0190 - IMPAIRED NEF'S ABILITY TO COUNTERACT SERINC5 BY IMMUNE-DRIVEN MUTATIONS

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1. Background

The host proteins SERINC 3 and 5 (SERINC3/5) are inhibitors of HIV-1 infectivity that are counteracted by Nef. Introduction of mutations to the highly conserved $FPD_{121-123}$ motif in Nef resulted in disruption of Nef's ability to counteract SERINC5 and enhance infectivity. Because this region encompasses HLA-restricted CTL epitopes, we hypothesized certain naturally arising HLA-driven mutations may impair Nef's ability to SERINC5 and affect patients' disease progression.

2. Correlation between HLA-associated polymorphisms and plasma viral load

HIV-1 chronically infected, treatment naïve individuals in Japan (n=375)

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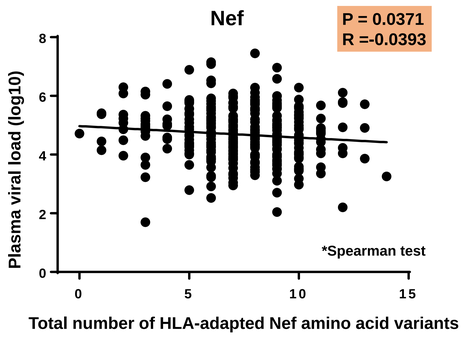
RT-PCR with plasma viral RNA

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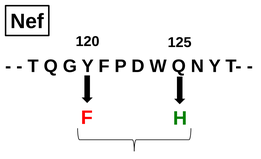
DNA sequences of accessory proteins

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Identify HLA-associated viral polymorphisms | Correlation with plasma viral load

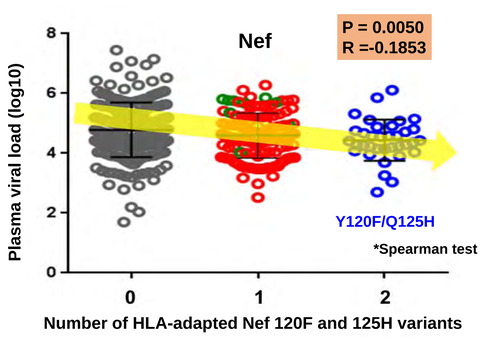


The total number of HLA-associated polymorphisms in Nef inversely correlated with plasma viral load



Overrepresented in HLA-B*51:01 and C*14:03 patients

Number of 120F and 125H in Nef inversely correlated with plasma viral load



3. Functional analysis of Nef

Japanese cohort	No of patients	%
120F only	147	39.2%
125H only	49	13.0%
Both	35	9.33%
Other	144	38.4%
Total	375	100%

Los Alamos (subtypeB)	No of patients	%
120F only	235	15.9%
125H only	83	5.6%
Both	28	1.9%
Other	1189	80.4%
Total	1479	100%

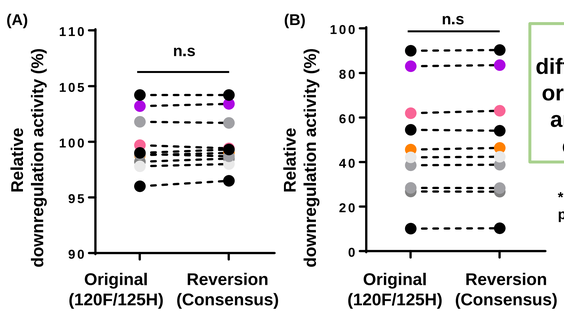
Cloning from patients who has 120F/125H mutations and introduce reversion (N=10)

	120					125				
Consensus (HXB2)	-	-	T	Q	G	Y	F	P	D	W	Q	N	Y	T	-	-
Patient clones	-	-	T	Q	G	F	F	P	D	W	H	N	Y	T	-	-

Test Nef functions

- Downregulation of CD4 and HLA-I
- Enhancement of virion infectivity

4. Downregulation of CD4 and HLA-A2

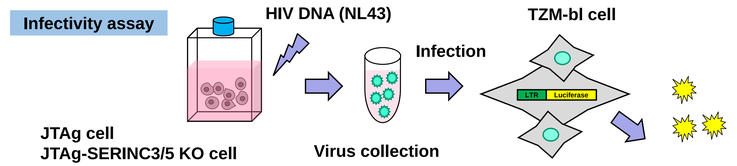


There are no difference between original sequence and reversion in each patients.

