

## SYMPOSIUM 09: A POTENTIAL ROLE OF BINDING/ADCC ANTIBODIES IN PROTECTION AGAINST HIV ACQUISITION

### S09.01

#### Modulation of IgG activity by differential glycosylation

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IgG antibodies are the primary mediators of protective humoral immunity against pathogens and have been used therapeutically for over a century. They were first used as antitoxins for the treatment of infectious diseases in the pre-antibiotic era. Today, hyperimmune sera from human donors recovering from infection with specific viruses, such as hepatitis B, cytomegalovirus, and varicella zoster, are used to provide protective immunity to susceptible populations. Moreover, tumor-specific antibodies have been successfully used in human cancer therapy. Besides these protective activities, IgG autoantibodies are the principal mediators of autoimmune diseases such as immune thrombocytopenia (ITP), autoimmune hemolytic anemia (AHA), and systemic lupus erythematosus (SLE). In addition to this pro-inflammatory activity, antibodies also are known to have an anti-inflammatory activity. If infused at high doses, IgG can effectively suppress autoimmune mediated inflammation (IVIg therapy). Recent evidence suggests that both the pro- and anti-inflammatory activity of IgG is regulated by the sugar side chain that is attached to the CH2-domain of all IgG subclasses. Subtle variations in the composition of this sugar moiety will either enhance or decrease the pro-inflammatory activity. The presentation will discuss which factors influence these opposing effects of IgG and how we can use this knowledge to enhance the therapeutic efficacy of immunoglobulins.

### S09.02

#### The role of Fc $\gamma$ Receptor mediated mechanisms in protection against HIV mucosal challenge

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Our earlier study, Fc Receptor but not complement binding is important in antibody protection against HIV (Hessel, et al., *Nature*, 2007), compared protection by wild-type and Fc variant b12 neutralizing IgG antibodies against SHIV<sub>SF162P3</sub> vaginal challenge in macaques. A higher rate of infection and a peak primary viremia similar to that of controls was observed for a b12 variant (LALA) that lacked leukocyte Fc $\gamma$  receptor (Fc $\gamma$ R) and complement binding. However, a variant (KA) that lacked only complement binding was as effective at protection as wild-type b12. Therefore, Fc $\gamma$ R binding was argued as important to antibody protection. When compared to wild-type b12 in a low-dose repeated mucosal SHIV challenge (Hessel, et al., *Nature Medicine*, 2009), macaques treated with the LALA variant became infected after fewer challenges than macaques treated with wild-type b12. A number of mechanisms may contribute to these observations. Among others, these include Fc $\gamma$ R-mediated activity against virions (e.g. phagocytosis), and Fc $\gamma$ R-mediated activity against infected cells (e.g. ADCC and phagocytosis).

Data from in vitro studies demonstrate the ability of wild-type b12 to mediate both ADCC and ADCVI while the Fc $\gamma$ R-crippled LALA fails to elicit responses from effector cells. To further probe the potential role of ADCC in protection, we are investigating a non-fucosylated variant of b12 with enhanced Fc $\gamma$ R binding capacity. In vitro studies to evaluate improvements in Fc-mediated effector functions confirmed a substantial improvement in ADCC. A summary of data from in vitro and in vivo studies examining the role of Fc $\gamma$ R in antibody protection will be presented.

**S09.03****Mechanisms of altered ADCC inducing antibodies in progressive HIV infection***C. Scanlan<sup>1</sup> and G. Alter<sup>1</sup>*<sup>1</sup>Ragon Institute, Charlestown, Massachusetts, USA

In addition to their ability to neutralize viruses, antibodies have a plethora of additional functions including the ability to recruit innate immune effector cell function, through Fc-receptors and induce complement deposition, that have been shown to be critical for the effective clearance and control of other viral, bacterial, and parasitic infections. Furthermore, elevated levels of antibody-dependent cellular cytotoxicity has been shown to be associated with the control of HIV in long-term non-progressors and correlate with delayed progression to AIDS in SIV-infected macaques. However, this activity has been shown to decline with disease progression, yet little is known about the changes in the humoral response that may account for the progressive loss of innate immune recruiting antibodies. Data from the therapeutic antibody community suggest that changes in the glycan attached to the CH-2 domain of an antibody can result in profound changes in the capacity of that antibody to bind to Fc-receptors and complement, thereby altering the innate immune recruiting properties of that antibody. Significant changes occur in the glycan structure of HIV-infected individuals resulting in particular patterns of glycosylated antibodies with altered innate immune recruiting function. Understanding how these glycans may be modulated following vaccination may provide novel insights by which poly-functional antibodies may be induced to gain more effective control of HIV infection.

