

Natural Resistance to HIV-1 by Select APOBEC3H Haplotypes

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1. Abstract

HIV-1 pathogenesis requires neutralization of innate immune defenses. For instance, its viral infectivity factor (Vif) triggers the degradation of several members of the APOBEC3 family of DNA cytosine deaminases. Without Vif, HIV-1 succumbs to lethal mutagenesis inflicted by APOBEC3D, F, G, and H. Overexpression studies have shown that polymorphisms in human APOBEC3H encode proteins that differ widely in their expression levels and degrees of HIV-1 restriction. The distribution of APOBEC3H haplotypes is variable in human populations, but expression and activity of endogenous APOBEC3H protein in primary T cells has heretofore been difficult to measure. A major question is therefore whether the natural variation of endogenous APOBEC3H haplotypes in human populations influences HIV-1 infection in primary cells. Naïve CD4+ T lymphocytes were isolated from 24 donors and genotyped to determine APOBEC3H haplotype. Subsets of these donors with haplotypes that encode putatively stable and unstable APOBEC3H proteins were subjected to immunoblotting to compare endogenous protein levels and to ex vivo spreading infections to monitor susceptibility to HIV-1 infection. These experiments used HIV(IIIB) separation-of-function Vif alleles that were all fully able to counteract APOBEC3F and G, but either unable (F39V) or fully able (N48H, G60E, D61K, A62G, K63E) to degrade APOBEC3H haplotype II. APOBEC3H mRNA levels increased with T cell activation and further with HIV-1 infection regardless of haplotype. In contrast, the protein levels of APOBEC3H haplotype II (which encodes a stable protein) but not haplotype I or III (which encode unstable proteins) increased during the course of the infection. APOBEC3H from individuals heterozygous for haplotype II was efficiently packaged into virus-like particles (VLPs) from Vif F39V mutant virus, to a lesser degree in wild-type HIV, and was absent in VLPs from the Vif N48H, G60E, D61K, A62G, K63E mutant virus. Importantly, packaging of stable APOBEC3H caused a near total restriction of the Vif F39V mutant virus, a delay in replication kinetics of the wild-type virus, and had no effect on the Vif N48H, G60E, D61K, A62G, K63E mutant virus. These data demonstrate that naturally occurring variants of APOBEC3H are expressed stably and capable of encapsidating and restricting HIV-1 replication in primary CD4+ T lymphocytes. Vif V39 exists in 25% of HIV-1 subtype B isolates suggesting a dynamic relationship between the evolution of Vif to antagonize APOBEC3H in populations in which some individuals express an active APOBEC3H and others do not. Future work will determine whether APOBEC3H haplotypes, along with viral variation in Vif, might explain some of the variation in HIV transmission or progression.

2. Introduction

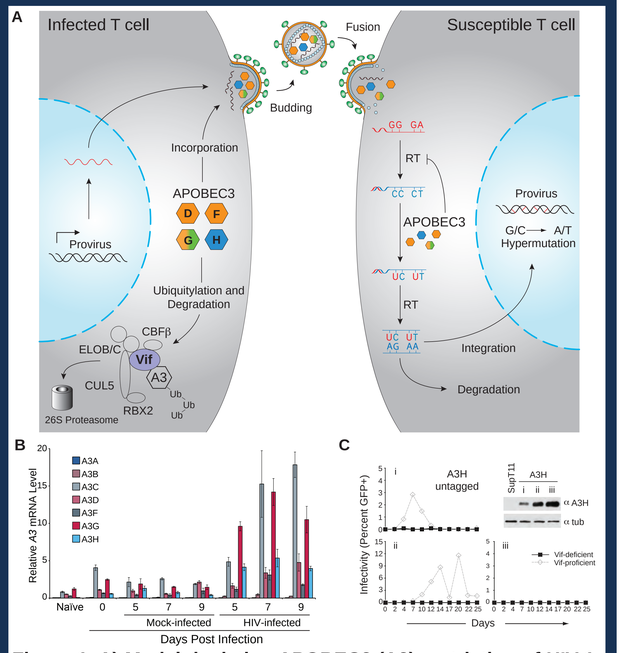


Figure 1. A) Model depicting APOBEC3 (A3) restriction of HIV-1. (Adapted from (1). B) *A3H* mRNA is induced upon stimulation and HIV infection. C) Untagged A3H restricts Vif-deficient HIV during a spreading infection when stably expressed in a T cell line (1).

