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Glycosylation and oligomeric state of envelope protein might influence HIV-1 virion capture by α4β7 integrin

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ABSTRACT

The α4ß7 integrin present on host cells recognizes the V1V2 domain of the HIV-1 envelope protein. This interaction might be involved in virus transmission. Administration of α4ß7-specific antibodies inhibit acquisition of SIV in a macaque challenge model. But the molecular details of V1V2: α4ß7 interaction are unknown and its importance in HIV-1 infection remains controversial. Our biochemical and mutational analyses show that glycosylation is a key modulator of V1V2 conformation and binding to α4ß7. Partially glycosylated, but not fully glycosylated, envelope proteins are preferred substrates for α4ß7 binding. Surprisingly, monomers of the envelope protein bound strongly to α4ß7 whereas trimers bound poorly. Our results suggest that a conformationally flexible V1V2 domain allows binding of the HIV-1 virion to the α4ß7 integrin, which might impart selectivity for the poorly glycosylated HIV-1 envelope containing monomers to be more efficiently captured by α4ß7 integrin present on mucosal cells at the time of HIV-1 transmission.

1. Introduction

The entry of viruses into a host cell is a complex and multistep process. While some viruses might “land and enter”, most viruses probably use a “land and seek” approach. The virus first attaches to a molecule that is either easily accessible or abundantly present on the cell surface, then seeks a primary receptor to which it binds specifically, and finally enters the cell (Boulant et al., 2015). This is perhaps best illustrated in the tailed bacteriophage T4, which contains six long fibers attached to the base of a tail. The tips of the fibers bind to lipopolysaccharide on the *E. coli* surface, allowing the virus to land on the host cell (adsorption). By reversible attachment and detachment of the tail fibers, the virus, still bound to the host cell, can move and scan for a specific and stable (irreversible) attachment to the primary receptor(s) on the cell surface. This strategy allows for high efficiency of infection which, in phage T4, reaches the theoretical maximum of one virus per host cell (Goldberg, 1983).

Although the components and mechanisms vary, the basic “land and seek” strategy appears well-conserved among viruses. Many mammalian viruses are known to move along the cell surface before binding to primary receptor and entering into the host cell. For instance murine leukemia virus (MLV) and vesicular stomatitis virus (VSV) have been described as “surfing” along cellular filopodia prior to entry (Lehmann et al., 2005). This strategy is also essential for cell-to-cell transmission, an important feature of HIV-1 life cycle. HIV-1 has been reported to interact with a number of surface molecules that might aid in its attachment and entry into T-cells or cell-to-cell transmission (Mothes et al., 2010). These include C-type lectin receptors such as dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) (Geijtenbeek et al., 2000) and dendritic cell immunoreceptor (DCIR) (Lambert et al., 2008), heparin sulfate proteoglycan (HSPG) (Mondor et al., 1998), sialic acid-binding immunoglobulin-type lectin-1 (Siglec-1) (Izquierdo-Useros et al., 2012; Jobe et al., 2016), and α4ß7 integrin (Arthos et al., 2008) (Fig. 1A).

The α4ß7 integrin is a particularly intriguing molecule. Projecting

Abbreviations: DC-SIGN, dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin; DCIR, dendritic cell immunoreceptor; HSPG, heparin sulfate proteoglycan; mAbs, monoclonal antibodies; MAdCAM-1, mucosal addressin cell adhesion molecule-1; Siglec-1, sialic acid-binding immunoglobulin-type lectin-1; T/F, transmitter/founder; gp16, gene product 16; MPER, membrane proximal external region; FV, founder virus; BnAb, broadly neutralizing antibody; RLU, relative light units; IMC, infectious molecular clones

