

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 want 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 contemporaneous isolates.

Aims:

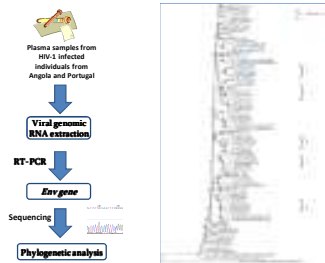
Produce new envelope glycoproteins_ truncated gp120 and C2-V3-C3 region_ from ancestral HIV-1 isolates circulating in Angola and characterize their antigenic structure.

Methods:

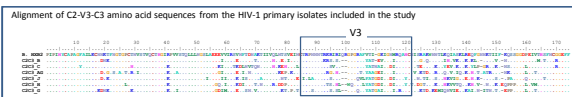
- ✓ Viral genomic RNA was extracted from the plasma of HIV-1 infected patients from Angola and the *env* gene was amplified using a nested RT-PCR method, sequenced and genotyped by phylogenetic analysis.
- ✓ gp120 fragments (1400bp) lacking 78 bases at the carboxyl terminus of the C5 region were amplified and cloned into the Vaccinia virus insertion vector pMJ601. 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 and quantified in an ELISA assay. Neutralizing activity was quantified using a single round infectivity assay with TZM bl cells.

Results:

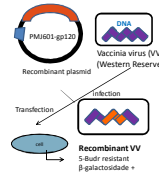
Full-length *env* genes were amplified, cloned and sequenced from HIV-1 subtypes B, C, G, H, J (n=2) and CRF02_AG. Full-length genomic sequence was obtained for the H virus.



HIV-1 env	XB2	SIAM01C28	SIAM01C31	SIAM01C35	RIPTC1N	SIAG04P	SIPT28A3
SIAM01C28	0.197						
SIAM01C31	0.205	0.205					
SIAM01C35	0.192	0.192	0.110				
RIPTC1N	0.211	0.209	0.221	0.213			
SIAG04P	0.202	0.202	0.226	0.226	0.215		
SIPT28A3	0.183	0.205	0.210	0.204	0.224	0.227	
XB2	0.217	0.185	0.221	0.193	0.197	0.231	0.214



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.



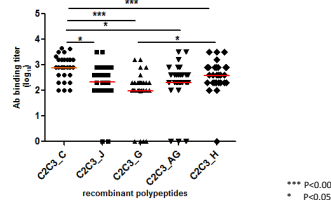
Antibodies present in plasma samples from HIV-1 infected patients from Angola react in Western blot analysis with gp120 expressed in Vaccinia virus (A) and with C2-V3-C3 polypeptides (B)



The majority of HIV-1 plasma samples from HIV-1 infected patients from Angola reacts with the C2-V3-C3 recombinant polypeptides in an ELISA assay

	C2C3-C	C2C3-AG	C2C3-J	C2C3-G	C2C3-H
Ab titer, mean (log ₁₀)	2.884	2.578	2.509	2.313	2.669
N (%)	28 (100%)	25 (89%)	26 (93%)	24 (86%)	27 (96%)

- Higher titer of binding antibodies against subtype C
- Lower titer of binding antibodies against subtype G



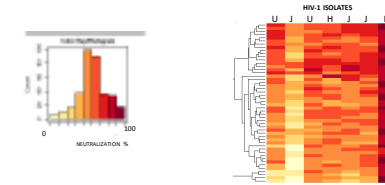
Acknowledgements:

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All plasma samples from Angolan HIV-1 infected patients neutralized the subtype B reference isolate HIV-1 SG3.1 at a 1:100 dilution; neutralization of primary isolates from Angola was observed in only 3.6 to 7.1% plasma samples.

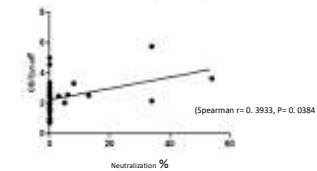
HIV-1 primary isolates from Angola (env subtype)	%plasma samples showing >50% virus neutralization
U	3.6
J	0.0
U	7.1
H	3.6
J	3.6
J	7.1
B (SG3.1)	100

The majority of the plasma samples neutralized >90% the reference isolate HIV-1SG3.1

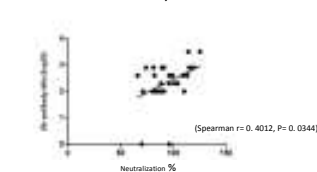


A positive correlation was observed between percent viral neutralization and C2-V3-C3 binding antibody response for different subtypes (cross reactivity)

Correlation between % neutralization of HIV-1 subtype H and C2V3C3-subtype C binding antibody reactivity



Correlation between % neutralization of HIV-1 SG3.1 and C2V3C3-subtype J binding antibody titer



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.