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Glycan Heterogeneity on HIV-1 Env Decides the Fate of Virus Within Antigen Presentation Cells

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ABSTRACT

HIV-1 envelope glycoprotein (Env) undergoes extensive post-translational glycosylation modifications cloaking the entire exposed Env surface with an assortment of high mannose, hybrid-, and complex-types of N-linked glycans (NLGs). This high density of NLGson HIV Env act as pathogen-associated molecular patterns (PAMPs) that enables the virion interaction with the host soluble and membrane bound carbohydrate-binding proteins (CBPs) particularly c-type lectin receptors (CLRs). CLRs with very fine and precise specificity to interact with the mannose rich glycans expressed on Env are expressed constitutively by various antigen-presenting cells (APCs) strategically located within the sexual route of virus transmission. Many of these CLRs like Langerin, DCIR, DC-SIGN, Mannose receptor (MR), BDCA2 and SIGLEC-1 play a very important role in the recognition, capture and dissemination of the virus within the host. The innate immune cells commonly use the CLRs to capture and internalize HIV for destruction and antigen presentation to T cells. However, HIV evades this CLR-mediated cellular degradation machinery and rather exploits it for transmission to CD4 T cells, the main cell types that host the robust and productive HIV replication. It is not known what determines the fate of virus whether being shuttled to degradative or trans-infection pathway of APCs. In this mini-review we address the relative importance of Env glycan composition and heterogeneity for HIV-1 transmission via the host membrane-associated carbohydrate-binding lectins particularly DC-SIGN and fate of virus being taken up by the cells expressing these CLRs.

Keywords: DC-SIGN, C-type lectin receptors (CLRs), Envelope glycoprotein, Viral degradation, Viral transmission, HIV-1 glycans

INTRODUCTION

Human Immunodeficiency Virus 1 (HIV-1) is the causative agent of Acquired Immunodeficiency Syndrome (AIDS). Despite continued and extensive research efforts, there is still neither a vaccine nor a cure for HIV/AIDS. This justifies a continuous search for novel and effective strategies for the effective treatment of this dreaded infection. The envelope glycoprotein (Env) like other enveloped viruses is the only molecular entity visible on the surface of HIV-1, embedded into the host derived lipid bilayer, mediating the first steps of cell attachment and entry through the primary receptor CD4 and co-receptor CCR5/CXCR4. The HIV-1 Env spike theoretically is the exclusive component of virus accessible to anti-HIV-1 neutralizing antibodies, thus, Env has been a target of intense research as a vaccine immunogen. Being central to viral pathogenicity and primary target of host immune response, the virus has developed extremely sophisticated mechanisms to shield Env spike glycoproteins, rendering it somewhat refractive to combating host defensive responses. HIV-1 Env is one of the known proteins with the highest genetic and antigenic variability. The distal most surface of HIV-1 Env is the main site of recognition by host receptors, which is decorated with poorly antigenic hypervariable loops (V1-V5). In addition, the Env glycoprotein (gp120/gp41 heterotrimer) is one of the most heavily glycosylated proteins found in nature, which is extensively covered with about 91 tightly packed potential N-linked glycosylation

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sites contributing approximately to 50% of the Env molecular mass [1-4]. The coating of the Env's most exposed and antigenically vulnerable surface with poorly antigenic V1-V5 hypervariable loops, together with coating by extraordinary dense glycans which further shield the underlying antigenic surface, play a key role in the determination of Env structure and epitope exposure, which consequently affect the antigenicity, immunogenicity, antibody neutralization, infectivity and receptor binding [5-11]. This type of architecture of the Env with higher levels of variability and glycan shield is the strategy to escape the host selection pressure. However, this unusual extensive degree of HIV-1 Env glycosylation, taken as an immunologically silent shield, masking the preserved functional sites on the HIV-1for more than a decade, has emerged as an amazing target for recognition by broadly neutralizing antibodies (bnAbs) [12,13].

HIV INTERACTION WITH INNATE CELLS

Besides protection by formation of immunologically silent glycan shield, the HIV-1 has evolved to use these glycans to facilitate its infection of host. There is growing evidence that Env also plays a major role in the viral capture, transmission and dissemination during early stages of HIV-1 infection by hijacking the natural functions of the cells of innate immune system, the Langerhans Cells (LCs), Dendritic Cells (DCs) and macrophages, which are strategically located at all the entry sites like epidermis, mucosa, sub-mucosa, lymphoid and circulatory system to facilitate optimal interaction of the virus during sexual transmission, which is the most common cause of HIV infection [14,15]. The enigmatic features of these innate cell interactions with Env are mediated primarily by the membrane bound c-type lectin receptor (CLRs) family like Langerin, DC immunoreceptor (DCIR), DC-specific ICAM-3 grabbing non-integrin (DC-SIGN), Mannose receptor (MR), blood DC antigen 2 (BDCA-2) and Sialic acid-binding immunoglobulin-type lectins (SIGLEC-1), the most important CLRs which recognize exclusively the glycans on the surface of HIV-1 Env [16-22]. The innate cells and the CLRs they express, are spatially and temporally distributed along the sexual transmission pathway to generally form the first line of defense and perform differential functions leading finally to viral internalization and endosomal degradation for efficient antigen presentation, and modulate TLR-induced cytokine expression to enhance the infection of HIV.

