Generation and characterization of neutralizing anti-V3 scFvs against HIV-1 clade C

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Background:
Majority of the HIV-1 viruses infecting Indian patients and more than 50% of the infections worldwide belong to clade C. An effective vaccine against HIV-1 should elicit bNAb responses against a series of diverse HIV-1 strains. The third variable region (V3) of HIV-1 is highly conserved and allows HIV-1 binding to host cells via the HIV-1 coreceptors. In this study we have successfully generated two neutralizing anti-V3 scFvs.

Methods:
An antigen specific scFv phage library was constructed from the enriched V3- positive antibody secreting EBV transformed cells of a drug naive HIV-1 clade C infected Indian patient whose plasma exhibited high neutralizing potential against a panel of viruses and also displayed cross-reactive anti-V3 antibodies. Two anti-V3 scFvs were selected after biopanning and checked for their antigen binding specificity in ELISA. The scFvs were checked for soluble expression and purified using metal chelate chromatography. The purified scFvs were checked for their neutralization potential in TZM-bl based assay. Preferential antibody gene usage of these scFv were determined by DNA sequencing

Results:
We generated two anti-V3 scFvs, 1E7B and F2C from an antigen specific phage library. The anti-V3 scFvs were expressed (32 kD) and confirmed by SDS-PAGE and Western blot. The soluble scFvs were highly specific to their antigens and did not show any reactivity against other unrelated peptides. The scFvs showed varying degrees of neutralization against 5/5 tier 1 and 7/12 tier 2 viruses. The two scFvs showed cross neutralizing activity against clade A, B and C viruses. The gene usage of scFv 1E7B and F2C was determined to be IGHV4-31*03 and IGHV4-31*02 genes in heavy chain and IGKV3-20*01 and IGKV2-28*01 in light chain respectively.

Conclusion:
Our study suggests that the anti-V3 scFv generated from clade C infected Indian patient display varying degrees of neutralization potential against tier 1 and 2 viruses. Further defining the epitope specificities of these anti-V3 scFvs will be helpful in identification of epitopes, unique to clade C or shared with non-clade C viruses, for immunogen design and will serve as a prerequisite for designing a polyvalent vaccine against a broad spectrum of HIV-1.