Immunofocusing to HIV’s V2 Loop C ß-Strand

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Background: The RV144 trial is the only HIV vaccine clinical trial to show evidence that a vaccine can prevent HIV infection. We aligned the V2 reagents with odds ratios (OR) lower than 0.5 reported in the RV144 immune correlates study, and we determined that at least some protective antibodies (Abs) from the RV144 trial target the peptide segment from positions 165–181 of the V2 loop (V2 165–181), which is centered on the V1V2 domain’s C ß-strand. Thus, vaccine immunogens that exclusively present V2 165–181 may preferentially elicit Abs that protect against HIV infection.

Methods: We fused a V2 165–181 peptide to a non-HIV scaffold in a manner that recapitulates the conformation of this segment in the native folded V1V2 domain. We tested this designed immunogen by immunization in rabbits using a DNA prime-protein boost approach.

Results: We found that IgG from the immunized rabbits’ sera bound a cyclic V2 peptide with high endpoint titers and neutralized Tier 1 HIV viruses from subtypes B, C and AG.

Conclusion: Our data demonstrate that Abs elicited from a synthetic peptide consisting of only the key C ß-strand-centered segment of the V1V2 domain can bind to V2 165–181 in its native context within the HIV virus, and in some cases can neutralize the virus. Our results provide proof of concept that a V2 165–181 immunofocused immunogen, which can be further designed to preferentially present the epitopes associated with protection in the RV144 trial, can elicit neutralizing antibodies.
Methods: We intend to engineer anti-HIV reagents that bind with high avidity to single spikes, overcoming potential problems with the low density of HIV spikes. Here, we demonstrate a strategy to use dsDNA as a rigid molecular ruler to map epitopes on the HIV envelope protein to gain insight into the relatively unknown spatial environment of the spike trimer. Upon determining the optimal separation distance between epitopes, the dsDNA linker will be replaced with a structured protein linker. This technique should allow for the development of a novel multivalent antibody reagent improving binding and increasing avidity.

Results: Optimal HIV binding proteins will be trimerized by attaching a trimerization motif, reducing the concentration required for stabilizing immunity. To date, we have several bispecific DNA reagents that have the ability to neutralize various strains of HIV with greater potency than its individual components.

Conclusion: These results demonstrate the promise for discovery of optimal anti-HIV reagents using this technology.

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Membrane Biophysical and Structural Investigations of HIV-1 Envelope Glycoproteins Epitopes in the Aim of Eliciting Liposomal Vaccine

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Background: A few stable regions have been discovered on the envelope glycoproteins of HIV-1 against which some patients produce neutralizing antibodies. The most promising epitopes are located in the membrane proximal external region and are probably exposed transiently during the fusion of cellular and viral membranes. Whereas the peptides isolated from this region failed to induce neutralizing immunogenicity. To date, we have several bispecific DNA reagents that have the ability to neutralize various strains of HIV with greater potency than its individual components.

Methods: We investigate these epitopes (one from gp120 and two from gp41) and their insertion into liposomal carriers by structural approaches (Nuclear Magnetic Resonance, Circular Dichroism: CD). This requires the production of the polypeptides by bacterial over-expression and their purification by using biochemical methods, and their reconstitution into liposomal carriers. In addition, the immunogenicity of these epitopes will be investigated by mice immunization and the immune response will be quantified by ELISA.

Results: The conditions for expression and purification of these polypeptides are established. The purified polypeptides solubilised in detergent are reconstituted overnight in liposomal carriers by a dialysis approach. Their secondary structure is investigated by CD, gp120 and gp41a adopt mainly an alpha-helical structure in the detergent. In contact with the lipids, gp41a maintains this helical structure and it is reinforced in liposomes having a similar composition of the viral membrane. In contrast the gp120 construct adopts a predominantly beta-sheets structure.

Conclusion: The comparative analysis of the potential for immunogenicity and structural results obtained will be confronted in order to better understand the fusion of the cellular and viral membranes and thereafter to help in the design of vaccines directed against HIV-1.