Latency reversal using specific antigens including those of HIV-1

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Background

Latently infected CD4-positive memory T cells are capable of producing replication competent virus and represent a barrier to cure. These cells were presumably infected while responding to a specific antigen.

Hypothesis

We hypothesized that latency is differentially established in cells with certain antigen-specificities.

We also hypothesized that such cells would respond again to their specific antigen and in particular to those from HIV-1 itself.

Methods

We stimulated 5 million primary CD8-depleted PBMC in quadruplicate (total of 20 million cells per condition) from HIV-1 infected patients (n=9) in whom plasma viremia was suppressed by cART for at least 1 year with the following for 3 days:

- DMSO
- anti-CD2/CD3/CD28-beads
- HIV-1 Gag peptide pool
- CMV pp65 peptide pool
- *C. albicans* MP65 peptide pool
- CEFT peptide pool (CMV, EBV, flu, C. tetani)
- HIV-1 Pol peptide pool
- HIV-1 Env peptide pool
- HIV-1 Nef peptide pool

After three days of stimulation, the cells were stained for CD4 and CD69, an activation marker, and analyzed by flow cytometry. The culture supernatant was cleared from cells by centrifugation, and virions were obtained by centrifugation through a 20% sucrose cushion. We isolated the virion-associated RNA and measured the copy number of Gag sequences via real-time RT-PCR (Figure 1).

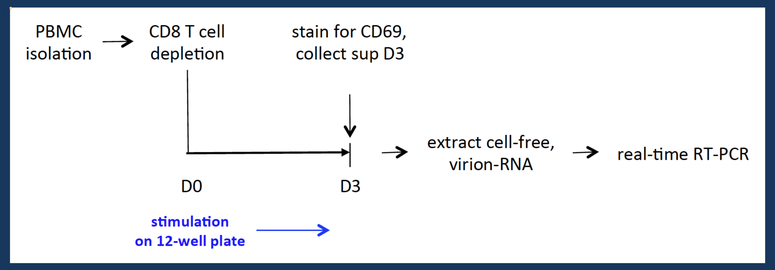


Figure 1: Protocol for detection of cellular activation and production of reactivated virus.

Results

Ten blood samples have been analyzed. The repeated analysis of donor 1 indicated a relatively consistent activation response to the antigenic peptides but some inconsistency in the reversal of latency. The virus-yield in response to Gag peptides was greater initially (Figure 2B) than subsequently (Figure 2D). Notably, the fraction of cells activated did not predict the virus yield.

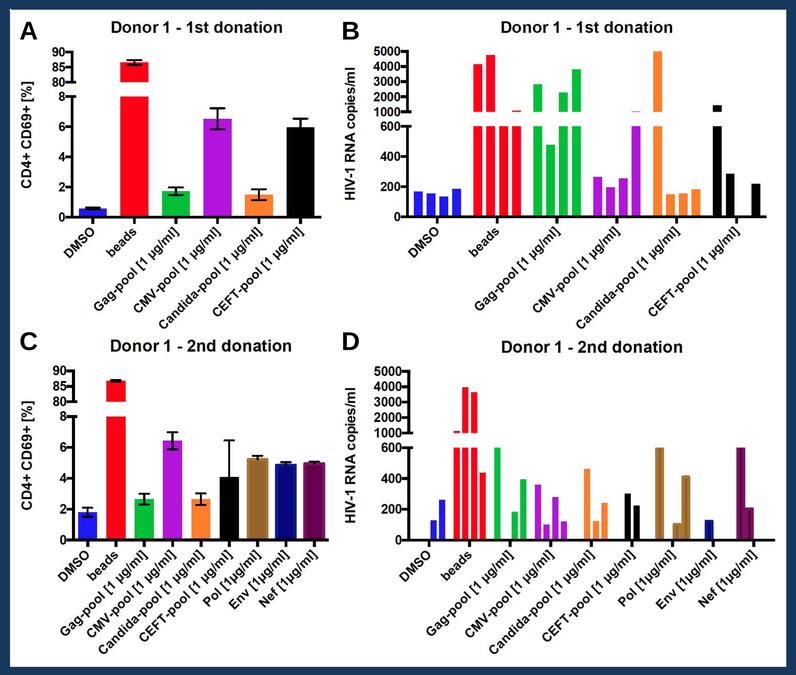


Figure 2: *Ex vivo* levels of latency reversal in CD8-depleted PBMCs in two longitudinal donations (nine months apart) from donor 1 after antigens stimulations. A & C: Levels of CD69 expression on CD4 T cells (measured by flow cytometry) and B & D: real-time RT-PCR detecting Gag RNA in released virions. The four replicates of each condition are shown as individual bars.