C-TYPE LECTIN RECEPTORS

Carbohydrates are the natural ligands of CLRs. The CLRs recognize specific carbohydrate structures by means of one or more carbohydrate recognition domains (CRDs) and are grouped on the basis of the presence of a conserved structural motif in their CRDs. Various CLRs have distinguishable carbohydrate specificity, which are related to their amino acid sequence in their respective CRDs. The innate (DCs and macrophages) cell CLRs primarily interact with pathogens via the recognition of mannose, fucose and glycan carbohydrate structures. The HIV Env is known to be having highest glycan content and in particular has a very high proportion, approximately 98% of glycans on native Env, as immature, unprocessed $Man_{5-9}GlcNAc_2$ type N-glycans, while the fully processed mature $GlcNAc_2Man_3GlcNAc_2$ and the $GlcNAcMan_5GlcNAc_2$ containing N-glycans representing only upto 2% [5,19]. Together, this makes HIV-1 a susceptible target of these CLRs that recognize mostly high mannose type glycans [19,23,24]. Several of the CLRs encountered by HIV-1 in the sexual route like DC-SIGN, MR, Langerin and BDCA-2, exclusively recognize the high mannose glycans on gp120 of HIV-1, thus enhancing the recognition and capture of HIV-1 on these cells. These cells are potent antigen presenting cells that up-take the pathogens, process them and readily migrate to lymph nodes to present the antigen in conjunction with MHC molecules to the naïve CD4+ T-cells. These events are anticipated to play essential role in the initial events of HIV-1 transmission by transporting the virus from the peripheral mucosa to lymph node, the place with concentrated number of CD4+ T-cells, making the interaction of virus with the T-cells favorable with its ultimate targets [25-27].

INTERACTION OF HIV-1 WITH ANTIGEN PRESENTING CELLS AND SUBSEQUENT FATE OF VIRUS

There are two ways DCs direct the transmission of HIV-1 to $CD4^+$T cells: DCs after capturing virus by CLRs, transfer captured virus in the absence of productive infection, which is referred to as in trans-infection or it endocytoses the virus within proteasome-resistant compartments in which the infectious virus is prevented from degradation and are released intact to infect the target cells **(Figure 1)** [28,29]. There are mounting evidences supporting the hypothesis that this hijacking of natural functions of DCs, degradation and presentation of antigens, by HIV-1 is determined by the glycan composition [30-33]. As mentioned, previously, the glycan composition on Env of different viruses is heterogeneous, as glycan maturation is primarily driven by the relative exposure of polypeptide, during folding, to Endoplasmic Reticulum (ER) and Golgi glycosylation modification enzymes. The specific constitution of Env between various HIV-1 strains may play a decisive role in a well-articulated CLR and DC-SIGN binding and transmission efficiency. A recent study showed the importance of the composition of HIV-1 glycans for DC-SIGN-mediated transmission [32-34]. The DC-SIGN as C-type (a type 2) lectin receptor (CLR) with a CRD, binds high-mannose glycans and fucose on the surface of viruses and bacteria, in a Ca2+-dependent manner. DC-SIGN displays enhanced affinity and specifically for high-mannose glycans with terminal Manα1-2 structures on HIV Env [25,35]. Although DC-SIGN serves as a PRR [15,36], yet the HIV-1 can evade the immune system mediated degradation machinery and exploit DC-SIGN for its

transmission to CD4 T cells [15,30,34,37,38]. DC-SIGN interaction with HIV-1 is thus implicated in the initial stages of virus acquisition and spread from the mucosal site of virus entry [39]. However, the relative importance of these CLR expressing cells particularly DC-SIGN in HIV-1 transmission remains controversial. In some studies, antibody blockage of DC-SIGN on DCs was found to reduce virus capture only up to 50% [40,41], but other studies have demonstrated 70%-75% inhibition of virus capture by immature monocyte derived dendritic cells (MDDCs) and almost complete blockage of transmission to CD4 T cells [31]. The siRNA-based knock down of DC-SIGN in immature dendritic cells also been able to decrease the HIV-1 transfer to CD4 T cells by 75% [42].

