Background: The X4 primary pediatric HIV-1 isolate PI-2 was found to be less pathogenic for developing thymocytes than the related primary isolate PI-2.1 and the X4-tropic NL-4.3 and R5-tropic NF-NSX laboratory isolates. HIV regulatory genes were analyzed for mutations and functions to determine the reason for a relatively low pathogenicity. Previously, Nef-mediated CD4 downregulation, but not MHC-I downregulation, has been linked to pathogenicity. We examined the nef gene of PI-2 to assess its role in pathogenicity.

Methods: We compared the nef gene of PI-2 with that of the more pathogenic strains (NL-4.3 and PI-2.1) and assessed its influence on MHC-I and CD4 expression in vitro infected thymocytes by flow cytometry. The PI-2 nef gene was placed in a HIV1 construct lacking env and rev to establish whether or not the lack of MHC-I downregulation was nef-mediated. Pathogenicity was assessed using in vivo infection of thymic implants in SCID-hu mice.

Results: We found previously undescribed mutations in 16 amino acids in nef, including a 7 amino acid deletion (DK72) in the PI-2 nef sequence. Thymocytes productively infected (KC57+) with PI-2 expressed higher levels of MHC-I (HLA-ABC) than the same cells infected with PI-2.1 or NL-4.3, but levels of CD4 expression were similar in all cases. PI-2 infected thymocytes were markedly less depleted of CD4+ cells than thymocytes infected with PI-2.1 or NL-4.3, suggesting that MHC-I downregulation may be related to cytopathicity. Less MHC-I downregulation was also observed in productively infected KC57+ thymocytes after infection with less cytotoxic pediatric isolate PI-2 in vivo thymic implants in SCID-hu mice. Nef, but not Env or Vpu-mediated MHC-I downregulation, was impaired when a VSV-pseudotyped virus containing the mutated nef gene from the less cytotoxic isolate PI-2.1.

Nef-mediated downregulation of MHC-I expression in thymocytes affects the pathogenicity of a CXCR4-tropic HIV-1 pediatric isolate.

Figure 1: Thymocytes infected with the less pathogenic PI-2 show lower CD4 depletion than thymocytes infected with PI-2.1 or NL-4.3. Thymocytes were infected in vitro with CXCR4-tropic pediatric isolates PI-2 (lanes 1 and 2), and the CXCR4-tropic molecular clone NL-4.3 or mock-infected with the supernatant from the same cells (CD4 T cell pool) and cultured in serum-free medium for 14 days with Interleukin 2 (IL-2) plus IL-4 as previously described (1). A. Fourteen days post-infection, thymocytes were stained with antibodies to CD4 and CD8. Apoptotic and dead cells were excluded using 7-Aminoactinomycin-D (7-AAD) and quadrant settings using the isotype controls. B. MFI of HIV-1-infected and PI-2.1 or NL-4.3 infected thymocytes was compared to mock infected PI-2. 2 PI-2.1 and PI-2.1 have similar levels of CD4 downregulation but differ in MHC-I downregulation.

Figure 2: Pediatric isolate 2 (PI-2) is impaired in MHC-I downregulation. Thymocytes were infected in vitro with CXCR4-tropic pediatric isolates (PI-2 and PI-2.1), and the CXCR4-tropic NL-4.3 and CCRTropic NF-NSX molecularly cloned HIV-1 isolates or mock-infected with the supernatant from the same cells (CD4 T cell pool) and cultured in serum-free medium with Interleukin 2 (IL-2) plus IL-4 as previously described (1). Following infection, MHC-I downregulation was measured to assess nef function by using the Mean Fluorescence Intensity (MFI). Geometric Mean) of HLA-A expression in KC57 (HIV-1 Gag) cells. The cells were surface stained with antibodies to HLA-ABC FITC and CD3 combined with intracellular staining for HIV-1 Gag proteins using the KC57 antibody. Isotype controls were used to set the cursors. A. Cells productively infected (KC57+) with CXCR4-tropic NL-4.3 with functional nef had decreased expression of HLA-ABC (MFI=363) compared to uninfected (KC7) cells (MFI=745) or Business. Cells infected with the R5-tropic virus NF-NSX with functional nef had a decreased expression of HLA-ABC (MFI=217) compared to uninfected cells (MFI=551). C. Cells infected with the pediatric isolate PI-2.1 with functional nef had decreased HLA-ABC expression (MFI=220) compared to uninfected cells (MFI=639); D. However, cells infected with the pediatric isolate PI-2.1 containing the nef mutation showed a smaller decrease in HLA-ABC expression (MFI=47) compared to uninfected cells (MFI=573).

Figure 3: PI-2 virus does not downregulate MHC-I in vivo. SCID mice implanted with human fetal thymus/liver (SCID-hu mice) were infected with mock uninfected, NL-4.3, or pediatric isolates PI-2.1 and PI-2. MHC-I downregulation was measured at 3 weeks post infection. The mean fluorescence intensity (MFI) values indicate levels of HLA-ABC on productively infected (KC57+) cells. Cells infected with NL-4.3 (n=5 mice) and PI-2.1 (n=5 mice) showed decreased expression of HLA-ABC compared to cells infected with PI-2 (n=5 mice), which was similar to basal levels of MHC-I expression in mock infection (n=4 mice, not shown).

Figure 4: Nef protein is detectable in PI-2 despite expression of previously undescribed mutations. A. Amino acid sequence comparison of Nef protein from CXCR4-tropic molecular clone NL-4.3 and pediatric isolates PI-2 and PI-2.1. Asterisks indicate common amino acids; hyphens indicate a deletion; colon indicates a similar amino acid substitution; space indicates non-similar substitution.
B and C. Western blot of virions from the CXCR4-tropic molecular clone NL-4.3 (lane 1), the CCRTropic pediatric isolates PI-2 (lane 1), and the CXCR4-tropic PI-2 containing the nef mutation (lane 3) were probed for expression of HIV-1 viral proteins Gag (B) and Nef (C). PI-2 expresses the Nef protein despite the presence of a mutated nef gene.

Figure 5: PI-2 Nef lacks functional MHC-I downregulation in the absence of Vpu, Env, or Vpr. To assess MHC-I downregulation mediated by Nef proteins from pediatric isolates PI-2.1 and PI-2 in the absence of other viral proteins, the nef genes were cloned out of the PI-2.1, PI-2, and NL-4.3 isolates and placed in a NL-4.3 vector lacking vpu, env, and vpr. VSV-G pseudotyped virus including a CD24 tag and one of the nef genes was prepared and used to infect T11 cells. HLA-A2 expression on T1 cells infected with (A) mock control, (B) VSV-G pseudotyped NL-4.3 nef lacking vpu, env, vpr, (C) NL-4.3 nef, (D) VSV-G pseudotyped PI-2.1 nef lacking vpu, env, vpr or (E) VSV-G pseudotyped PI-2.1 nef lacking vpu, env vpr. While the wild-type nef from NL-4.3 and PI-2.1 downregulated HLA-A2, the mutated Nef protein from PI-2.1 did not downregulate HLA-A2. Mean Fluorescence Intensities (MFI) of HLA-A2 in infected (CD24+) cells are shown below each representative plot.

Figure 6: PI-2 Nef lacks functional MHC-I downregulation in the absence of Vpu, Env, or Vpr.