells.

CONCLUSIONS

Those results show that myeloid cells, and specially mast cells, represent a great potential candidate for driving viral genetic variability necessary for the reservoir to rebound after treatment interruption.

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PLAIN LANGUAGE SUMMARY

HIV can mutate and recombine (“mix up” its genes) very quickly, which helps it easily adapt to survive in the body, even during therapy, and come back if treatment is stopped. We found that two types of immune cells—monocytes and mast cells—are especially likely to help HIV mutate and recombine. If we can target these specific cells, we may be able to reduce the hidden virus that remains during treatment and lower the chances of the virus returning—one of the biggest challenges to curing HIV.