Induction of high titers HIV-1 neutralizing antibodies against Tier 1 and Tier 2 viruses using a DNA prime heterologous protein trimer boost strategy in rabbits

Emma Bowles[1]*, Hannes Uchtenhagen[2]*, Leo Heyndrickx[3], Celia LaBranche[4], Marianne Jansson[6], Adnane Achour[2], Anders Fomsgaard[5], David Montefiori[4], Guillaume Stewart-Jones[1]*, Anna-Lena Spetz[2]*

[1]Human Immunology Unit, Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Oxford University, [2]Center for Infectious Medicine, Karolinska Institutet, Stockholm [3]Institute of Tropical Medicine, Antwerpen, [4]Laboratory for AIDS Vaccine Research & Development, Duke University, [5]Statens Serum Institute, Copenhagen, [6]Department of Laboratory Medicine, Lund University, Lund.

Summary

During the last years, we have gained considerable increased knowledge about the binding sites for antibodies capable of neutralizing a broad range of HIV-1 strains. However, there is still a gap in our knowledge/understanding on how to focus the B-cell responses to these vulnerable conserved sites within HIV-1 Env. One immunological problem is that the variable parts of the virus are more immunogenic than the conserved. Here we describe an immunization regimen that was set up with the goal to teach the immune system to recognize subdominant conserved elements. We used a mixture of three different *env* from clade B during a DNA priming phase and boosted with gp140 trimeric Env from SIVmac239 based on the hypothesis that an Env protein from a virus infecting non-human primates, which still binds human CD4 and utilizes CCR5 for entry, would increase the possibility for expansion of antibody responses towards structurally conserved critical sites. We show induction of high titers heterologous neutralization to tier 1 viruses in the TZMBl assay and against tier 2 viruses in the A3R5.7 assay. Peptide inhibition demonstrated, in the majority of animals, that neutralization against the SF162 virus was significantly competed with a peptide against the V3 crown containing a highly conserved region. These results provide a proof of concept for a HIV-1 DNA prime -SIV protein boost principle, to target conserved elements of HIV-1 Env that can be exploited further for vaccine design.

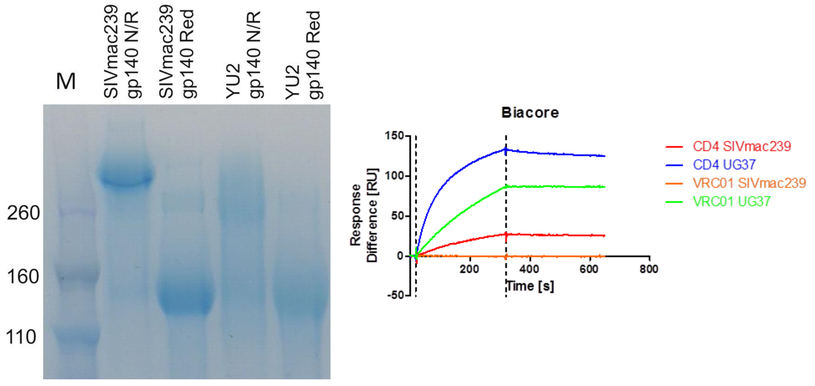

Figure 1: Reducing and non-reducing SDSPAGE analysis of SIVmac239 and HIV-1 (YU2) gp140s, showing the disulphide bridging between the three gp140 chains (left). Biacore analysis (right) of the binding of soluble human CD4 and VRC01 mAb to SIVmac239 gp140 and UG37 gp140. Demonstrating measurable and specific binding of the SIVmac239 gp140 trimer to human CD4.

Conclusions

Induction of **heterologus neutralizing** antibodies to;

•Tier 1: SF162, MN.3 (mean 2818, median 705, **range 174-20000**) in TZMBl assay

•Tier 2: SC22, RHPA (mean 511, median 328, **range 142-2200**) in A3R5.7 assay

•Cross-clade against C and AE

after immunization with a DNA-HIV **clade B *env* 3 mix** followed by **SIVmac239** gp140 **trimer** in rabbits.

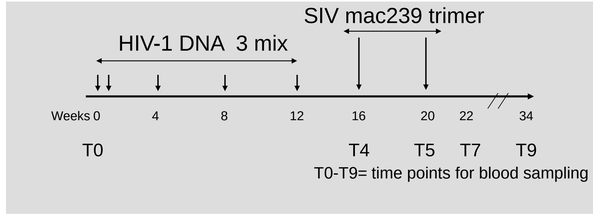

Figure 2: A schematic representation of the immunization regimen.

Arrows depict an immunization. Sera were collected before immunization (**T0**). All animals (New Zeeland white rabbits) received a mix of three clade B *env*-DNA plasmids at weeks 0 (twice first week),4,8 and 12 (200mg DNA by electroporation i.d. (mix of three clade B env-DNA gp140). Sera were collected 2 weeks after last DNA immunization (**T4**). All animals then received a heterologous protein trimer SIVmac239 by i.d. injection (no adjuvant) and sera were collected 4 weeks after first protein boost (50μg) (**T5**). Animals received a second protein boost with SIVmac239 gp140 trimer 8 weeks after the first protein immunization and sera were collected 2 wks after second protein boost (50μg) (**T7**). Three months later sera were analysed for memory responses (**T9**).

