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Poster presentation

P20-08. Glycosylation: an important factor in Env diversity

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Background

Approximately 50% of the Env mass is glycan. Env glycans shield immunogenic epitopes, and incorporation of glycans provides the virus with mechanisms of immune escape. In addition, at least one broadly neutralizing antibody, 2G12, has as its target a conformational glycan epitope. To characterize the glycan antigenicity and heterogeneity of expressed recombinant envelopes, we have analyzed glycosylation profiles on clade B and clade C Env gp140 oligomers, and characterized Env glycosylation diversity.

Methods

Six gp140 cleavage (C) deficient and fusion (F) region deleted oligomers were expressed either as recombinant vaccinia virus produced proteins or as recombinant proteins following transfection of 293T epithelial cells. Env gp140CF proteins were denatured, reduced, and alkylated prior to digestion with trypsin. The glycopeptides were fractionated by HPLC and analyzed using mass spectrometry. The spectral data was interpreted with the aid of the web-based program GlycoPepDB.

Results

The number of glycans present did not correlate with the number of potential glycosylation sites on each protein. All recombinant Envs had unutilized glycosylation sites, and Envs with more potential glycosylation sites generally had more unutilized sites. The glycan profiles were mapped at each glycosylation site on the six proteins. Comparison of the profiles for conserved glycosylation sites among the proteins showed a broad range of glycan diversity at sites in C2, V3, C3, V4, C4, and in the gp41 membrane proximal region. This glycan diversity could modulate exposure of potential immunogenic epitopes and as well impact overall Env folding.

Conclusion

Glycosylation diversity was extensive on recombinant Env oligomers, and the sites of greatest glycan diversity did not correlate with regions of largest diversity in the gene sequence. The technology described here provides a new way to antigenically characterize recombinant envelope proteins being developed as experimental HIV-1 immunogens.

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