antibodies to the SIV Env differ from the antibody responses to the HIV-1 Env. Thus, NHP vaccine studies may require inclusion of HIV-1 envelope sequences, instead of SIV envelope, to accurately reflect antibody specificities induced by HIV-1 vaccination in human clinical trials.

OA04.04

Co-administration of Rapamycin with a DNA/MVA SIV Vaccine Improves Memory CD8 T Cell Response and Enhances Control of SIV251 Infection

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Background: Recent studies in mice and macaques demonstrated generation of higher frequency and better quality memory CD8 T cells by inhibiting the mTOR pathway using rapamycin. In this study, we tested the effect of rapamycin on the immunogenicity and efficacy of a DNA/MVA SIV vaccine in rhesus macaques.
Methods: Macaques were primed with a DNA/SIV vaccine on weeks 0 and 8, and boosted with a MVA/SIV vaccine on weeks 16 and 24 in the absence (DM) or presence of rapamycin either during MVA boosts or during primes and boosts. Rapamycin treatment was given for a period of 32 days starting from day -3 of each immunization where used. Macaques were challenged with SIV251 intrarectally at about 21–24 weeks after the final MVA boost.
Results: Rapamycin treatment did not increase the magnitude but reduced contraction of effector CD8 T cell response that resulted in higher frequencies of SIV-specific memory CD8 T cells with enhanced quality as determined by higher expression of CCR7 and BCl-2 ex vivo and higher proliferative capacity in vitro. Rapamycin treatment did not significantly alter the memory CD4 T cell response. Post SIV251 infection, rapamycin-treated macaques demonstrated a marked expansion of SIV-specific CD8 T cells reaching up to 50% in blood and 25% in gut and this enhanced expansion in rapamycin-treated groups was associated with a markedly lower (2-logs compared to unvaccinated and 1-log compared to DM) set-point viral loads, better preservation of central memory T cells in blood and enhanced survival compared to unvaccinated and DM-vaccinated macaques.
Conclusion: Inhibiting mTOR pathway during MVA boosts of a DNA/MVA vaccine regimen enhances the functional quality of vaccine-elicited memory CD8 T cell response that is capable of markedly restricting a highly pathogenic SIV replication.

OA04.05

Protection of Cynomolgus Macaques from Pathogenic SIV Following Vaccination with Varicella-Zoster Virus Based Vaccines

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Background: Varicella Zoster virus (VZV) is a persistently replicating virus with the potential to deliver life-long immunity in an HIV vaccine setting. As a recombinant vaccine vector, the ability to self-boost may overcome the limited capacity of pox- and adenoviral vectors to generate long-lived immunogenicity.
Methods: We describe a comprehensive preclinical non-human primate challenge trial with recombinant VZV-SIV constructs expressing codon-optimized and safety augmented antigenic targets from SIV as candidate prophylactic HIV vaccines. As a strict evaluation of our approach, a cohort of MHC-typed cynomolgus macaques were administered only a single vaccination with VZV-SIV vaccine or vector alone, and subsequently challenged intra-rectally using a weekly low-dose exposure to highly pathogenic SIV.
Results: Vaccine-induced durable humoral and cellular immune responses were observed with evidence to suggest VZV replication and subsequent reactivation driving progressive increases in anti-VZV IgG titers. There was no significant difference in SIV acquisition rates or challenge doses to infection between vaccine and control groups over the course of multiple low-dose mucosal challenges. Importantly, the vaccine group showed lower peak and set point viral loads (VL) with a subset of vaccinees ($\sim$35%) with persistent undetectable VL over 1.5 yrs of follow-up. We saw no evidence of accelerated disease course or increased SIV replication in vaccinees.
Conclusion: A single dose of human VZV-based SIV vaccines was sufficient to provide substantial benefit to vaccinees with apparent early disease modification and preliminary evidence to suggest a similar capacity to induce viral clearance as recently demonstrated by Louis Picker's related RhCMV-based approach. Future work will be directed to determine the extent of this SIV clearance with subsequent NHP trials designed to augment the immunogenicity and capacity of this approach. The well documented clinical and safety evaluations of VZV vaccines in humans, provides a substantial advantage in developing this platform for human clinical trials.

OA05.01

Structural Basis for HIV-1 gp120 Recognition by a Germ-line Version of a Broadly Neutralizing Antibody

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Background: Following infection by HIV-1, the host immune response is unable to clear the virus due to a variety of factors, including rapid viral mutation and the establishment of latent reservoirs. The only target of neutralizing antibodies is the trimeric envelope (Env) spike complex, but HIV-1 can usually evade anti-spike antibodies due to rapid mutation of its two spike glycoproteins, gp120 and gp41, and structural features that allow the spike to hide conserved epitopes. Efforts to design an effective antibody-based vaccine against HIV-1 would benefit from understanding how germ-line B-cell receptors recognize the gp120/gp41 envelope spike. Recently isolated from several infected individuals, potent VRC01-like (PVL) HIV-1 antibodies derived from the VH1–2*02 germ-line allele target the CD4 binding site on gp120. These antibodies have unprecedented potency and breadth for HIV-1 neutralization and some protect against HIV-1 infection in animal models. Since the VH1–2*02 germ-line allele is present in up to 95% of the population, it may be possible to elicit by vaccination similar CD4 binding site antibodies in other