The hierarchy of CD69 expression in the 9 donors in response to the stimuli was similar: beads > CMV/CEFT > HIV-1 > *C. albicans* (Figure 3)

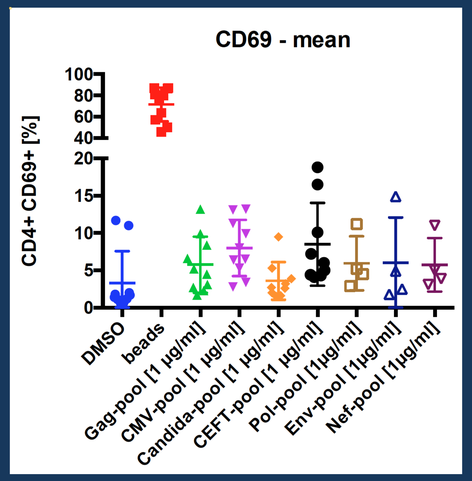


Figure 3: Levels of CD69 expression among nine HIV-infected donors. Stimulated CD8-depleted PBMC stained for CD4 and CD69 after three days of stimulation with different agents. Shown is the replicates mean CD69 expression on CD4 T cells of each agent.

Real-time RT-PCR detected recoverable cell-free virion-associated RNA in 7/9 donors after stimulation of the CD8-depleted PBMC. Cells from two donors yielded no virion-associated RNA even after maximal stimulation with antibody coated beads. For the seven analyzable donors, the response to the various antigenic peptides was different and in almost all instances substantially less than that of antibody coated beads (Figure 4).

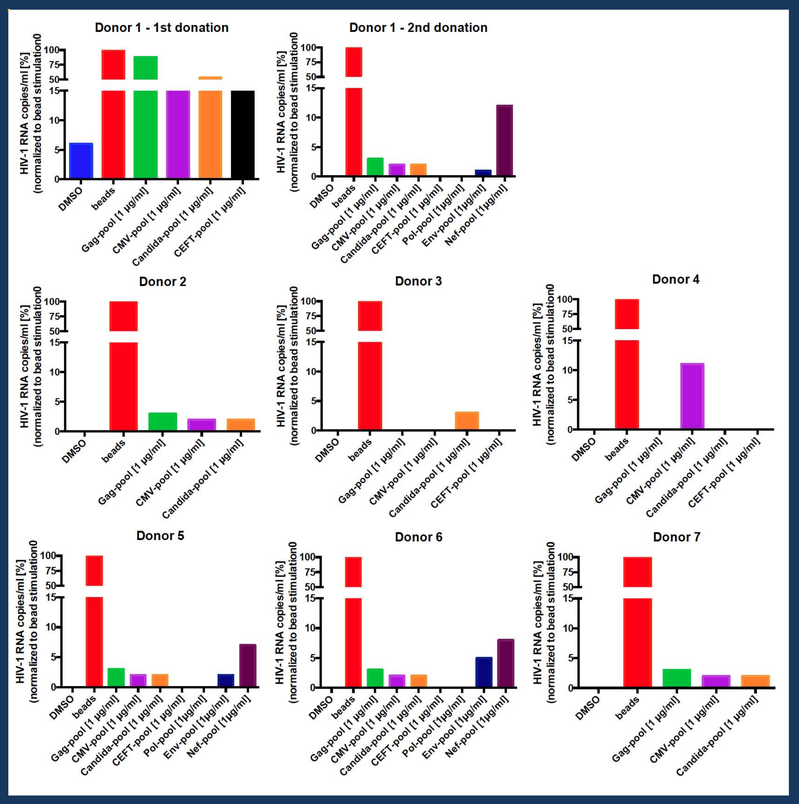


Figure 4: Cell-free, virion-associated Gag-RNA recovery from seven donors after stimulation with the various antigenic peptides: different degrees of latency-reversal. Copy-numbers of Gag RNA were combined for the quadruplicates of each condition and normalized to the antibody-coated beads as 100%.

HIV peptide pools, particularly those derived from Gag and Nef, showed sporadic latency reversal activity (donors 1, 5 & 6) as did peptides from CMV (donor 4).

Limitations

Our data suggest difficulty in measuring latency-reversal in a consistent manner. This could be due to variation in reservoir size among donors and the number of cells tested. Here, 5 million CD8-depleted PBMC in quadruplicate for each condition. The use of peptide pools might be a suboptimal way of presenting antigens to CD4 cells, use of antigen loaded dendritic cells might be more effective.

Conclusions

Our preliminary results suggest the potential for latency to be differentially established in cells with different antigen specificities.

This could explain why the fraction of cells activated by specific antigens did not predict virus-yield.

By defining the specific antigens that can reactivate viral expression in latently infected cells, we aim to inform models of latency and in the best case derive an antigenic mixture capable of reversing latency in the majority of cells.

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