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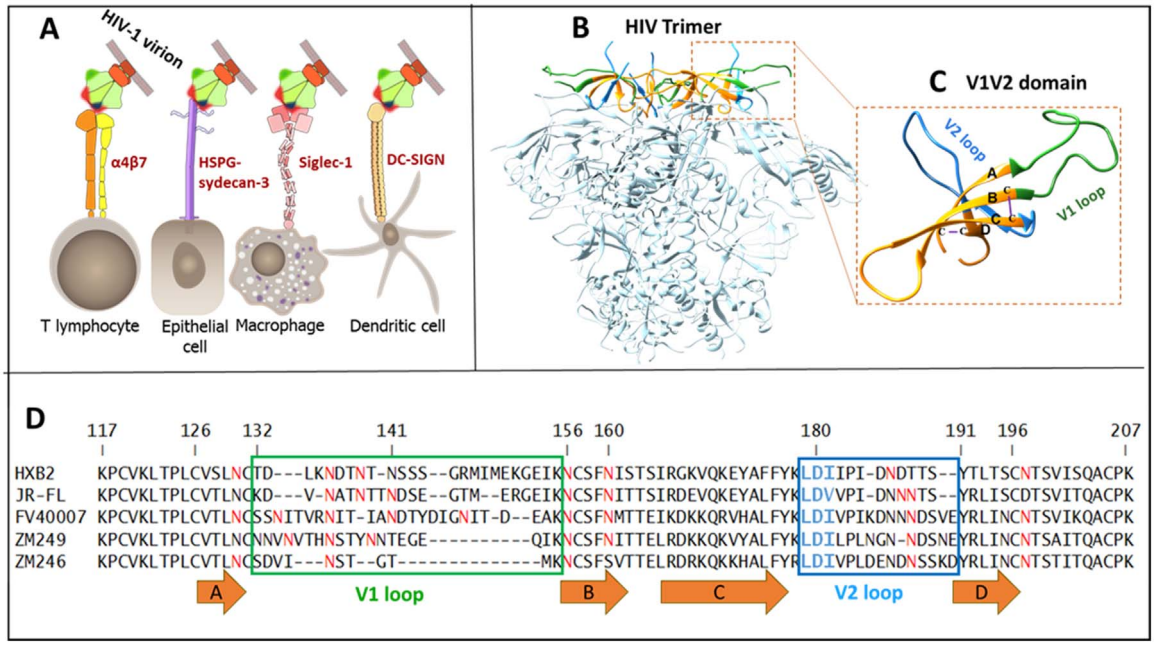

Fig. 1. Structure and function of the V1V2 domain of HIV-1 envelope glycoprotein. *A,* Attachment of HIV-1 virion to host cells may be mediated by interaction of Env V1V2 domain with different surface molecules (attachment factors) present on different host cells. Siglec-1 binds sialic acid moieties on glycans in the V1V2 region. HSPG presented on sydecan-3 binds the V3 loop and has a binding site in the C-strand of the V1V2 region. DC-SIGN binds glycans on gp120 and enhancement of virus infection can be modulated by the V1V2 length, the overall V3 charge, and N-linked glycosylation patterns. One Env trimer of HIV-1 virion is shown; V1V2 domain is shown in red, CD4 binding site in green, and V3 domain in blue. *B,* Structure of the HIV-1 trimer (PDB: 4NCO (Ringe et al., 2013) showing the V1V2 domains at the apex. *C,* V1V2 domain is enlarged. V1 loop is shown in green, β-strands labeled A-D in orange, and V2 loop in blue. The residues missing in the structure are modeled using Swiss-Model web-server by homology with PDB:4NCO. *D,* Sequence alignment of the V1V2 domains used in this study. The β-strands are denoted as orange arrows. Potential N-linked glycosylation sites (NXT/S) are shown in red. The variable loops are boxed (colors correspond to *C*). The conserved LDI/V tripeptide is highlighted in blue. The numbers correspond to amino acids in HXB2 envelope glycoprotein.

