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Plasmablast Phenotype and Mucosal Antibodies to V2 in Vaccine-induced Protection Against SIVmac251

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Background: We have recently recapitulated the RV144 vaccine efficacy in a SIV_{mac251} model. In our study, rectal anti-cyclic V2 IgG antibodies correlated with a decrease risk of SIV_{mac251} acquisition ($p = 0.0063$). Analysis of the homing markers on plasmablast (PB) resulted in a higher frequency of $alpha_4beta_7^+$ PBs in animals with higher levels of IgG and IgA to cyclic V2 in rectal mucosal.
Methods: We investigated the homing potential in 5 different vaccine strategies by measuring $alpha_4beta_7$ and CXCR3 as markers for gut mucosa and inflammatory sites, respectively in a total of 118 macaques. The immunoglobulin (Ig) expression on PB and the mucosal antibody responses were assessed in all groups. We studied a cohort of macaques immunized 4 times with ALVAC-SIV and twice with gp120/alum or gp120/MF59. A second cohort was immunized twice with DNA-SIV/gp120/alum or once Ad26-SIV, and boosted with ALVAC-SIV/gp120/alum twice. Finally, an additional group was immunized 4 times with NYVAC-SIV boosted twice with gp120/alum.
Results: Both ALVAC-SIV/gp120/alum and DNA-SIV/gp120/alum immunized animals were protected from SIV_{mac251} mucosal acquisition ($p = 0.029$ and $p = 0.014$, respectively). However, no protection was observed with ALVAC-SIV/gp120/MF59, NYVAC-SIV/gp120/alum and Ad26 primed regimens. DNA-SIV/gp120/alum and ALVAC-SIV/gp120/alum immunizations increased the frequency of $alpha_4beta_7$ plasmablasts ($p = < 0.0001$ and $p = 0.015$) while ALVAC-SIV/gp120/MF59, NYVAC-SIV/gp120/alum increased the frequency of CXCR3 + plasmablasts ($p = 0.0001$ and $p = 0.004$ respectively). We are completing Ad26-SIV/gp120 and DNA-SIV/gp120/alum studies as well as the analysis of Ig expression and correlations with mucosal antibody responses.
Conclusions: Preliminary results suggest that different vaccine modalities are able to alter the plasmablast homing and the effector functions of the antibody response at mucosal sites that may correlate with protection.

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Use of Enzyme-digested Virus-like Particles as Probes for Flow Cytometric Sorting of HIV-specific Neutralizing Ab-producing B-cells

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Background: Isolating new broadly neutralizing antibodies (bnAbs) provides fresh insights for rational HIV-1 vaccine design. Several sites of vulnerability to bnAbs are now well defined, and it is likely that additional targets exist. Virus-like particles (VLPs) that express Env trimers display all of the natural epitopes and are therefore attractive candidates for use as probes. However, VLP surfaces are also decorated with nonfunctional forms of HIV Env. Exposing VLPs to proteases clears nonfunctional Env, leaving native trimeric Env intact. The resulting ''trimer-VLPs'' preferentially bind to nAbs compared to non-neutralizing antibodies and therefore may have gained sufficient selective power for use as B cell probes to identify new bnAbs.
Methods: PBMC from HIV-infected patients were stained with the enzyme-digested trimer-VLPs, and VLP-positive memory B cells were sorted by flow cytometry. Multiplex RT-PCR was performed on cell lysates to amplify kappa, lambda, and heavy chain genes. Cloned antibodies were assessed for neutralization breadth and potency against a panel of Env-pseudoviruses using the TZM-bl neutralization assay.
Results: VLPs were used to sort B cells from the donor of the broad CD4bs antibody VRC13. The sort yielded relatives of VRC13, as well as unrelated sequences that exhibited high sequence divergence from germline VH genes and/or long CDR3 regions, which are characteristics of known bnAbs. Cloning and characterization of these Abs is ongoing.
Conclusions: Unlike many previously used B cell probes, VLPs express multiple epitopes including those for trimer specific antibodies which allows for simultaneous sorting of B-cells of different specificities. Additionally, these probes are devoid of non-functional forms of Env and therefore preferentially bind to neutralizing Ab-producing B cells. VLPs therefore hold great promise for isolating novel HIV-specific bnAbs that can better inform vaccine design.

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Viral Escape Pathways from Broadly Neutralising Antibodies Targeting the HIV Envelope Cleavage Site Enhance MPER Mediated Neutralisation

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Background: A preventative HIV-1 vaccine will likely elicit broadly neutralising antibodies (bNAbs). The diverse modes of neutralisation by bNAbs leads to variable escape pathways in each epitope. Documenting escape and identifying common elements