individuals. VH1–2*02 germ-line B-cell receptor interactions with gp120 were uncharacterized, which was a bottleneck for designing immunogens to elicit PVL antibodies.
Methods: We determined the structures of a VH1–2*02 germ-line antibody alone and a germ-line heavy chain/mature light chain chimeric antibody complexed with gp120 by X-ray crystallography and characterized them through surface plasmon resonance and neutralization experiments.
Results: VH1–2*02 residues make extensive contacts with the gp120 outer domain, including all PVL-signature and CD4-mimicry interactions, but not critical CDRH3 contacts with the gp120 inner domain and bridging sheet that are responsible for the improved potency of NIH45–46 over closely-related clonal variants, such as VRC01.
Conclusion: Our results provide insight into initial recognition of HIV-1 by VH1–2*02 germ-line B-cell receptors and may facilitate the design of immunogens tailored to engage and stimulate broad and potent CD4-binding site antibodies.

OA05.02
Structural Characterization of Potent, Longitudinally-Defined HIV-1 V1/V2-Directed Antibodies

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Background: Approximately 20% of individuals infected with HIV-1 develop neutralizing antibodies that are cross-reactive with a variety of HIV-1 strains. These antibodies generally exhibit extensive affinity maturation and are slow to appear. Antibodies targeted against the V1/V2 region of Env display lower levels of somatic mutation than those targeting other sites of vulnerability and are among the most frequently elicited. Comprehensive analysis of B cell ontogeny of effective V1/V2 antibodies would provide valuable insight into vaccine development aimed at eliciting this class of antibody.
Methods: Broadly-neutralizing V1/V2-directed antibodies isolated from donor CAP256 at various time points following infection were isolated, and evolution of antibody development was longitudinally analyzed through deep sequencing and structural characterization.
Results: Here we present crystal structures of the antigen-binding fragments from several V1/V2-directed antibodies isolated from donor CAP256. The antibodies contain long heavy chain 3rd complementary determining regions (CDR H3)s, a common feature among V1/V2-directed antibodies. The structures reveal a disulfide bond at the base of an extended CDR H3 loop and show that tyrosine sulfation contributes to a negatively charged surface extended away from the framework of the Fab. Deep sequencing data reveal that the length of the CDR H3 is the result of a recombination event and does not extend over time, however specific features of the mature loop such as the disulfide bond are not found early in the lineage.
Conclusion: Combinatorial studies with structural characterization and deep sequencing reveal paths to the development of potent and broad V1/V2 directed antibodies. While some structural components appear immediately such as the long CDR H3, affinity maturation plays an important role in stabilizing specific features. The ontogeny of the antibodies presented here reveal that breadth can be achieved rapidly and extensive affinity maturation is not a necessary feature of broadly neautralizing HIV-1 antibodies.

OA05.03
Interplay Between Broadly Cross-Neutralizing V2 Monoclonal Antibodies and Autologous Viral Evolution

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Background: CAP256, an HIV-1 subtype C superinfected individual, developed potent broadly cross-neutralizing (BCN) V2 plasma antibodies that were critically dependent on R166 and K169. Eleven related BCN mAbs targeting V2 have recently been isolated from this individual after 30 months post-infection (p.i.). Here we investigated the relationship between these mAbs, longitudinal CAP256 viruses and previously characterized escape variants.
Methods: CAP256 primary transmitted/founder (t/f) (N160 glycan -), superinfecting t/f (N160 glycan +) and 6-, 12-, 21- and 39-month viruses were tested for neutralization by the 11 mAbs. The role of escape mutations at positions 160–171 in V2 and 437 in C4 was investigated using 15 autologous mutants and 3 chimeric viruses.
Results: While the primary t/f was only neutralized by 1 mAb, all 11 mAbs potently neutralized the superinfecting t/f. All mAbs were unable to neutralize the 21- and 39-month viruses. mAbs showed differential dependence on individual V2 mutations, however the neutralizing activity of all mAbs was abrogated by R166S or K169E mutations. Deleting the N160 glycan only slightly affected the neutralizing capabilities of each mAb. Finally, a sensitive V1V2 chimeric virus was made resistant to all the mAbs through a P437A mutation in C4.
Conclusion: The potent neutralization of the superinfecting t/f but not the primary t/f by all mAbs suggested that the superinfecting t/f was responsible for eliciting these BCN antibodies. The inability of these mAbs (isolated after 30 months p.i.) to neutralize autologous clones after 21 months p.i. suggests they arose before this time point and that this memory B cell family persisted despite viral escape. The presence of circulating viruses with and without the N160 glycan after superinfection explains the independence of the mAbs on this glycan. The drastic effect of the P437A mutation on mAb activity indicates that in the context of the envelope trimer, C4 may modulate V2 conformation.

OA05.04
A Diverse Antibody Response to the Gp120 N332 Glycan Epitope in an HIV-Infected Donor

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