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## P04-17. The N-glycosylation sites N295, N332 and N392 of gp120 are necessary but not sufficient for HIV-1 to be neutralized by 2G12

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### Background

The human monoclonal antibody 2G12 has shown a broadly neutralizing activity *in vitro* and has contributed to protection towards virus challenge in animal models. It binds to a carbohydrate-dependent epitope. Three major N-linked glycosylation sites (PNGs) for 2G12 binding (N295, N332 and N392) were identified by site directed mutagenesis of prototype strains. We analyzed the sensitivity to 2G12 of pseudoviruses carrying envelope proteins issued from HIV-1 clade B infected patients and analyzed the data according to the corresponding *env* sequences.

### Methods

We studied four long-term non-progressors infected by HIV-1 subtype B variants. Two of them developed broadly neutralizing antibodies, defined as the capability to neutralize four primary isolates of various clades. Starting from 27 *env* clones, twelve functional HIV-1 pseudoviruses were generated by co-transfection of 293T cells with a plasmid expressing the relevant *env* gene and the pNL4.3Δ*env* plasmid carrying the luciferase gene reporter. Sensitivity to 2G12 was analyzed in TZM-bl cells.

### Results

The 3 PNGs known to be essential for 2G12 binding were present in the 3 most sensitive pseudoviruses, derived from a single patient. At least one of these major sites was absent in 8 of 9 clones that were resistant to neutralization by 2G12. However one clone, although harboring the 3 PNGs, was not neutralized by 2G12. Its sequence analysis revealed a long and heavily glycosylated V1/V2 loop (86

amino-acids and 9 PNGs) and an additional PNG at position 302 on V3 loop.

### Conclusion

Our study confirms, on naturally occurring functional *env* genes, the essential role of the PNGs N295, N332 and N392 for 2G12 binding, but also suggests that an extended V1–V2 loop or additional PNGs on V1–V2 and V3 loops might limit the accessibility of the 2G12 epitope. This biological material should be useful to better characterize the 2G12 epitope and analyze its role in virus entry.