out to ~22 nm from the cell surface, it has been implicated in enhancing the transmission competency of HIV-1 at the site of exposure during sexual transmission (Cicala et al., 2011). The V1V2 domain, present at the apex of the HIV-1 envelope spike, is the site for α4ß7 binding (Fig. 1B and C) (Jelicic et al., 2013). Although not essential for HIV-1 infection, the α4ß7:V1V2 interaction has been reported to enhance the efficiency of infection *in vitro* and *in vivo* (Ansari et al., 2011; Li, 2015; Tjomsland et al., 2013). The RV144 trial, the only HIV-1 vaccine trial that showed a modest 31% efficacy, demonstrated correlation between elicitation of V2-specific antibodies and protection against HIV-1 infection (Haynes et al., 2012). In a macaque model of repeated low-dose vaginal challenge, animals treated with anti-α4β7 antibodies were >60% less likely to become infected at any one challenge than the untreated animals (Byrareddy et al., 2014). Furthermore, a direct correlation between the expression of α4ß7 and susceptibility and progression of disease has been observed (Byrareddy et al., 2015). In the most recent study (Byrareddy et al., 2016), blocking of α4ß7 in SIV infected monkeys maintained undetectable viral loads and normal $CD4^+$ T cell counts even after all anti-retroviral therapy (ART) treatment was withdrawn.

The primary receptor and co-receptors of HIV-1 are CD4 and CCR5 (or CXCR4), respectively (Klatzmann et al., 1984; Rizzuto et al., 1998). Both receptors are reported to co-localize with α4ß7 on the surface of CD4+ T-cells in the mucosa (Cicala et al., 2009). The trimeric envelope spike on the virion surface is the entry machine (Kwon et al., 2015; Pancera et al., 2014). Each subunit (protomer) of the spike is a hetero-dimer of the glycoproteins gp120 and gp41 that are produced by cleavage of the envelope glycoprotein (Env) precursor, gp160. gp120 interacts with the CD4 receptor which causes a conformational change that exposes the CCR5 binding site in the V3 domain. Upon binding to CCR5, a series of conformational changes occur in the protomer, resulting in the insertion of the gp41 fusion peptide into the host cell membrane (Klasse, 2012; Weissenhorn et al., 1997). The viral and host lipid bilayers fuse, resulting in entry of the nucleocapsid core into the target cell.

The interacting regions of Env, CD4, and CCR5 have been elucidated in atomic detail (Diskin et al., 2010; Kwon et al., 2015; Kwong et al., 1998), leading to the design of vaccine candidates and antiviral therapeutics that can interfere with these interactions. However, it is unlikely that the HIV-1 virus lands on the CD4/CCR5 receptors. Nor is it likely that the virus can find the CD4 receptor by random interactions with the cell surface, which contains an intricate maze of molecules in which CD4 is probably buried. Therefore, attachment of HIV-1 virion to cell surface molecules such as α4ß7 through the V1V2 domain probably provides a mechanism for virus capture, and to reach the CD4 receptor at the timescales of virus infection. However, very little is known about these putative pre-CD4 V1V2: α4ß7 interactions. The highly conserved LDI/V tripeptide present at the junction of the C β-strand and the V2 loop (Figs. 1C and D) is thought to be important for binding to α4ß7 (Arthos et al., 2008). But it is not clear if LDI/V is part of the binding site or if it is important for the conformation of the V1V2 domain (O'Rourke et al., 2010). Other residues of the V2 loop (Tassaneetrithep et al., 2014), and of the V3 loop (Peachman et al., 2015), were also linked to α4ß7 binding. Complicating the issue is the glycosylation state of the V1V2 domain which contains a cluster of protein N-glycosylation sites. Furthermore, the number of glycosylation sites vary greatly in different strains and subtypes, and also depending on the length of the highly variable V1 and V2 loops. One or a very few transmitter/founder (T/F) viruses that establish the initial infection during HIV-1 transmission are reported to be poorly glycosylated (Derdeyn et al., 2004; Ping et al., 2013). How these T/F viruses are selected remained as an open question. If α4ß7 were to be important for transmission of HIV-1 at the mucosa, as the evidence implicates (Byrareddy et al., 2014), a detailed understanding of the α4ß7:V1V2 interactions is critical to elucidate the mechanisms and to design effective HIV-1 vaccines.

Here, we examined the α4ß7 interaction of a series of HIV-1 envelope proteins (gp140) from different subtypes and strains, in