Naturally occurring polymorphisms in HIV-1 Nef impair its functions and decrease viral replication capacity

Vollbrecht T.[1, 2], **Bornfleth L.**[3], **Frohnen P.**[3], **Lewis M. J.**[3]

[1] Department of Medicine, UC San Diego, La Jolla, CA; [2] The Veterans Affairs San Diego Healthcare System, San Diego, CA; [3] Division of Infectious Diseases, David Geffen School of Medicine at UCLA, Los Angeles, CA

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Background

The HIV-1 Nef protein plays a pivotal role in immune modulation during acute infection through several different mechanisms.

- increases the number of potential target cells through cellular activation
- increases the efficiency of viral release and provides protection from ADCC by down-regulation of CD4
- evades cytotoxic T lymphocyte (CTL) killing by down-regulation of MHC class-I

The high error-prone reverse transcriptase results in a high number of mutations in the Nef protein.

We have previously described ten *in vivo* polymorphisms in Nef quasispecies (N52A/S, A84D, Y135F, G140R, S169I, H171N/P/G, V180E) with no previous association with Nef function from plasma samples of nine subjects with chronic, untreated HIV-1 infection that reverted under CTL pressure *in vitro*[1].

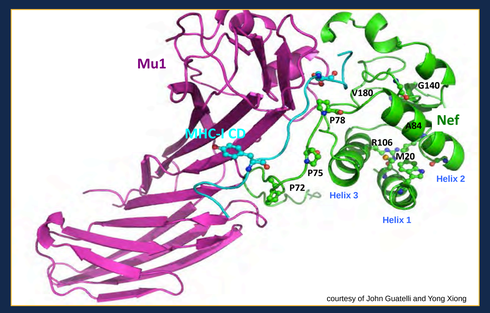


Figure 1: Structure of HIV-1 Nef, MHC-I and AP1. Crystal structure of HIV-1 Nef (green) bound to the cytoplasmic domain of MHC class-I (cyan) and the Mu1 domain of the adaptor protein complex 1 (AP1) (magenta).

Here we investigate the functional characteristics of these polymorphisms in the HIV-1 Nef protein in regards to down-regulation of CD4 and MHC class-I as well as viral replication capacity.

Methods

We introduced ten Nef polymorphisms (N52A/S, A84D, Y135F, G140R, S169I, H171N/P/G and V180E) into a NL4-3 *nef* sequence by site directed mutagenesis. For functional testing, each mutated *nef* sequence was subcloned into a modified replication defective NL4-3 based full genome Δ*env* HSA reporter vector (Figure 2).

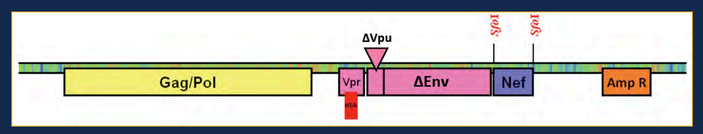


Figure 2: Map of the cloning vector AA1305 highlighting the key features:
1.) the reporter murine CD24 (HSA) is located in the *vpr* reading frame
2.) a deletion in *vpu* eliminates its contribution to CD4 down-regulation
3.) a deletion in *env* eliminates its ability to down-regulate CD4
4.) the unique *SfoI* restriction sites for subcloning of the mutated *nef* alleles

[1] **M. J. Lewis et al.**, Immune Selection In Vitro Reveals Human Immunodeficiency Virus Type 1 Nef Sequence Motifs Important for Its Immune Evasion Function *In Vivo*, J Virol. 2012

HEK 293T cells were transfected with these constructs in combination with a plasmid containing the VSV-G envelope protein. The pseudo-typed virus was collected with the culture supernatant (Figure 3).

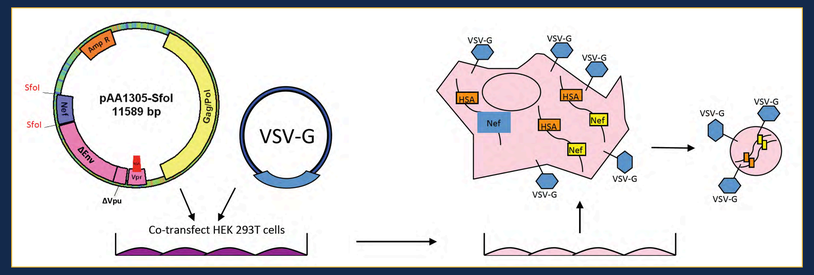


Figure 3: Generation of VSV-G pseudo-typed reporter viruses.

174xCEM.T1 cells were subsequently infected with these VSV-G pseudotyped reporter viruses. Three days after infection T1 cells were stained for the HSA reporter to identify infected cells. Reporter positive cells were further stained for CD4 and HLA-A*02 (representing MHC class-I) expression in order to analyze the impact of the different Nef quasispecies or control alleles on expression of CD4 and HLA-A*02 expression (Figure 4).

