Envelope C2-V3-C3-specific antibodies correlate with neutralization activity in plasma from HIV-1 infected patients from Angola

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Background:
Development of immunogens that induce broadly neutralizing antibodies (bNAbs) is a major goal in HIV-1 vaccine field. Recently, we found that bNAbs can be elicited in Balb/c mice against HIV-2 by using a prime-boost vaccination strategy combining recombinant Vaccinia virus expressing a truncated form of the SU glycoprotein and a polypeptide comprising the C2, V3 and C3 envelope regions. We went on to test the hypothesis that a similar vaccination strategy can also be effective for HIV-1. We also want to test the hypothesis that envelope glycoproteins derived from ancestral HIV-1 isolates from Angola may induce a broader neutralizing antibody response compared to envelope glycoproteins derived from contemporary isolates.

Aims:
Produce new envelope glycoproteins truncated gp120 and C2-V3-C3 region from ancestral HIV-1 isolates circulating in Angola and characterize their antigenic structure.

Methods:
- Viral genomic RNA was extracted from the plasma of HIV-1 infected patients from Angola and the env gene was amplified using a nested RT-PCR method, sequenced and genotyped by phylogenetic analysis.
- gp120 Fragments (1400bp) lacking 78 bases at the carboxyl terminus of the C5 region were amplified and cloned into the Vaccinia virus insertion vector pMKS01. To obtain recombinant vaccinia virus expressing the glycoprotein gp120 plasmids were transfected by the calcium-orthophosphate method and cells were simultaneously infected with Vaccinia Virus WR.
- C2-V3-C3 coding region from HIV-1 gp120 was amplified by PCR and cloned into a bacterial expression vector. Expression, purification and quantification of the recombinant polypeptides were performed.
- Antigenic reactivity of the purified polypeptides and gp120 was analysed by Western blotting and quantified in an ELISA assay. Neutralizing activity was quantified using a single round infectivity assay with TZM-bl cells.

Results:
- Full-length env genes were amplified, cloned and sequenced from HIV-1 subtypes B, C, G, H, J (n=2) and CRF02_AG. Full-length genomic sequence was obtained for the H virus.
- Recombinant Vaccinia virus expressing the surface glycoprotein from subtypes B, C, CRF02_AG, J and H were produced. Recombinant polypeptides comprising the C2-V3-C3 regions from the same isolates were also produced.

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Conclusions:
- We have produced an extensive new set of recombinant vaccinia viruses expressing the envelope surface glycoproteins from ancestral HIV-1 isolates from Angola, recombinant polypeptides comprising the C2, V3 and C3 envelope regions were also produced.
- The antigenic structure of the new antigens is preserved as determined in ELISA and WB assays.
- The positive correlation between binding antibodies to C2-V3-C3 region and antibody neutralization suggests that the C2-V3-C3 region comprises a conserved NAb epitope.
- This new set of expression constructs and proteins will be used to immunize Balb/c mice to determine whether they can lead to the production of potent neutralizing antibodies against different subtypes of HIV-1.