



Chinks in the armor of the HIV-1 Envelope glycan shield: Implications for immune escape from anti-glycan broadly neutralizing antibodies



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ABSTRACT

Glycans on HIV-1 Envelope serve multiple functions including blocking epitopes from antibodies. We show that removal of glycan 301, a major target of anti-V3/glycan antibodies, has substantially different effects in two viruses. While glycan 301 on Du156.12 blocks epitopes commonly recognized by sera from chronically HIV-1-infected individuals, it does not do so on CAP45.G3, suggesting that removing the 301 glycan has a smaller effect on the integrity of the glycan shield in CAP45.G3. Changes in sensitivity to broadly neutralizing monoclonal antibodies suggest that the interaction between glycan 301 and the CD4 binding site differ substantially between these 2 viruses. Molecular modeling suggests that removal of glycan 301 likely exposes a greater surface area of the V3 and C4 regions in Du156.12. Our data indicate that the contribution of the 301 glycan to resistance to common neutralizing antibodies varies between viruses, allowing for easier selection for its loss in some viruses.

1. Introduction

Existing approaches used in the development of vaccines have not worked for HIV-1 (Haynes, 2015) and therefore novel approaches are needed for the development of a successful HIV-1 vaccine. One method is the modeling of immunogens that are based upon target sites of broadly neutralizing antibodies (bnAbs) (Burton et al., 2012; Burton and Mascola, 2015; Haynes, 2015) including the use of the structures of the antibodies themselves to model immunogens (Zhou et al., 2014). The glycans of the V3 and C3 regions form an important part of a target of bnAbs (Sok et al., 2014; Walker et al., 2011) and are therefore of substantial interest as vaccine immunogen models (Pantophlet and Burton, 2006; Walker et al., 2011).

The HIV-1 Envelope (Env) contains a large number of potential N-linked glycosylation sites (PNGs) on its outer surface, with glycans accounting for ~50% of its molecular weight (Korber et al., 2001). These PNGs are usually characterized by an Asn-X-Ser/Thr motif, where X is any amino acid except for proline (Gavel and von Heijne, 1990; Marshall, 1974). Glycans are added to the HIV-1 Env by the host cell protein machinery. Therefore, the nature of the glycan found at a

particular PNG is dependent on the type of host cell it originates from, the processing that takes place at both the endoplasmic reticulum and Golgi apparatus and on the density of the glycans nearby, which may limit access of processing enzymes (Bonmelli et al., 2011; Hioe et al., 2014). Recent evidence suggests that glycans on Env trimers are consistently under-processed and a high proportion of glycans are Man₈₋₉ GlcNAc₂ structures that are important for formation of a compact Env spike (Panico et al., 2016; Pritchard et al., 2015b).

The glycans of the HIV-1 Env protect the virus from neutralization (Moore et al., 2012; Pantophlet and Burton, 2006; Townsley et al., 2016; Wei et al., 2003; Wyatt et al., 1998; Zolla-Pazner et al., 2016), packing together to form a so-called “glycan shield” (Wei et al., 2003). Epitopes protected from neutralization include those on the V3 loop (Binley et al., 2010), a region that is highly susceptible to neutralization by common, mostly narrowly-neutralizing antibodies (Davis et al., 2009; Krachmarov et al., 2001; Vogel et al., 1994), and the CD4 binding site (CD4bs) to which antibody access is heavily constrained by glycans (Binley et al., 2010; Chen et al., 2009; Koch et al., 2003). Removal of particular glycans increases antibody access to the CD4bs (Binley et al., 2010; Koch et al., 2003; Li et al., 2008; McCaffrey et al.,

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2004; Townsley et al., 2016; Zolla-Pazner et al., 2016) and the V3 loop (Binley et al., 2010; Koch et al., 2003; Li et al., 2008; McCaffrey et al., 2004; Townsley et al., 2016; Zolla-Pazner et al., 2016). Addition/shifting of glycans in the V1, V2, C2, V4 and V5 regions occur in macaques infected with simian/human immunodeficiency virus (SHIV), suggestive of antibody-mediated selection for viruses in which glycans block access to antibody epitopes (Blay et al., 2006).

