NEF-mediated down-regulation of MHC I expression in thymocytes may affect the pathogenicity of a pediatric isolate of X4 HIV-1

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Background:
An X4-tropic 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 laboratory isolates NL-4-3 and NF-NSX. HIV regulatory genes were analyzed for mutations and function 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 more pathogenic strains (NL4-3 and PI-2.1) and assessed MHC-I and CD4 expression in infected thymocytes by flow cytometry previously transfected. Pathogenicity was assessed using in vivo infection of thy/liv implants in SCID-hu mice. PI-2 nef gene was placed into a HIV-1 genome construct lacking env and vpu to establish that differential modulation of MHC expression by PI-2.1 was nef-mediated.

Results:
Novel mutations affected 16 amino acids in nef, including a 7 amino acid deletion (ΔK7S) in PI-2. KC57+ thymocytes productively infected with PI-2 expressed higher levels of MHC-Class I (HLA-ABC) than the same cells infected with PI-2.1 or NL4-3, but levels of CD4 expression were similar in all cases. PI-2 infected thymocytes were less markedly depleted of CD4+ cells than thymocytes infected with PI-2.1 or NL4-3, suggesting that MHC-Class I downregulation may be related to cytopathicity. MHC-Class I downregulation was also not observed in productively infected (KC57+) thymocytes after infection with less cytopathic pediatric isolate PI-2 in vivo thy/liv implants in SCID-hu mice. Nef, but not Env or Vpu-mediated MHC-Class I downregulation, was impaired when a VSV-pseudotyped virus contained the mutated *nef* gene from the less cytopathic isolate PI-2.

Conclusions:
The less pathogenic pediatric isolate (PI-2) contains novel mutations in *nef.* Our *in vitro* and *in vivo* data with primary isolates suggest that MHC-I downregulation plays a larger role in pathogenicity than observed using highly cytopathic laboratory strains such as NL4-3 The impact of mutations should therefore be investigated in primary isolates rather than in molecularly cloned isolates.