No.227 Dynamic range of down-regulation of HIV-1 entry receptors by primary Nef isolates

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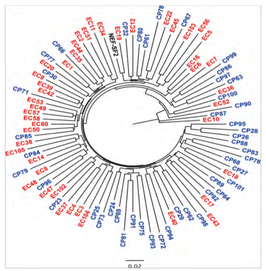
Background

HIV-1 Nef is a key factor for pathogenesis and is known to down-regulate functionally important molecules, including viral entry receptors CD4, CCR5 and CXCR4, from the surface of HIV-infected cells. This activity is thought to prevent superinfection of cells and impair neutralizing antibody responses by preventing the presentation of CD4-induced antigenic surface of viral envelopes.

Although sequence motifs responsible for these functions have been well documented by in vitro mutagenesis studies with limited laboratory strains, it remains elusive whether the same sequence motifs could be applied to primary isolates with various disease status.

To clarify this issue, Nef clones from 45 elite controllers (EC) and 46 chronic progressors (CP) were examined for down-regulation activity of CD4, CCR5, and CXCR4.

Study design



Elite Controller (EC)
45 samples (Untreated)
CD4 count : median 811 (IQR612-1022)
viral load copy/ml: all <50

Chronic Progresser (CP)
46 samples (Untreated)
CD4 count : median 292.5 (IQR72.5-440)
viral load copy/ml: median 90850 (IQR25121-221250)

Phylogenetic tree analysis revealed no specific lineage or clustering.
Genetic analysis show no common difference in Nef clones between EC and CP.

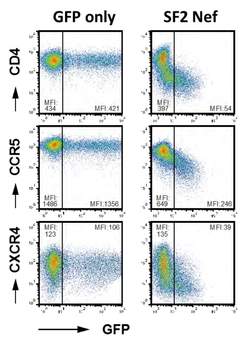
We then went to functional analysis of Nef; Down-regulation of CD4, CCR5 and CXCR4

Strategy-1

Patient-derived nef genes were amplified from plasma HIV RNA and cloned into the pIRES-GFP Vector.

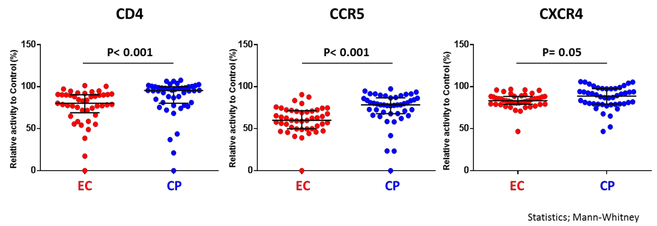
Those plasmids were transfected into TZM-bl cells.

Stained by CD4, CCR5,CXCR4 mAb and analyzed by flow cytometry



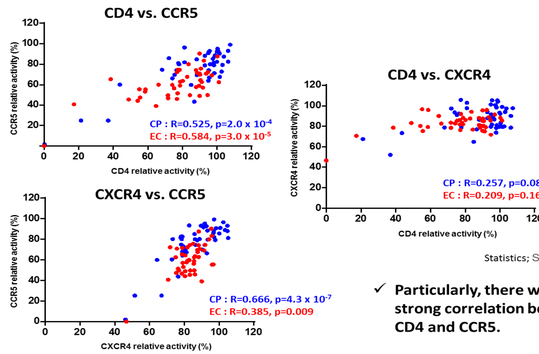
The down-regulation activity was calculated as relative to that of SF2 Nef, which was arbitrarily set 100%

Differential receptor down-regulation activity
Controller vs. Progressor



- ✓ There was a statistically significant difference between EC and CP in CD4 and CCR5.
- ✓ EC Nef showed lower activity in all viral receptor down-regulation activities compared to CP Nef.

Pairwise correlations of Nef-mediated viral receptor down-regulation activities

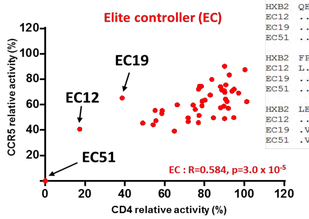


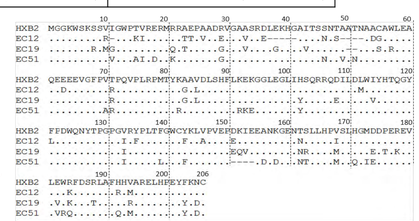
- ✓ Particularly, there was a strong correlation between CD4 and CCR5.

In previous mutational studies…

Responsible motifs of CD4 down-regulation	Responsible motifs of CCR5 down-regulation
Diglutamic acid motif (EE:154-155)	Myristoylation domain (G2)
Dileucine motif (ExxxLL:160-165)	Acidic cluster (EEEE:62-65)
Diaspartic acid motif (E/DD:174-175)	Proline-rich motif (PxxP:72-75)

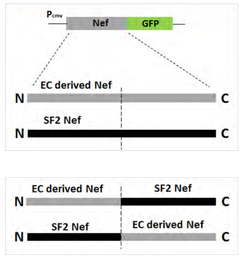
In our cohort…





To further investigate naturally-occurring polymorphisms responsible for Nef-mediated viral receptor down-regulation, we focused on three EC-Nef clones, EC12, EC19, and EC51 because they showed severely impaired CD4 and CCR5 down-regulation activity.