Initially, the glycans of HIV-1 Env were thought to be almost uniformly unrecognized by antibodies with the exception of the 2G12 epitope (Sanders et al., 2002), leading to terms such as the "silent face" of Env (Burton et al., 2005; Wyatt et al., 1998). However, more recently, it has become clear that glycans are very important for the formation of key epitopes such as the V2-glycan site (PG9/16 class of broadly neutralizing antibodies) (Doores and Burton, 2010; Doria-Rose et al., 2015), the epitope targeted by 3BC315 and 3BC176 in gp41 (Lee et al., 2015) and the gp120/gp41 interface epitope (Falkowska et al., 2014; Huang et al., 2014; Scharf et al., 2014). The anti-CD4bs antibody HJ16 is also glycan dependent (Balla-Jhaghoorsingh et al., 2013). Glycans are also the primary targets for the anti-V3/glycan class of broadly neutralizing antibodies (Julien et al., 2013b; Mouquet et al., 2012; Sok et al., 2014; Walker et al., 2011) that are the main focus of this report.

The anti-V3/glycan class of antibodies primarily target the conserved (Gao et al., 1996; Louwagie et al., 1995; Travers, 2012) glycans at positions 301, 332 and 334 (HXB2 numbering), around the base of the V3 loop (Julien et al., 2013b; Mouquet et al., 2012; Pejchal et al., 2011; Sok et al., 2014; Walker et al., 2011; Ward and Wilson, 2015). Some moderately broad neutralizing anti-V3/glycan antibodies, such as early antibodies in the PGT121–123 lineages, require both the 301 glycan and the 332 glycan to neutralize viral variants but as the antibodies mature and gain breadth they lose their dependence on the 301 glycan, presumably due to higher affinity binding to the 332 glycan (Sok et al., 2013). This indicates that the dependence of epitopes on the presence of particular glycans can change over time with the evolution of the antibodies. In aggregate, the primary targets of the anti-V3/glycan class of antibodies are the glycans at 301 and 332 (Walker et al., 2011), although other glycans or arrays of glycans (including 334 to a lesser extent) are recognized in some viruses (Doores et al., 2015; Sok et al., 2014). These secondary targets are not restricted to glycans on the V3 loop but may include glycans in the V1/V2 loops, such as at position 137 (Sok et al., 2014).

Narrowly-neutralizing antibodies which recognize underlying epitopes may select for viruses expressing glycans to protect their V3 loops while glycan-recognizing bnAbs may select for loss of their target glycans (Moore et al., 2012; Wei et al., 2003). Illustratively, HIV-1 populations in two individuals first evolved to exhibit a PNG at position 332 to escape early neutralizing antibodies, and then induced anti-V3/glycan broadly neutralizing antibodies. Subsequently, the virus populations lost the PNG at 332, likely to evade neutralization by those anti-glycan antibodies (Moore et al., 2012). Little is known about how the construction of the glycan shield may affect such concurrent antibody-mediated selection for or against the V3/glycans.

Therefore, in this study, we investigated the role of key glycans around the base of the V3 loop, at positions 301, 332 and 334, to understand the balance between their role in blocking antibody responses (glycan shield) and being a target of bnAbs. Using *in vitro* neutralization assays and energy minimization models of fully glycosylated Env trimers, we show that dependence on the glycan at 301 for maintenance of the "glycan shield" differs in two subtype C viruses, Du156.12 and CAP45.G3 that share approximately 90% sequence identity. In particular, we used monoclonal antibodies (mAbs) to help elucidate exposure of new epitopes and a large set (n=64) of sera from chronically HIV-infected individuals in order to study antibodies that are commonly found in natural infection.

