Poster #222

Mechanistic Differences in Interactions of HIV-1 and HIV-2 with Dendritic Cells

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BACKGROUND

HIV-2 infection has been restricted predominantly to West Africa; in contrast HIV-1 has spread rapidly and accounts for 95% of all HIV infections globally. Interestingly, HIV-2 infected individuals maintain low viral loads and have lower mortality rates than HIV-1 infected individuals. The mechanistic basis for viral control and slower progression to AIDS in HIV-2 infected individuals remains unclear. We hypothesized that reduced interaction of HIV-2 with CD169, the primary HIV-1 attachment factor on myeloid dendritic cells (DCs) that targets captured virus particles to the trans infection pathway, plays an important role in its restricted pathogenesis.

METHODS

We constructed an HIV-2 proviral plasmid that encodes GFP between MA and CA and flanked by protease cleavage sites (HIV-2-iGFP), such that infectious virions contain GFP, similar to the previously described HIV-1-iGFP proviral clone (*J. Virol. 2007, 81:12596*). To examine interactions of HIV-2 with CD169, THP1 cells constitutively expressing CD169 (THP1/CD169) were pulsed with HIV-1iGFP or HIV-2iGFP virions and virus capture in the presence or absence of αCD169 blocking antibodies was determined by flow cytometry. Intra-cellular localization of HIV-1-iGFP or HIV-2-iGFP within mature DCs was determined by immunofluorescence microscopy. To determine access of HIV-2 to the CD169 mediated trans infection pathway, mature DCs were exposed to GFP-expressing single-cycle of replication competent HIV-2 virus pseudotyped with HIV-2 Env, and then co-cultured with autologous CD4⁺ T cells.

Comparing HIV-1 & HIV-2

Similarities:
- **same modes of transmission**
- **associated with similar opportunistic infections and AIDS**
- **30-60% sequence homology**

Differences: HIV-2 is associated with
- **Lower viral loads**
- **Longer asymptomatic stage**
- **Slower clinical progression to AIDS**
- **Slower decline in CD4 count**
- **Lower mortality**
- **Concentrated in West Africa; the United States has few reported cases.**

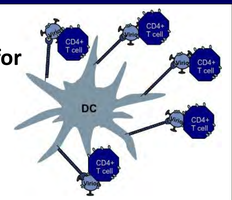
HYPOTHESIS

HIV-2 is less efficient than HIV-1 at exploiting CD169 on dendritic cells for dissemination to CD4+ T cells.

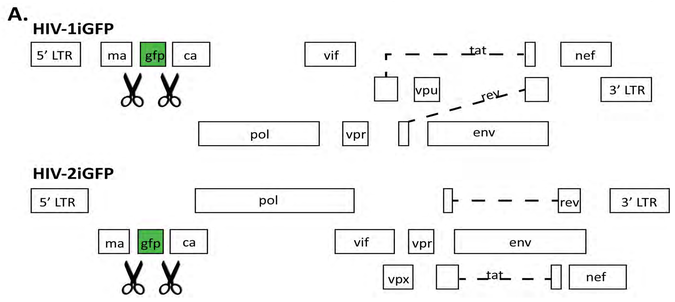
RESULTS

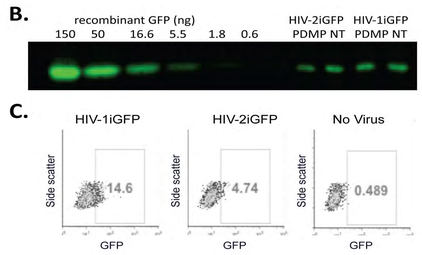
We have previously shown that HIV-1 evades cell-intrinsic restrictions in DCs by exploiting CD169 for enhanced transfer to CD4⁺ T lymphocytes.

(Puryear & Akiyama *et al.*: *PLoS Pathog* 9(4), e1003291 (2013), Izquierdo-Useros N *et al.*: *PLoS Biol* 10(12), e1001448 (2012))



HIV-2 is Captured Less Efficiently by CD169 than HIV-1





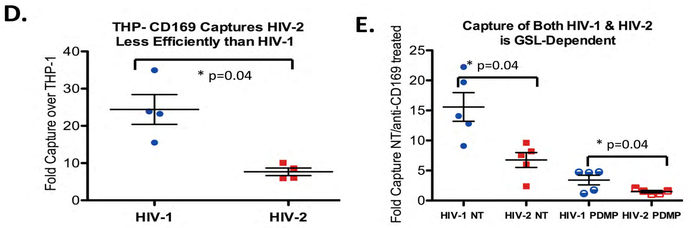
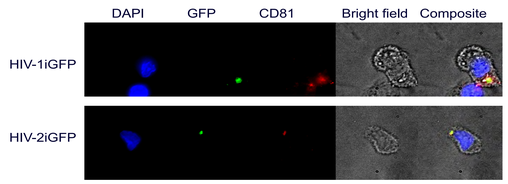