3. Hypothesis

Stable APOBEC3H haplotypes constitute a natural barrier to HIV-1 infection.

4. APOBEC3H variation

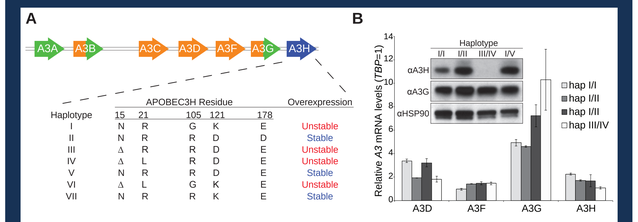


Figure 2. Humans encode seven A3H haplotypes. A) Five polymorphisms in A3H result in stable or unstable protein when overexpressed (2-3). B) *A3H* mRNA is expressed in cells from the indicated donors. Inset, A3H protein expression is dependent on donor haplotype.

5. APOBEC3H-specific viral probes

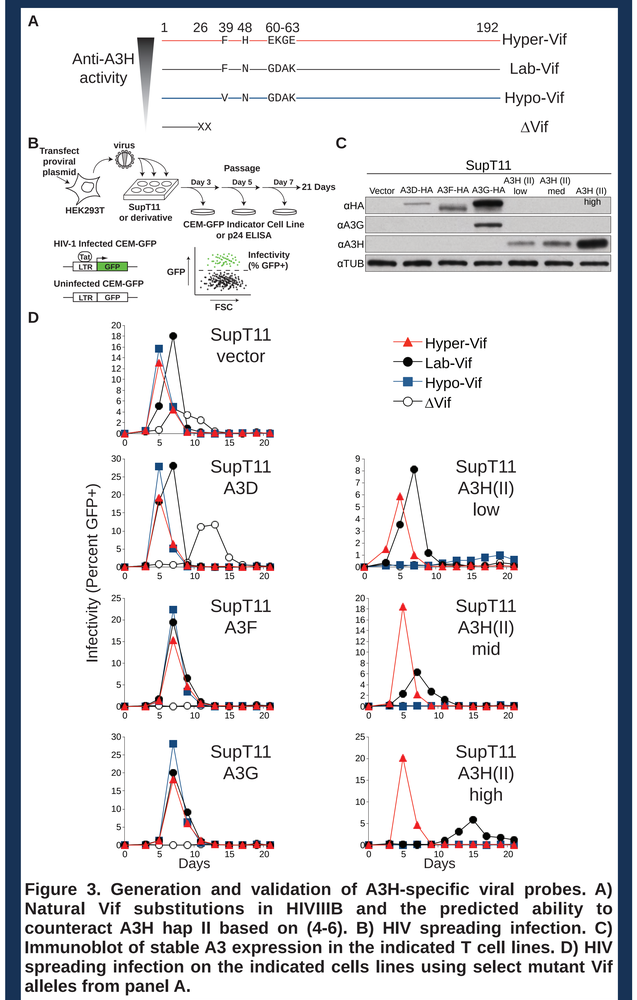


Figure 3. Generation and validation of A3H-specific viral probes. A) Natural Vif substitutions in HIVIIIB and the predicted ability to counteract A3H hap II based on (4-6). B) HIV spreading infection. C) Immunoblot of stable A3 expression in the indicated T cell lines. D) HIV spreading infection on the indicated cells lines using select mutant Vif alleles from panel A.

6. Restriction in primary CD4+ T cells

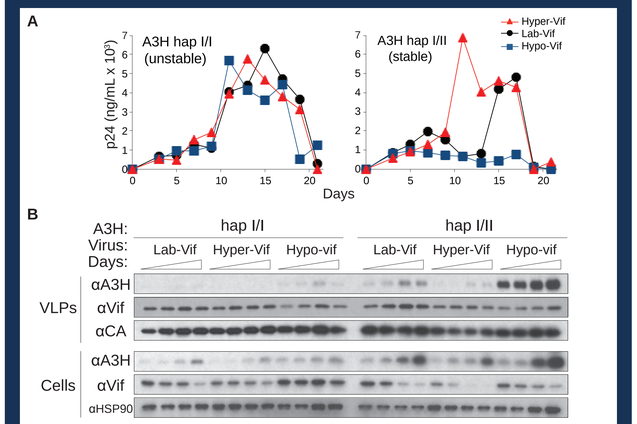


Figure 4. Stable A3H inhibits HIV replication in primary cells. A) HIV replication kinetics of the indicated HIV variants in CD4+ T lymphocytes from healthy donors encoding unstable or stable A3H. B) Immunnoblot of the indicated proteins in viral like particles (VLPs) and cells from days 3, 5, 7, and 9 in the spreading infection in panel A.