**S09.04****Antibody-Dependent Cell-Mediated Virus Inhibition (ADCVI) Antibodies Elicited by Vaccination***D. Forthal<sup>1</sup>*<sup>1</sup>University of California, Irvine School of Medicine, Irvine, California, USA

ADCVI is a measure of the ability of antibody, in the presence of Fc $\gamma$  receptor (Fc $\gamma$ R)-bearing cells, to inhibit viral yield from infected cells. ADCVI assays offer a summation of various anti-viral activities triggered by Fc-Fc $\gamma$ R interactions, including antibody-dependent cellular cytotoxicity (ADCC), chemokine/cytokine release, and phagocytosis. We have measured ADCVI in serum or plasma from humans and non-human primates vaccinated with a number of HIV vaccines. In the case of humans vaccinated with rgp120 (Vax004 trial), we demonstrated an inverse correlation between the level of vaccine-elicited ADCVI antibodies and the rate of HIV infection. ADCVI antibody levels were weakly associated with gp120-specific IgG1 and IgG3 levels. On the other hand, there was an inverse correlation between ADCVI antibody and gp120-specific IgG2. We also found that individuals with high IgG2 responses to the vaccine had a higher rate of HIV infection than those with lower IgG2 responses. Moreover, the association between IgG2 and infection risk was strongest for individuals with the Fc $\gamma$ RIIIa FF or Fc $\gamma$ RIIa RR genotypes. Further analyses revealed that enzymatic deglycosylation of gp120 resulted in a small increase in IgG1 and IgG3 binding and a large decrease in binding to IgG2 and IgG4.

Together, these results indicate that Fc-Fc $\gamma$ R interactions might mediate protective effects of anti-Env antibodies in humans. In addition, both the IgG subclass response to an Env-containing vaccine and a vaccinee's Fc $\gamma$ R genotype may impact vaccine efficacy.

## S09.05

## Unravelling Mechanisms Underlying Protective NK Cell Responses to HIV-1 Peptides: More than ADCC?

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Host genetic studies suggest an important role for NK cells in protective immunity to HIV-1 and attenuation of disease progression via recognition by KIR receptors of specific HLA class I molecules. We have shown, using a whole blood intracellular cytokine assay, that mothers and infants with NK cell responses to Reg (Tat, Rev, Vif, Vpu, Vpr peptide pools combined) and/or Env HIV-1 peptides are substantially less likely to transmit and acquire infection, respectively. Vpu and Tat were the regions targeted in the Reg peptide pool. These peptide-specific NK cell responses were detected in 49.4% (39/79) of individuals with chronic HIV-1 infection (and associated with lower viral loads and higher CD4 counts), 88.9% (8/9) of LTNPs and in 29.5% (13/44) of exposed-uninfected infants, but not in uninfected mothers or their infants. One mechanism responsible appears to involve HLA class I molecules and, in the individuals tested, required a non-specific plasma factor, suggesting specificity afforded directly by cell-cell interactions. ADCC antibodies have been shown to account for another mechanism of peptide-specific NK cell activation in the same assay. It is therefore likely that different mechanisms may be demonstrated in different patients or population groups or even at different stages of infection. Thresholds required for NK cell activation may differ in such a way as to allow one mechanism to take precedence over another.

KIR-HLA gene analyses of mothers and matched infants concordant and discordant for HIV-specific NK cell responses, NK responders and nonresponders across KIR genotypes and those that share similar KIR genotypes among HIV-1 infected individuals, and association studies of KIR-HLA and maternal-infant HIV-1 transmission have allowed us to identify several targets for further functional studies and overall implicate KIR and HLA class I interactions in the development of NK cell responses to HIV-1 peptides.