P34.08
Strain Specific Anti-HIV Antibody Evolution during Acute Infection and Viral Escape

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Background: We previously identified an individual (CAP88), from the CAPRISA 002 cohort, with potent strain-specific neutralization. This response mapped to the C3 region of gp120 and was detected at 11 weeks post-infection (wpi) and then later waned co-incident with viral escape. A monoclonal antibody (CAP88-CH06) was isolated at 34 wpi which utilized the IGHV4-39*01, D3-3*01 and J4*02 genes, had 5.9% divergence from germline, and CDRH3 length of 17 amino acids. This study examined the evolution of the CAP88-CH06 heavy chain immunoglobulin genes over 121 weeks, starting from 11 weeks of infection.
Methods: RNA and DNA were extracted from PBMCs from donor CAP88 at four time-points (11, 17, 38 and 121 wpi). The heavy chain VDJ regions of IGHV4-39 gene were PCR amplified and sequenced by Illumina MiSeq. The resulting sequences were blasted against a database of germline IGHV and IGHJ sequences from IMGT and compared to the CAP88-CH06 sequence.
Results: We detected ~3,500 sequences that were highly related to CAP88-CH06. The majority (75%) of these were from RNA at 11 wpi (1,051 sequences, 39%), 17 wpi (1,625, 61%) and 1 sequence each in 38 wpi and 121 wpi. This corresponded to the antibody response which first appeared at 11 wpi, peaked at 26 wpi and by 54 weeks had declined. Most of the DNA sequences were from 11 wpi (n=761) followed by 38 wpi (n=113) and fewer than 10 from 17 wpi and 121 wpi. The 38 wpi DNA sequences were closely related to the 11 wpi sequences of both RNA and DNA.
Conclusions: We have identified clonally related antibody sequences from 4 different time-points from CAP88 in both RNA and DNA. The frequency of sequences in RNA corresponded with plasma neutralizing antibody titres. DNA-derived sequences from later time-points clustered in phylogenetic trees with RNA-derived sequences from earlier time-points, suggesting that they were from the memory B cell compartment.

P34.09
Engineering Antibodies to Enhance Activity and Increase Half-life

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Background: HIV/AIDS remains one of the most serious current threats to global public health. Although anti-HIV drugs have been effective among the wealthiest populations, a vaccine and/or new methods to prevent infections are needed to control HIV globally. Strategies to combat HIV-1 require structural knowledge of how antibodies recognize HIV envelope proteins and how the immune system eliminates viruses. Until recently, only a small number of broadly neutralizing antibodies against HIV-1 had been characterized, and the immunological basis for their breadth and potency remains poorly understood. However, it was recently demonstrated that antibodies could be engineered to greatly enhance their breadth and potency (Diskin et al., Science 2011). Unfortunately, this and other engineering efforts have resulted in a decrease in antibody half-life in mouse and non-human primate models. This decrease in half-life correlates with an increase in reactivity to a variety of antigens, termed polyreactivity.
Methods: In order to make better targets for passive delivery therapies, we are working to increase the half-life of antibodies while maintaining their breadth and potency using a variety of computational and structure-based techniques. One technique involves reducing the spatial aggregation propensity, in which an algorithm finds dynamically exposed hydrophobic patches on the surface of proteins (Chennamsetty et al., PNAS 2009). To this end, we have constructed several mutations in regions that have been predicted to have high aggregation propensities, and have tested them for polyreactivity and potency in neutralization assays.
Results: Initial results show that these novel reagents have reduced polyreactivity, yet they still maintain their potency in *in vitro* neutralization assays.
Conclusions: We are currently pursuing *in vivo* experiments in mice to further understand the relationship between antibody potency, polyreactivity, and half-life.

P34.10
Antibody-based PrEP and Cross-reactivity

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Background: Broadly neutralizing antibodies (bNAbs) are being developed for topical and systemic pre-exposure prophylaxis. Since some bNAbs (e.g. 4E10) have been reported to interact with non-viral epitopes, the cross-reactivity of bNAbs is an important safety parameter to be documented in regulatory submissions. The objective of this study was to determine the cross-reactivity of Nicotiana (-N) manufactured anti-HIV bNAbs 4E10-N and VRC01-N, and anti-HSV glycoprotein D bNAb HSV8-N with cryosections of human tissues.
Methods: In order to detect binding, the antibodies were biotinylated and applied to cryosections of normal human tissues (3 donors per tissue) at two concentrations (2-20 μg/ml). Commercially available Synagis was biotinylated and used as a control. The study was GLP compliant.
Results: 4E10-N variably stained a variety of tissue elements in the human tissue panel. VRC01-N also produced staining of tissue elements; however, the staining with VRC01-N was generally present in fewer tissues and with reduced intensity and frequency. No staining with HSV8-N or Synagis was observed in the human tissue panel examined.
Conclusions: The majority of observed staining was cytoplasmic in nature, which is of little toxicologic concern since the cytoplasmic compartment is generally considered to be inaccessible to