7. Hypermutation

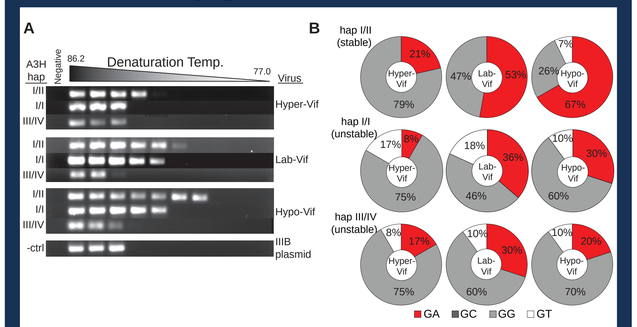


Figure 5. Stable A3H alleles inflict HIV mutagenesis. A) 3D-PCR on a region of *gag/pol* proviral DNA isolated on day 15 of a spreading infection of primary cells. B) Dinucleotide sequence analysis of the *gag/pol* regions of integrated proviruses.

8. Distribution

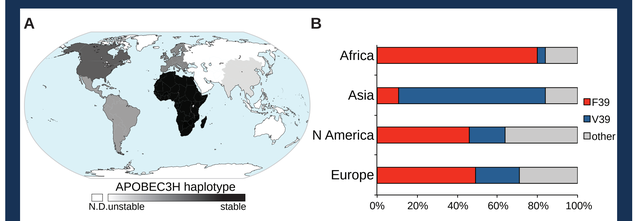


Figure 6. Global distribution of A3H haplotypes and hypo-Vif alleles. A) Estimation of the prevalence of A3H stable alleles based on the 1000 Genomes Project (7). B) HIV sequence data of Vif residue 39 from the Los Alamos National Laboratory (8).

9. APOBEC3H-Vif interaction

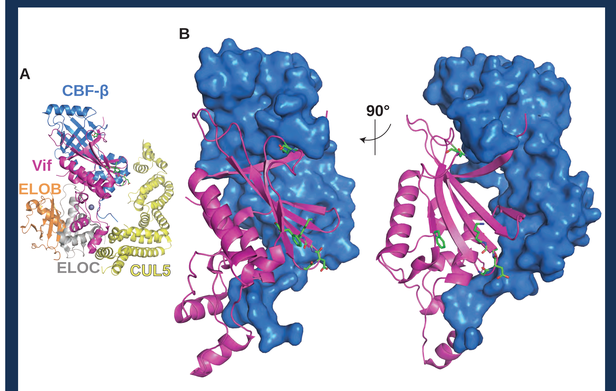


Figure 7. A) Crystal structure of Vif-CBF-β-CUL5-ELOB-ELOC (PDB 4N9F) (9). B) Residues of Vif altered here are highlighted in green.

10. Conclusions

Results from A3H overexpression experiments extend to primary CD4+ T lymphocytes.

V39 confers a high degree of sensitivty to A3H hap II.

Natural H48 E60 K61 G62 E63 Vif residues render it resistant to A3H hapII restriction.

Endogenous A3H is induced upon HIV infection, encapsidates into viral particles, and restricts natural HIV variants.

Stable A3H hypermutates HIV hypo-Vif variants.

Unstable A3H alleles are found in locations more likely to have HIV isolates with hypo-Vif alleles.

Residues affecting A3H neutralization are found on a solvent exposed surface of Vif.

11. Future Directions

Evaluate the viral fitness of the hyper- and hypo-Vif HIV variants.

Investigate how Vif adapts to A3H pressure in long-term cell culture experiments.

Explore whether A3H haplotype could partly explain some of the observed differences in HIV transmission and/or disease progression.

12. Acknowledgements and Selected References

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