LENTIVIRAL DENDRITIC CELL VACCINE INDUCES CTLS AND ACTIVATES LATENT HIV

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ABSTRACT

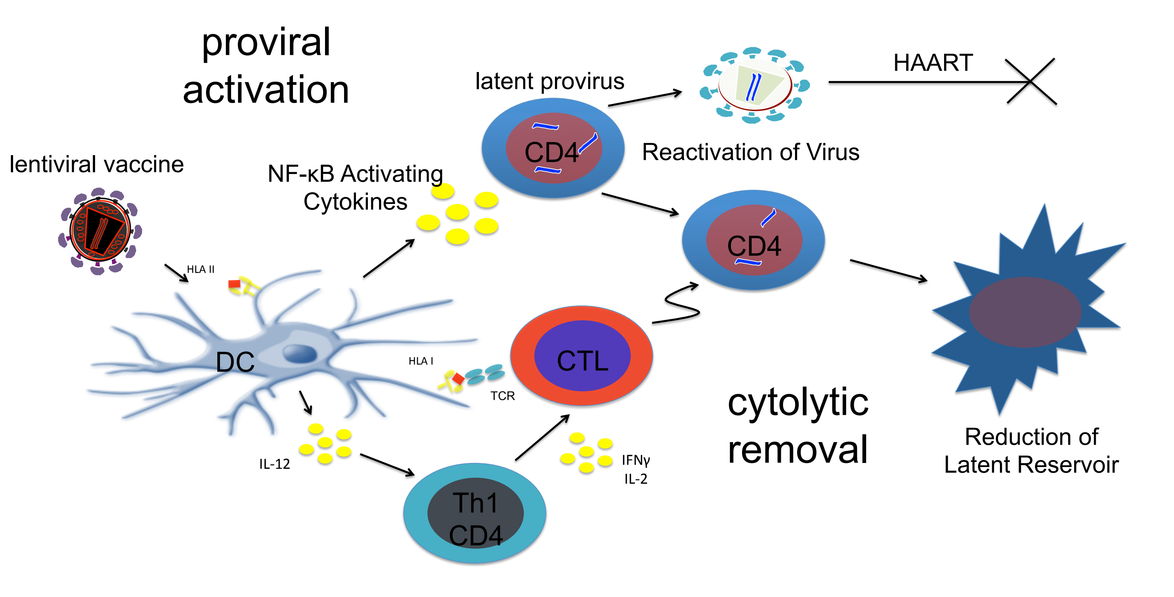
Background
Dendritic cells (DCs) orchestrate immune responses to viral pathogens and have been used in vaccine strategies against cancer, infections, and autoimmune diseases. To further develop this approach we have established lentiviral vectors that package the lentiviral accessory protein Vpx. Vpx increases DC transduction by two-logs allowing for long-term antigen expression, a significant improvement over peptide-pulsing. To test this approach, we generated vectors that express the lymphocytic choriomeningitis virus (LCMV) GP33 epitope or the major HLA-A2-restricted HIV Gag epitope SL9. The vectors co-express CD40L, increasing their ability to stimulate antigen-specific T cells. Cytokines released by transduced DCs reactivated latent HIV-1 provirus in latency models, supporting their use as a two-pronged approach for activating and targeting the latent reservoir in HIV+ patients. (Norton, *et al*., 2015). We have now tested the lentiviral DC vaccine strategy in HIV-infected humanized mouse and LCMV mouse models.

Methods
Bone marrow, liver, thymus (BLT)-humanized mice expressing transduced SL9-specific T cell receptor (TCR) CD8 T cells were injected with CD40L-SL9 expressing vector-transduced DCs and then challenged with HIV-1. Expansion of SL9 TCR+ CD8 T cells was quantified by flow cytometry and peripheral viral loads measured by RT-PCR. Wild-type mice were similarly injected with CD40L-GP33 LV-transduced bone marrow-derived from SAMHD1$^{-/-}$ mice and then challenged with LCMV. GP33 TCR+ CD8 T cells were quantified by flow cytometry and viral loads were determined by plaque assay. Survival was also monitored following a lethal challenge with LCMV.