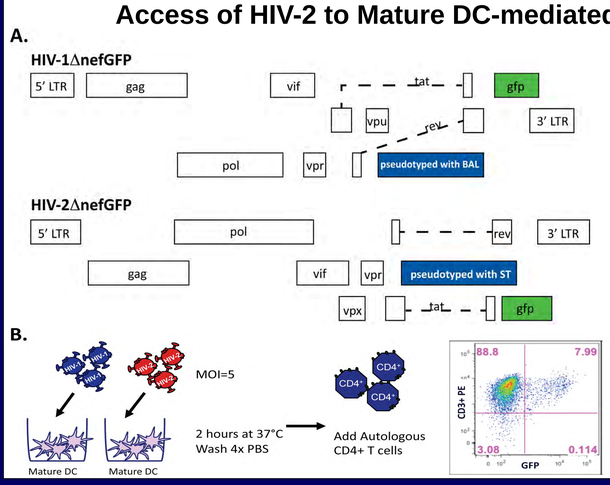
Figure 1. A. A model of the proviral plasmids used in this study, which encode GFP between MA and CA, flanked by protease cleavage sites, such that infectious virus particles contain GFP. **B.** Representative quantitative western blot analysis of sucrose gradient purified-virus particles for measuring GFP content of virions. **C.** Mature DCs Capture HIV-2 less efficiently. Monocyte derived DCs were matured with LPS for 48hrs and challenged with 10ng either HIV-1iGFP or HIV-2iGFP. Representative FACS plots are shown. **D.** THP1 cells constitutively expressing CD169 (THP1/CD169) were pulsed with HIV-1iGFP or HIV-2iGFP virions and virus capture in the presence or absence of αCD169 blocking antibodies was determined by flow cytometry. **E**. PDMP treatment of virus-producer cells decreases the level of GSL incorporated in virions and results in decreased capture of both HIV-1 and HIV-2 by LPS-matured DCs. Data is normalized to anti-CD169 Treated Cells to measure CD169 specific capture. The data shown is the mean (± SE) virus capture by DCs derived from 4 independent donors.

Both HIV-1 and HIV-2 Traffic to a CD81+ Compartment in Mature DCs

Figure 2 Immunofluorescent microscopy shown with Dapi staining the nuclei of mature DCs, either HIV-1iGFP or HIV-2iGFP in green, CD81 (AF594) in red, Brightfield and the merged image. Mature DCs were pulsed with 25ng GFP virus input of either HIV-1iGFP or HIV-2iGFP for 2 hours at 37° C, washed, fixed, permeabilized and stained for CD81.



Access of HIV-2 to Mature DC-mediated trans infection Pathway is Attenuated



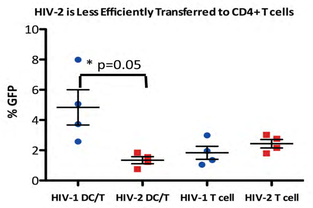


Figure 3. A. HIV-1 and HIV-2 proviral constructs containing GFP in the place of nef. **B**. Mature DCs were infected at an MOI=5 with single cycle viruses encoding GFP (pseudotyped with HIV-1/BaL or HIV-2 ST env). Virus-exposed mDCs were washed and cultured with autologous CD4+ T cells. Additionally, CD4+ T cells were infected directly (cell-free infection) in parallel. %GFP positive CD4+ T cells were quantified via flow cytometry **C.** The data from 4 independent infection with cells from 4 donors is shown (mean ±SE).

SUMMARY

- HIV-1 evades cell-intrinsic restrictions in DCs by exploiting CD169 for enhanced transfer to CD4⁺ T lymphocytes.
- There was a 2-3 fold reduction in capture of HIV-2 compared to HIV-1 by THP1/CD169 cells and mature DCs.
- Both HIV-1 and HIV-2 virions were trafficked to a CD81+ non-lysosomal compartment following capture.
- Though HIV-1 and HIV-2 are equally infectious for CD4⁺ T cells, there was a 7-fold decrease in mature DC-mediated HIV-2 trans infection of CD4⁺ T cells compared to HIV-1
- HIV-2 might interact with additional receptors on mature DCs, which might inhibit access of HIV-2 to the CD169-mediated trans-infection pathway.

CONCLUSIONS

We conclude that a reduced interaction of HIV-2 with CD169 inhibits access of HIV-2 to the DC-mediated trans infection pathway and might result in attenuated dissemination in vivo.

ACKNOWLEDGMENTS

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