Strategy-2



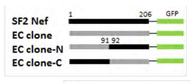
In this study, we used Nef-GFP fusion constructs.

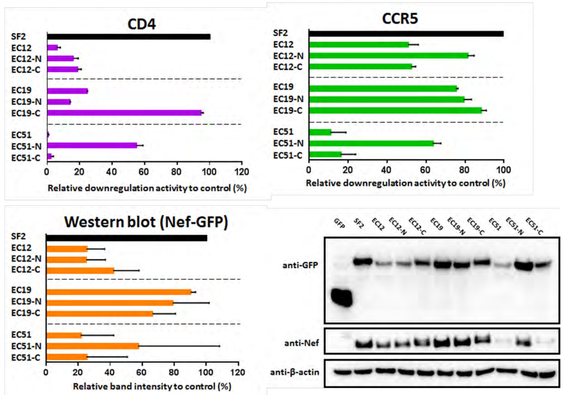
HIV-1 SF2 Nef and EC derived Nef fused to GFP were cloned into pcDNA Vector

We generated the chimeric constructs to identify the regions impaired CD4 and CCR5 down-regulation activity.

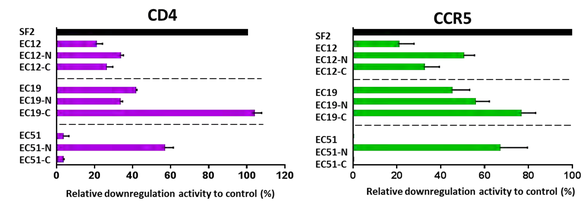
Those plasmids were transfected into TZM-bl cells (or human T cell line CEM.)
Stained by CD4, CCR5 mAb and analyzed by flow cytometry.
GFP and Nef protein level were examined.

Result of TZM-bl



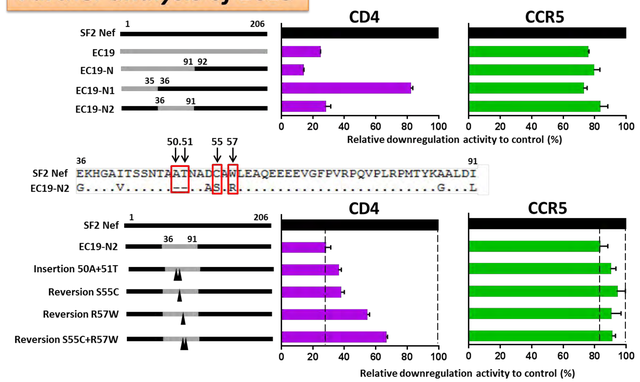


Result of CEM



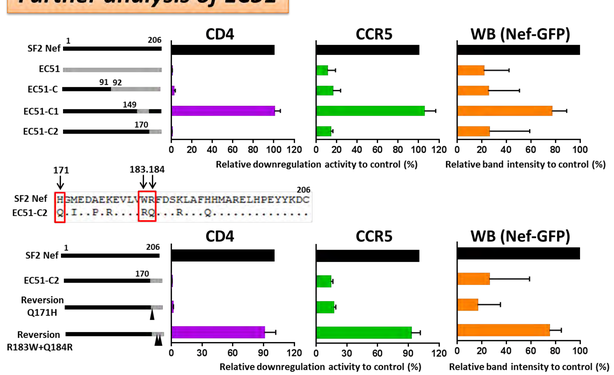
- ✓ In all three EC clones and their chimeric constructs, down-regulation of CD4 and CCR5 in these CEM cells showed data consistent with those obtained with TZM-bl cells

Further analysis of EC19



- ✓ Insertion and S55C restored CD4 down-regulation activity about 10%.
- ✓ R57W had great effect for restoration of Nef mediated CD4 down-regulation activity.

Further analysis of EC51



- ✓ R183W and Q184R restored Nef mediated CD4 and CCR5 down-regulation activity and protein expression level in EC51

Conclusion

- EC Nef showed lower activity in all viral receptor down-regulation activities compared to CP Nef.
- Pairwise correlations of Nef-mediated viral receptor down-regulation activities significantly showed positive relationships between CD4 vs CCR5, and CXCR4 vs CCR5 in both EC and CP cohorts. Particularly, there was a strong correlation between CD4 and CCR5.
- One Nef clone restored the down-regulation activity of CD4, but not CCR5, by the reversion of the rare polymorphism W57R.
- And, reversion of the rare polymorphisms W183R and R184W, with no previously known functional role, restored both CD4 and CCR5 down-regulation activity and protein expression level.

Taken together, modulation of Nef-mediated viral receptor down-regulation was mediated by several different functional motifs in primary isolates and, in some cases, naturally rare mutations in EC played a role in functional attenuation in vivo.