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4th International AIDS Society Conference on HIV Pathogenesis and Treatment

Sydney, Australia - July 22 - 25, 2007

EVIDENCE FOR CRITICAL DIFFERENCES IN HIV-1 ENV GP120 N-LINKED GLYCOSYLATION PATTERNS IN PLASMA AND DIVERSE BLOOD LEUKOCYTE COMPARTMENTS *IN VIVO*

IAS Conf HIV Pathog Treat 2007 Jul 22-25;4th: Abstract No. MOAA202

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OBJECTIVES: N-linked glycosylation is present on the HIV envelope glycoprotein and is a major mechanism for minimizing virus neutralizing antibody response. Although it is known that the glycosylation changes can dramatically influence virus recognition by the host antibody, the actual contribution of compartmental differences in N-linked glycosylation patterns remains unclear. Given that *in vivo* HIV survives as cell-associated and cell-free entities, a clear understanding of N-linked glycosylation patterns and single amino acid differences will provide deeper insights into viral survival, evasion and adaptation *in vivo*.

METHODS: We PCR amplified the *env* gp120 C2-V5 region and analysed 305 clones derived from plasma, whole peripheral mononuclear cells, monocytes, CD4+ and CD8+ T cells from 15 HIV-1 patients receiving HAART, experiencing different levels of plasma viremia. Bioinformatics and Bayesian network analyses was used to examine N-linked glycosylation differences between compartments.

RESULTS: Evidence for cell-specific single amino acid residues was seen in monocytes, which may be involved in cellular adaptation of HIV. Variations were found in the total number of N-linked glycosylation sites between patients ($P < 2.2 \times 10^{-16}$) confirming that glycosylation is driven by the host immune system. However, significant differences in the number of glycosylation sites were observed between plasma and cellular compartments ($p = 0.02281$) demonstrating compartmental differences in HIV. Bayesian network analyses showed interdependency between N-linked glycosylation sites found in our study, which may have immense functional relevance.

CONCLUSIONS: Our analyses have demonstrated single cell/compartment-specific amino acid changes and critical differences in N-linked glycosylation patterns between plasma and diverse blood leukocytes. Bayesian network analyses showed associations inferring possible glycosylation pathway. We believe that these studies will provide crucial insights into the host immune response and its ability in controlling HIV replication in vivo. These findings could have relevance in shielding and evasion of HIV-1 from neutralizing antibodies and may provide insights into future designs of vaccine strategies.

2007-07-22

MOAA202

HIV Diversity, Tropism and Compartmentalization

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