Results
In mice injected with transduced DCs, the number of SL9 TCR+ and GP33 TCR+ CD8 T cells expanded 30-fold and 10-fold, respectively. BLT-mice injected with SL9 expressing LV-transduced DCs and then infected with HIV-1 dramatically suppressed virus loads 2-6 weeks post-infection. Mice injected with GP33 expressing LV-transduced DCs were protected against LCMV challenge. No infectious virus was detected in 4/5 immunized mice and the survival rate was close to 100%, while in mice injected with Control LV-transduced DCs, virus loads reached > 2 X 10^6 PFU/mL and resulted in death.

Conclusions
This lentiviral DC vaccine approach induces antigen-specific CTLs that protect against LCMV and HIV infection. This is a promising approach to restore CTLs and reduce the latent reservoir in chronically infected individuals.

DC-targeted lentiviral vaccine approach for treating HIV-1 infection



Schematic. DC transduction with Vpx-lentiviral vector expressing CD40L and HIV epitopes targets DCs to (i) induce secretion NF-κB cytokines that activate provirus from latently infected cells and (ii) efficiently present HIV antigens to expand HIV-specific CTLs that, in the setting of enhanced DC activation and secretion of Th1 skewing cytokines (IL-12), facilitate an HIV specific Th_1 response to reduce the latent reservoir and control viral loads.

Figure 1: Lentiviral vector virions that contain SIVmac Vpx infect DCs with high efficiency.

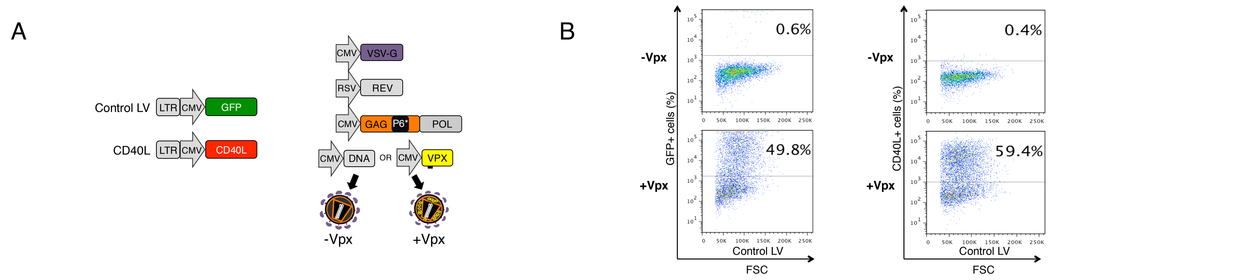


Figure 1. A. Lentiviral vectors expressing GFP (Control LV) or CD40L were produced as VSV-G pseudotypes containing or lacking SIVmac Vpx. **B.** Human monocyte derived dendritic cells (MDDCs) were transduced at MOI = 2 with the indicated lentiviral vector that contained (bottom) or lacked (top) Vpx. 72 h post-transduction the number of GFP+ and CD40L+ cells was quantified by flow cytometry.

Figure 2: DCs transduced with Vpx-vectors expressing CD40L and HIV antigen secrete pro-inflammatory cytokines, reactivate latent HIV, and stimulate HIV-specific CTLs.

