



V4 Mutations and Neutralization Escape in SIV

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Abstract

Background: The progression to disease in SIV infection has been associated with the emergence of neutralization-resistant variants with mutations previously mapped to the V4 region. The goal of this study was to address the role of V4 variation on the neutralization of SIV/17E-CL (an attenuated, neutralization-sensitive clone).

Methods: Mutations were introduced into the envelope gene of SIV/17E-CL using overlapping PCR and site directed mutagenesis and mutations were confirmed by sequencing. Mutant viruses were generated by transfection of 293T cells and tested in neutralization assays using a panel of rhesus monoclonal antibodies (MAbs).

Results: Introduction of single potential N-linked glycosylation sites in the V4 region of SIV gp120 resulted in replication competent viruses with no difference in the neutralization compared to the parental SIV/17E-CL virus. In contrast, partial deletion of the SIV V4 loop resulted in a replication competent virus with a marked reduction in neutralization sensitivity to MAbs that recognize discontinuous epitopes in the C-terminal half of SIV gp120 as well as enhanced neutralization by V1/V2 MAbs.

Conclusions: Partial deletion of the V4 region likely caused conformational changes in Env that resulted in disruption of the discontinuous antibody binding site. Further, these results suggest that potential interactions between the V1/V2 and V4 regions of Env in its trimeric form on the virus particle exist, and that these interactions may involve conformational epitopes important for recognition of neutralizing antibodies.

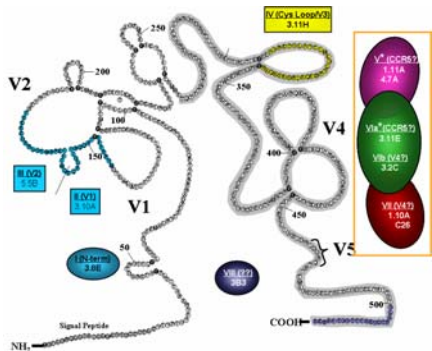


Figure 1: Binding properties of Rhesus Monoclonal Antibodies Generated From Monkeys Infected With Attenuated SIV/17E-CL. The locations of the epitope clusters defined by nine groups of rhesus MAbs are shown superimposed on a two-dimensional model of SIV/17E-CL gp120. All rhesus MAbs were screened for reactivity to synthetic, overlapping 20mer peptides by ELISA and the linear epitopes (listed in rectangles) recognized by six MAbs are shaded. The putative binding domains of the remaining MAbs that recognize conformational epitopes (listed in ovals) are placed in the regions of gp120 thought to involve antibody binding. N-term, amino-terminus. (Cole et al. *Virology* 290:59, 2001).

Methods

SIV/17-CL genome

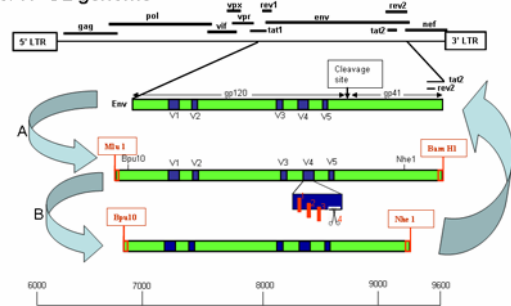


Figure 2. Construction of mutant viruses. (A) The SIV/17E-CL Env gene was PCR amplified using primers with 5' MluI and 3' BamHI restriction sites and cloned into a plasmid vector. (B) Using site directed mutagenesis and overlapping PCR the following mutations were introduced into the V4 region: 1, CN89 contains the N415S mutation; 2, CN92 contains the A417T mutation; 3, CN95 contains a chimeric envelope in which part of the SIV V4 has been replaced with the homologous region of HIV-1_{SF162}; 4, CN103 contains a deletion of amino acids 421-425; (C) Env was digested with unique cutters, Bpu10 and Nhe1, and cloned back into the SIV/17E-CL provirus backbone. (D) Amino acid sequence of SIV/17E-CL wild type and mutant Env V4 regions; (*) indicate amino acid homology; (-) indicates a deletion; amino acids highlighted in red indicate potential N-linked glycan sites.

