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

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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 TZMBI 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.

Conclusions
Induction of heterologous neutralizing antibodies to;
•Tier 1: SF162, MN.3 (mean 2818, median 705, range 174-20000) in TZMBI 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 1: Reducing and non-reducing SDS-PAGE analysis of SIVmac239 and HIV-1 (YU2) gp140s, showing the disulfide bridge between the three gp140 chains (left). Blaocore analysis (right) of the binding of soluble human CD4 and VRC01 mAb to SIVmac239 gp140 and LK37 gp140. Demonstrating measurable and specific binding of the SIVmac239 gp140 trimer to human CD4.

Figure 2: A schematic representation of the immunization regimen. Arrows depict an immunization. Sera were collected before immunization (T0). All animals (New Zealand 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 plg40)). 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 (50ug) (T5). Animals received a second protein boost with SIVmac239 gp140 trimer 8 weeks after the first protein immunization and sera were collected 2 weeks after second protein boost (50ug) (T7). Three months later sera was 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 TZMBI assay against tier 1 MN.3 (A) and SF162 (B). IgG were purified by a protein G column and tested in a TZMBI assay against SF162 and BX08 (C and D). Percent neutralization is shown using身旁omg/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 virus clades B RHPA (E) and SC22.32C (F) and in TZMBI assay against clade C (MW9505.26) (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 >1% 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) IgG from individual animals (indicated on each figure) were assessed for their capacity to neutralize SF162 in the TZMBI assay in the absence (no peptide) or following incubation with a peptide against the V3 region (7919 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.

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