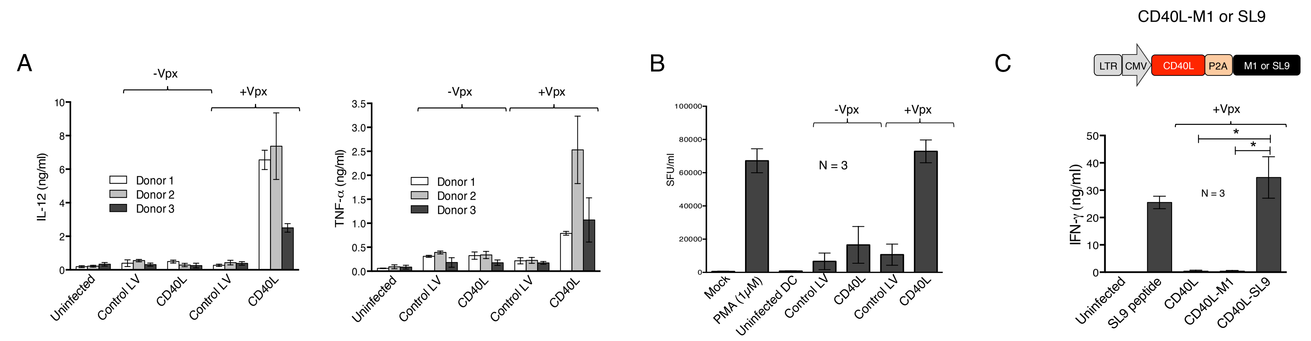


Figure 2. DCs from the three donors were transduced with Control LV or CD40L vectors. **A**. 72 h post-transduction, supernatant IL-12 and TNF-α levels were quantified by cytokine bead array (CBA). **B.** 24 h post-transduction, transduced DCs were cocultured with latently infected ACH-2 cells and after two days, infectious virus in the culture supernatant was quantified with a TZM-bl reporter assay. **C.** DCs were transduced with vectors expressing CD40L alone or fused to antigenic epitopes, including an irrelevant HLA-A2 epitope from influenza M1 (CD40L-M1) and an HLA-A2 epitope from HIV-1 Gag SL9 (CD40L-SL9). 72 h post transduction, the DCs were cultured with a SL9-specific CTL clone and supernatant IFNγ was quantified by CBA. DCs pulsed with SL9 peptide served as a control.

Figure 3: Lentiviral vector transduced DCs raise HIV-specific central and effector memory CD8 T cells in humanized mice.

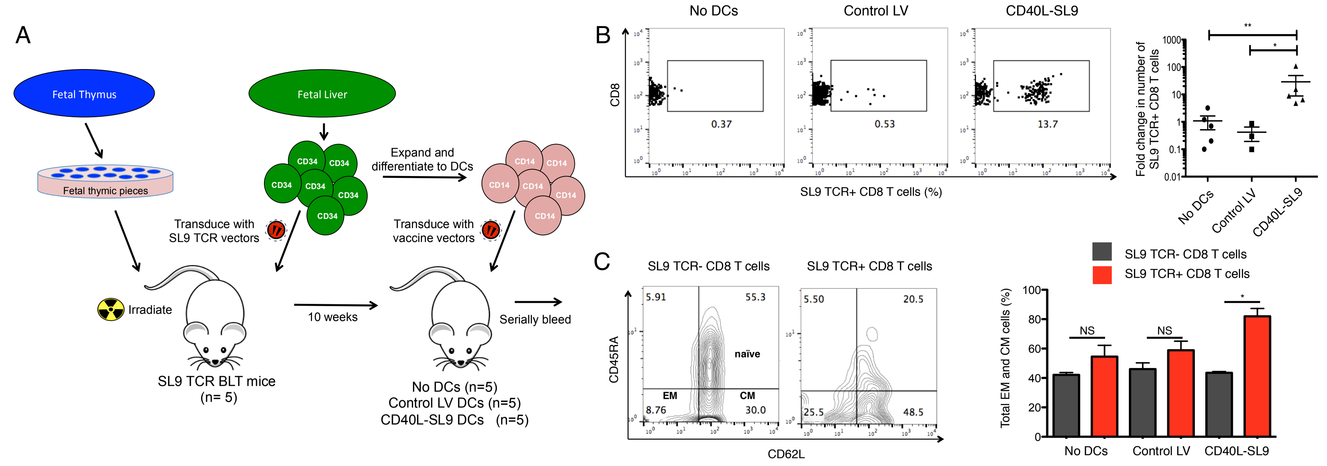


Figure 3. A. Humanized BLT mice were constructed with SL9 TCR vector-transduced CD34+ stem cells. Unmodified autologous CD34+ stem cells were used to generate DCs that were transduced with Control LV or CD40L vectors and injected into the SL9 TCR BLT mice. **B.** Mice were bled at baseline and 1 week post-injection and the number of SL9 TCR+ CD8 T cells was quantified by flow cytometry. Representative blots one week post-injection (left) and summary fold changes from baseline (right) are shown. **C.** One week post DC injection, naïve (CD45+/CD62+), effector memory (EM; CD45-/CD62-), and central memory (CM; CD45-/CD62+) SL9 TCR- and SL9 TCR+ CD8 T cell populations were identified by flow cytometry. Representative blots from a CD40L-SL9 vaccinated mouse (left) and the aggregate combined EM and CM T cell percentages (right) are shown.