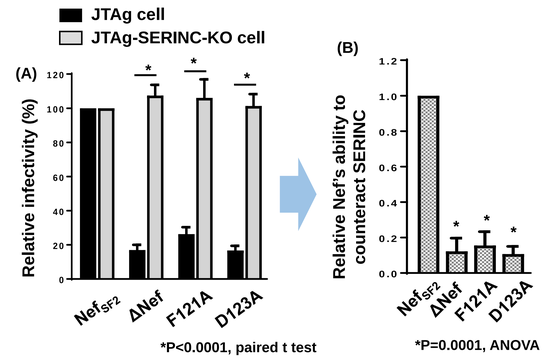
* Wilcoxon matched-pairs signed rank test

5. Effects of SERINC3/5 on virion infectivity

SERINC3/5 — **SERINC3/5 is host-cell restriction factor that suppresses the infectivity of HIV-1 particles, and is counteracted by Nef.**

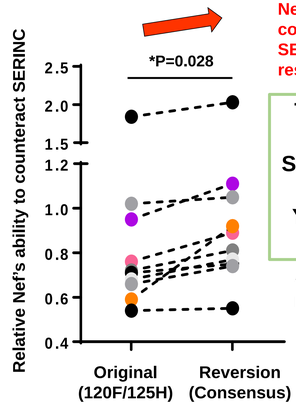


6. Effects of Nef_{SF2} and FPD motif on SERINC3/5



Defective Nef and FPD mutations showed disruption of Nef's ability to counteract SERINC3/5

7. Effects of 120F and 125H mutations on SERINC3/5

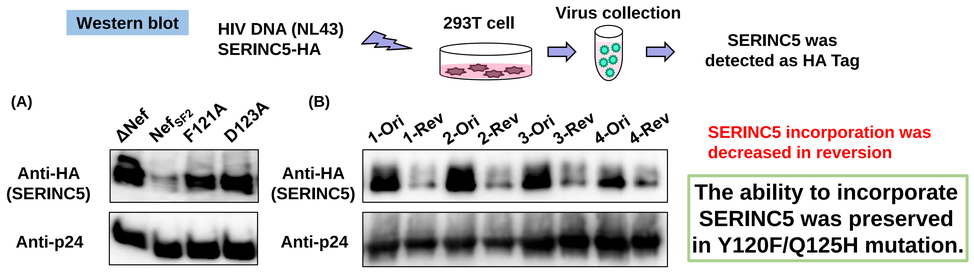


Nef's ability to counteract SERINC3/5 was restored in reversion

The ability to counteract SERINC3/5 was impaired in Y120F/Q125H mutations.

* Wilcoxon matched-pairs signed rank test

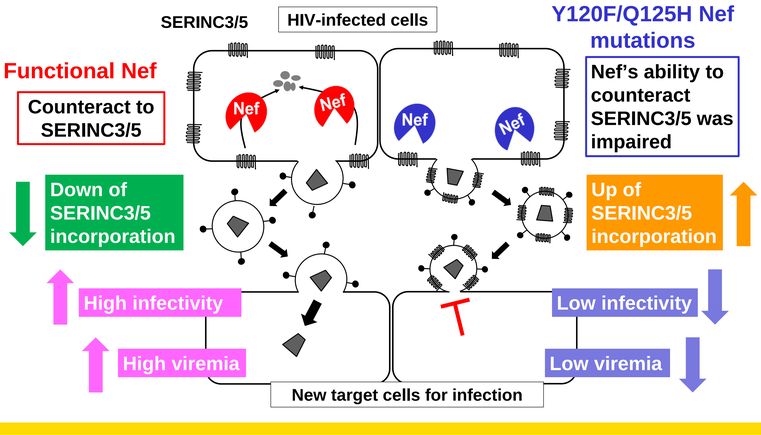
8. Incorporation of SERINC5 into virions



SERINC5 incorporation was decreased in reversion

The ability to incorporate SERINC5 was preserved in Y120F/Q125H mutation.

9. Summary



10. Conclusion

Taken together, certain naturally occurring HLA-associated Nef mutations can impair Nef's ability to counteract SERINC3/5 and are associated with lower plasma viral load in HIV-infected hosts.