We show that Du156.12 requires the glycan at position 301 for effective blocking of commonly neutralizing antibodies, similar to

previous work with other viral isolates (Binley et al., 2010; Koch et al., 2003; Li et al., 2008; McCaffrey et al., 2004; Townsley et al., 2016; Zolla-Pazner et al., 2016). In contrast, CAP45.G3 depends very little on the presence of this glycan for resistance to neutralizing antibodies commonly found in immune sera. Our data therefore indicate that the contribution of the glycan at 301 to resistance to common neutralizing antibodies varies between viruses. The PNG at position 301 is highly conserved (Gao et al., 1996; Louwagie et al., 1995; Travers, 2012) and is found across all subtypes, although it is less common in subtypes D (Gao et al., 1996; Louwagie et al., 1995; Travers, 2012) and CRF01_AE (Travers, 2012). This suggests that, with sufficient pressure, certain viruses with the properties of CAP45.G3 may more easily simultaneously evade both anti-V3/glycan antibody responses and antibody responses normally blocked by the same glycans. The ability to simultaneously evade broadly neutralizing anti-V3/glycan antibodies and antibodies blocked by V3/glycans may impair the efficacy of anti-V3/glycan antibodies passively infused as therapy or those induced by a future vaccine.

2. Methods

2.1. Blood samples

Blood samples were collected between February 2010 and July 2011 from donors who were > 18 years old, chronically HIV-1 infected (> 1 year) and were not exposed to antiretroviral therapy (ART), except for ART given for prevention of mother-to-child transmission (> 3 months prior). Study participants were recruited from (i) caregivers of patients at the paediatric HIV clinic at Groote Schuur Hospital (n=26) and (ii) attendees of the HIV wellness clinic at the Khayelitsha Site B clinic (n=38). CD4 counts were available for 57/64 participants. The median CD4 count was 416 (interquartile range (IQR) 286, 545). Viral loads were measured on 20/64 of the samples; median viral load was 31500 (IQR 8825, 105000). Both clinics are in Cape Town, South Africa. Written informed consent was received from study participants. This study was approved by the Human Research Ethics Committee, Faculty of Health Sciences of the University of Cape Town.

2.2. Pseudovirus constructs, cell lines and mAbs

The *env* constructs for CAP45.2.00.G3 (Li et al., 2006) (referred to as CAP45.G3) and Du156.12 (Williamson et al., 2003) were obtained from Dr. Lynn Morris, National Institute for Communicable Diseases, and Dr. Carolyn Williamson, University of Cape Town. The *env* constructs for QH343.A10 (Blish et al., 2009) and 253-11 (Kulkarni et al., 2009) were obtained from through the NIH AIDS Reagent Program, Division of AIDS, NIAID. Other mutants of CAP45.G3, Du156.12, QH343.A10 and 253-11 were generated using the GeneArt® Site-Directed Mutagenesis PLUS Kit (Invitrogen, Germany). Collectively, the CAP45.G3 and Du156.12 variants expressed each of the possible different combinations of the PNGs at positions 301, 332 and 334 on both viruses, except the variant with none of these PNGs (Table 1). All mutants were confirmed by

Table 1

Nomenclature used for pseudovirus mutants containing different combinations of glycans at positions 301, 332 and 334.

Virus Name		PNG 301	PNG 332	PNG 334
CAP45 ^{301.332}	Du156 ^{301.332*} ^a	Y	Y	–
CAP45 ^{301.334*} ^a	Du156 ^{301.334}	Y	–	Y
CAP45 ³⁰¹	Du156 ³⁰¹	Y	–	–
CAP45 ³³²	Du156 ³³²	–	Y	–
CAP45 ³³⁴	Du156 ³³⁴	–	–	Y

^a An asterisk (*) indicates that the glycan pattern corresponds to the wild-type CAP45.G3 or Du156.12 virus.

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