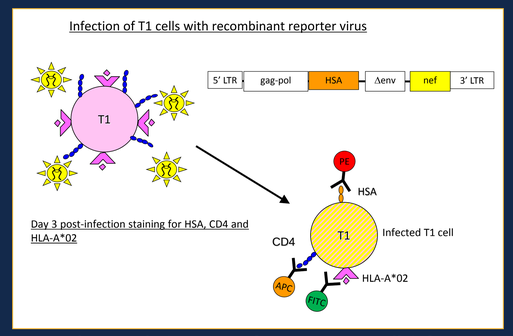


Figure 4: Flow analysis setup of 174xCEM.T1 cell infection with VSV-G pseudo-typed recombinant reporter virus.

Results

Flow analysis of the stained cells revealed the impact of the different Nef alleles on surface expression of CD4 (Figure 5) and MHC class-I (Figure 6), represented by HLA-A*02, on infected T1 cells.

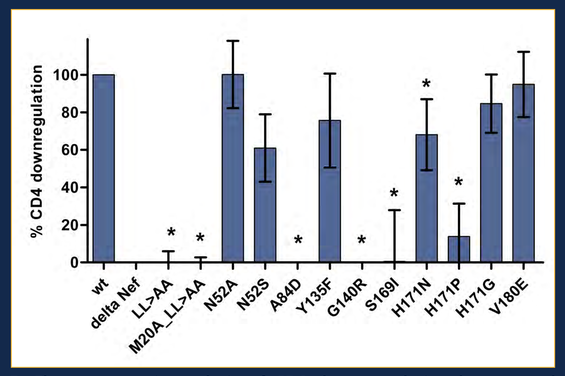


Figure 5: Flow cytometric analysis of CD4 down-regulation by mutated *nef* alleles. Asterisk indicates significance.

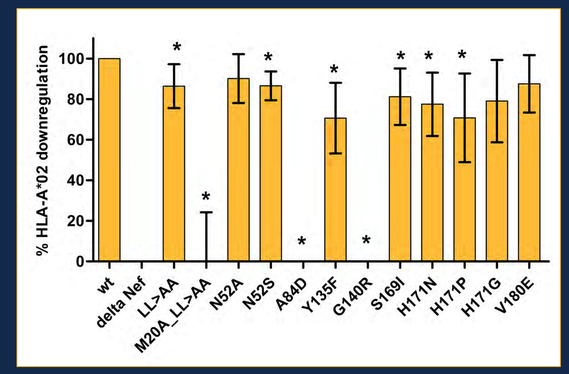


Figure 6: Flow cytometric analysis of HLA-A*02 down-regulation by mutated *nef* alleles. Asterisk indicates significance.

Additionally, we performed viral replication assays in primary cells and found a significant reduction in viral replication capacity for the G140R mutation compared to wild type NL4-3 Nef (Figure 7).

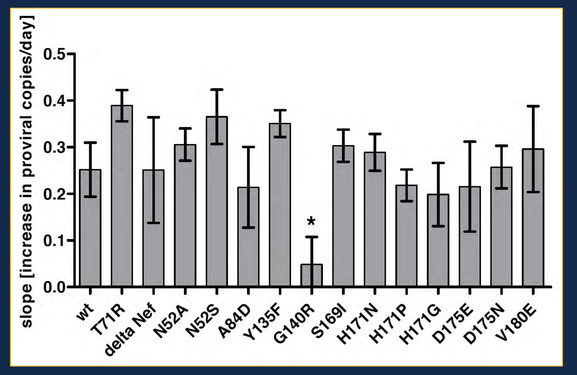


Figure 7: Summary of the viral replication capacity of *nef* mutants in primary cells measured by qPCR. Asterisk indicates significance.

In order to exclude any influence of altered protein expression by these *nef* mutants we analyzed the Protein expression by Western blotting. We found no difference in Nef expression in any of the examined polymorphisms (Figure 8).

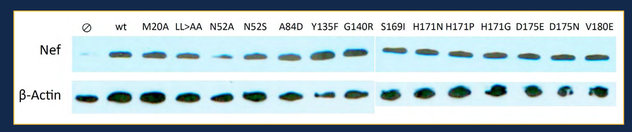


Figure 8: Western blot analysis of Nef expression in the examined polymorphisms.

Conclusions

The polymorphisms A84D and G140R show an inhibitory effect on CD4 and MHC class-I down-regulation by Nef. Additionally, the G140R polymorphism reveals a significant reduction in viral replication capacity in primary cells.

Studying the effects of polymorphisms within circulating Nef quasispecies, such as the G140R, may reveal targets for the design of pharmacologic or immunotherapeutic agents that can disable Nef's capacity for immune evasion.

Contact: Thomas Vollbrecht, Ph.D., Department of Medicine, Stein Clinical Research Building, MC 0679, 9500 Gilman Drive, UC San Diego, La Jolla, CA 92093, Phone: (858) 552 - 7439, Email: tvollbrecht@ucsd.edu

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