Figure 4: DC vaccine protects against HIV-1 challenge in humanized mice.

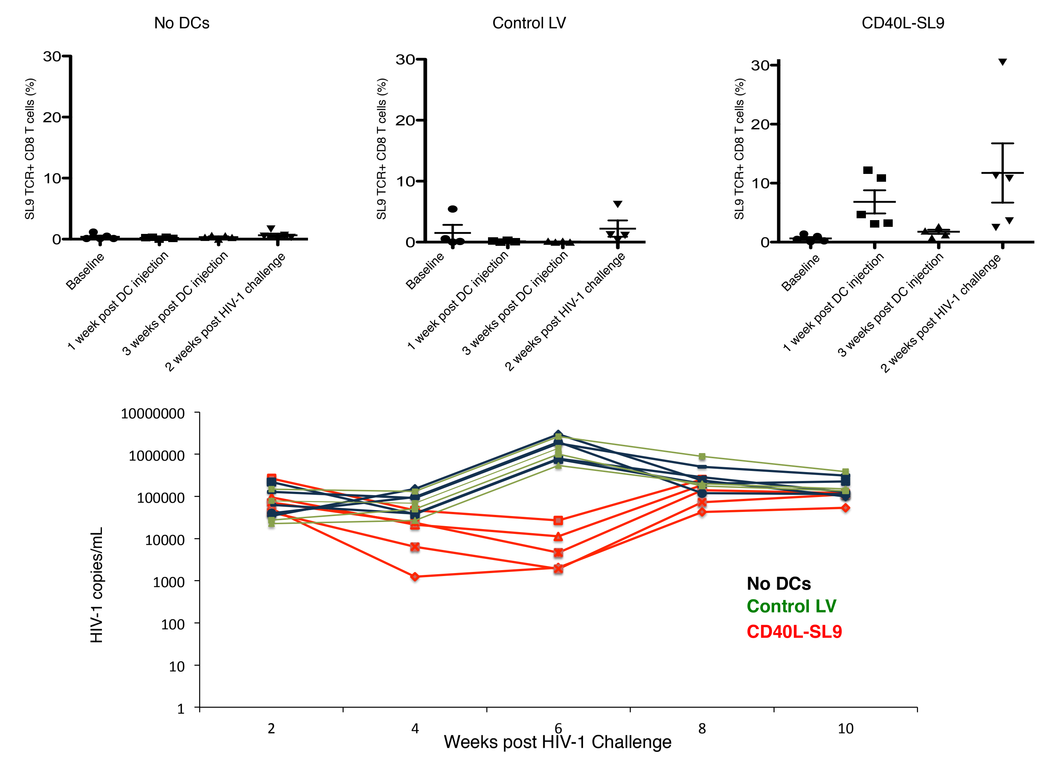


Figure 4. SL9 TCR BLT mice were injected with lentiviral vector transduced DCs and challenged 3 weeks later with HIV-1 NL43 SL9. **A.** Mice were bled at baseline, 1 and 3 weeks post DC vaccination, and 2 weeks post-HIV-1 challenge and the number of SL9 TCR+ CD8 T cells was quantified by flow cytometry. **B.** Peripheral HIV-1 viral loads were quantified by RT-PCR for up to 10 weeks post HIV-1 challenge.

Figure 5: Lentiviral DC vaccine against LCMV stimulates a strong CD8 T cell response *in vivo* and protects against high titer viral challenge.