Results

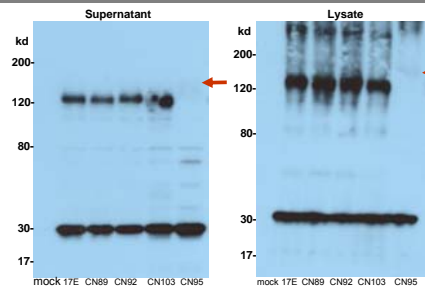


Figure 3. Expression of viral proteins in transiently transfected 293T cells. 293T cells were transfected with 3 µg proviral DNA in a 6-well plate; supernatants and lysates were harvested at 48hrs. 3% of supernatant and 8% of lysate was analyzed by SDS-PAGE / Western blot and probed with SIV/mac251 polyclonal serum at 1:1000. All the recombinant virus constructs expressed gag protein (p27). WT 17E-CL, CN89, CN92 and CN103 all expressed similar amounts of envelope while CN95 had little detectable envelope (indicated by the red arrows).

Competition Group*	MAb	17E-CL	CN89	CN92	CN103
I	3.8E	>25	>25	>25	>25
II (V1)	3.10A	3.000	1.875	3.515	0.114
III (V2)	5.5B	0.154	0.114	0.088	0.016
IV (V3)	3.11H	0.016	0.030	0.015	0.003
V (CCR5?)	1.11A	0.016	0.028	0.028	0.003
V (CCR5?)	4.7A	0.007	0.005	0.007	0.001
VIA (CCR5?)	3.11E	0.005	0.006	0.005	0.002
Vib (V4?)	3.2C	0.018	0.012	0.009	>5
Vib (V4?)	E31	0.009	0.023	0.013	>5
VII (V4?)	1.10A	0.003	0.002	0.002	>5
VII (V4?)	C26	0.002	0.001	0.002	>5
VIII	3B3	>25	>25	>25	>25

Figure 4. Neutralization assay results for SIV/17-CL and mutants viruses. The concentration of MAb (µg/ml) required to achieve 50% neutralization of viral infectivity (IC₅₀) is shown for each virus; values represent the mean of six independent experiments. CN89=N415S, CN92=A417T, CN103=Δ421-425 mutants. CN95 did not make infectious virus and therefore was not tested. Green numbers represent enhanced neutralization sensitivity compared to SIV/17E-CL; red numbers indicate loss of neutralization by MAb (i.e., neutralization similar to SIV/mac239). *Variable loop corresponding to epitope is indicated in brackets, see figure 1.

Summary

Point mutations N415S and A417T (previously associated with disease progression in SIV) did not affect the neutralization profile of SIV/17E-CL by a panel of Rhesus MAbs

Deletion of amino acids 421-425 resulted in the abrogation of neutralization by Rhesus MAbs in groups V1b and V1c but not groups V or V1a. Further enhancement of neutralization by MAbs that recognized linear epitopes in the V1/V2 region was also observed.

Specific replacement of SIV/17E-CL amino acids 405-417 with the homologous region of HIV-1 SF162 resulted in the abrogation of normal Env expression.

Implications

Deletions in V4 resulted in abrogation of neutralization, either by removal of the MAb binding site or by altering the conformation of the envelope and disrupting conformational MAb epitope. Elucidation of the residues involved in conformational MAb binding will provide insight into the structure of Env and will identify important neutralizing epitopes of SIV/17E-CL.

Neutralization results with the deletion mutant suggests potential interactions between V1/V2 and V4 regions of Env in its trimeric form, and that these interactions may involve conformational epitopes important for recognition of neutralizing antibodies.

Understanding the effect of Env mutations that lead to immune escape on Ab-virus interactions will be important in the design and evaluation of potential vaccine candidates.

Acknowledgements

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