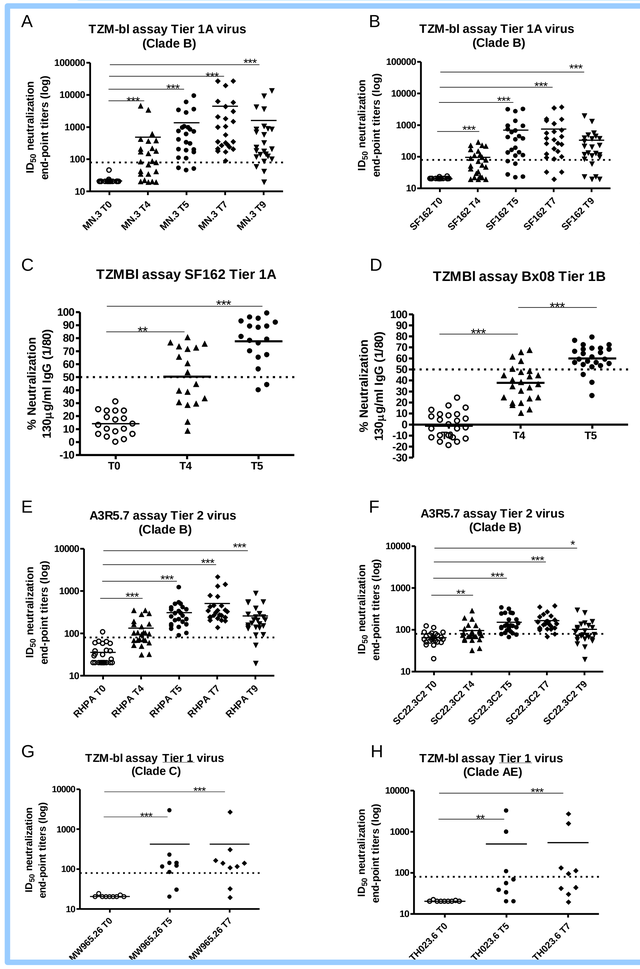

Figure 3: Heterologous neutralization against tier 1 and tier 2 viruses.

Data are shown for individual animals after intradermal application without using a specific adjuvant according to schedule depicted above. High titers ID50 neutralizing activities were detected in a TZMBl assay against tier 1 MN.3 (A) and SF162 (B). IgG were purified by a protein G column and tested in a TZMBl assay against SF162 and BX08 (C and D). Percent neutralization is shown using 130mg/ml IgG approximately corresponding to a 1/80 serum dilution. Endpoint neutralization titers (ID50) in sera were tested in A3R5.7 assay against tier 2 viruses clade B RHPA (E) and SC22.3C2 (F) and in TZMBl assay against clade C (MW965.26) (G) and clade AE (TH023.6) (H) viruses. Significant values are indicated by *** p<0.001, **p<0.01 and *p<0.05 using paired t-test. Dotted line shows an arbitrary 1/80 serum dilution (A,B,E-F= or %50 cut-off of neutralization (C,D).

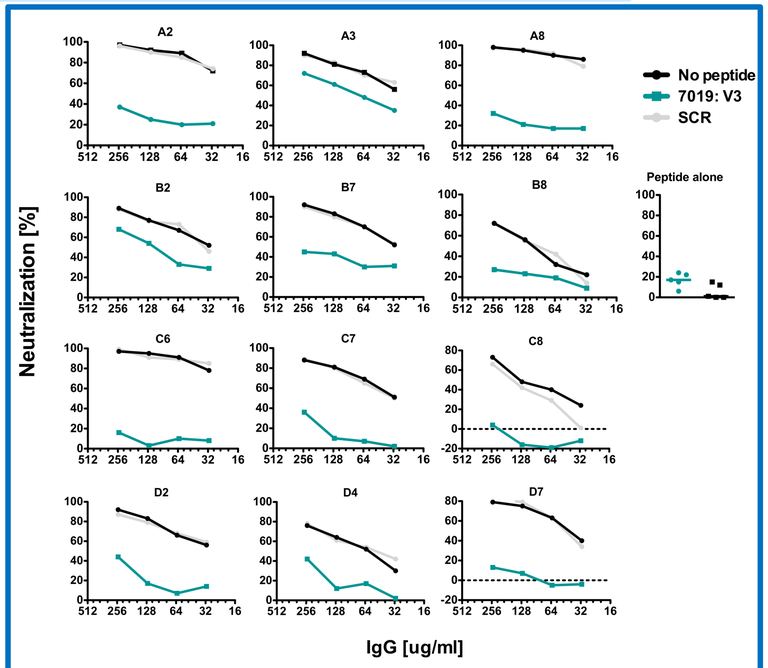

Figure 4. Neutralization against the SF162 virus is targeted to the V3 loop in the majority of animals.

(A) IgGs from individual animals (indicated on each figure) were assessed for their capacity to neutralize SF162 in the TZMBl assay in the absence (**no peptide**) or following incubation with a peptide against the V3 region (**7019 V3**: a cyclic peptide spanning the entire V3) or a scrambled control peptide (**SCR**) at 16 μg/ml. The IgG were purified from sera obtained at the T7 time point two weeks after the second protein boost. On-going characterization further indicates a focus of the neutralization capacity on the GPGR-tip of the V3.

Karolinska Institutet
Anna-Lena Spetz
Center for Infectious Medicine (CIM)
Karolinska University Hospital Huddinge F59
S-141 86 Stockholm Sweden

anna-lena.spetz@.ki.se
+46 8 58582272
www.ki.se/cim

Karolinska Institutet