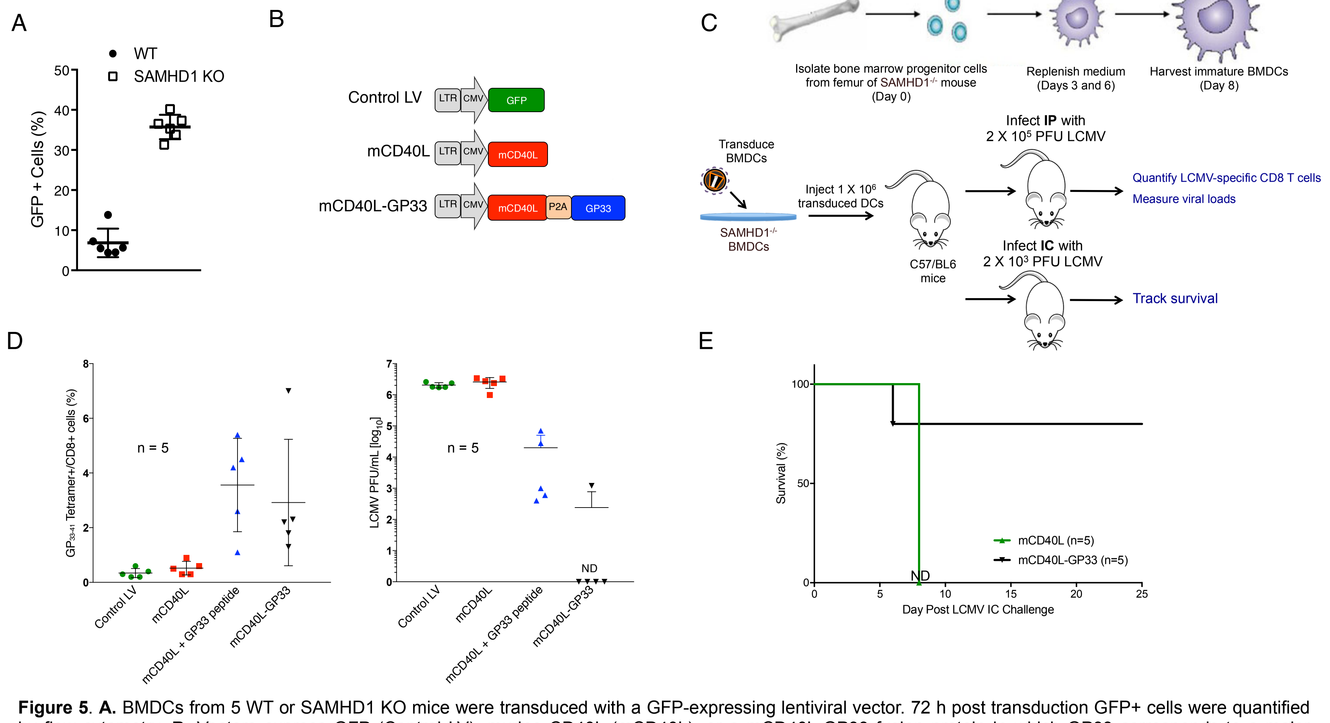


Figure 5. A. BMDCs from 5 WT or SAMHD1 KO mice were transduced with a GFP-expressing lentiviral vector. 72 h post transduction GFP+ cells were quantified by flow cytometry. **B.** Vectors express GFP (Control LV), murine CD40L (mCD40L), or a mCD40L-GP33 fusion protein in which GP33 corresponds to a major epitope from the LCMV glycoprotein. **C**. BMDCs prepared from SAMDH1 KO mice were transduced with the panel of lentiviral vectors and injected into syngeneic mice to determine whether DC vaccination protected against subsequent high titer LCMV challenge. **D**. Immunized mice challenged with LCMV via intraperitoneal injection were sacrificed and the number of splenic GP33 Tetramer+ CD8 T cells was quantified by flow cytometry (left). Splenic LCMV viral loads were quantified by LCMV plaque assay (right). **E**. Immunized mice challenged with a lethal dose of LCMV via intracranial injection were monitored for survival.

Summary

- Vpx-containing lentiviral vector-transduced DCs reverse HIV latency in cell culture model systems.
- Vpx-containing lentiviral vector-transduced DCs induce T cell responses to HIV epitopes in human cells.
- Lentiviral DC vaccination protects BLT-humanized mice against HIV-1 infection.
- Lentiviral DC vaccination protects mice against LCMV infection.
- In HIV-infected patients, this approach may enhance HIV-specific CTL responses against the virus while reversing latency. This would reduce the latent reservoir and suppress viral rebound upon discontinuation of antiretroviral drugs.