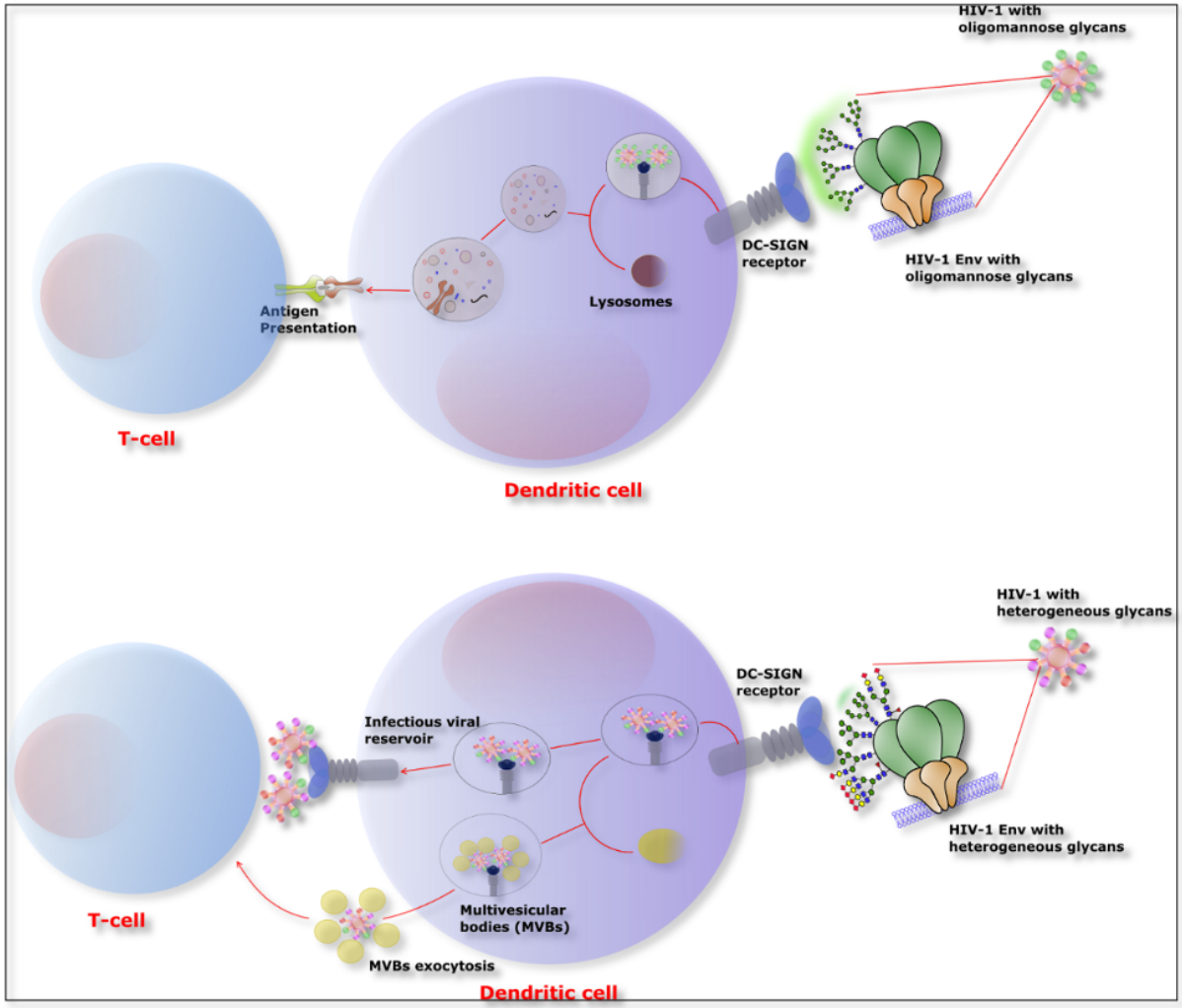

Figure 1. Schematic representation of HIV-1 Env glycan composition governing the balance between virus capture and transmission vs. degradation by antigen presenting cells (APCs). The innate immune cells (macrophages and dendritic cells) recognize HIV-1 via CLRs expressed by these cells lining the mucosal surface of the host. DC-SIGN expressed by mucosal innate immune cells has a very high affinity to bind to oligomannose glycans. It captures the virus, and the downstream processing and fate of virus within these APCs whether directed to viral degradation and presentation pathway towards CD4 cells or transmission and dissemination within the host is determined by the glycan composition of the virus envelope glycoprotein. (A)HIV-1 with Env expressing homogenous oligomannose type of glycans are taken by the APCs via DC-SIGN and endocytosed. Viruses expressing abundantly $Man_{5\text{-}9}GlcNAc_2$ oligomannose type of glycans bind with high affinity to DC-SIGN rendering their release to other cells unlikely. These viruses are shuttled to lysosomal degradative pathway, processed and presented through MHC II to TCR. (B)Viruses with heterogenous glycans of different levels of complexities with complex, hybrid and oligomannose glycans representing the heterogeneity bind to DC-SIGN relatively with lower affinity and avidity. These weakly bound viruses are released from the DC-SIGN and transferred directly through trans-infection pathways. Within APCs the weakly bound viruses are preserved within the endosomal compartments to prevent degradation and protect the insidious reservoir of viral particles. The infectious viral particles within the APCs along with MVBs are transferred to target CD4 cells or the virus is recycled with or without DC-SIGN to the cell surface and transferred to CD4 cells through free virus particles or trans-infection.

The glycan composition expressed on HIV Env is a key factor determining DC-SIGN-mediated virus transmission and fate of virus being taken up by the cells expressing these CLRs. Previously, it has been shown that HIV-1 enriched with high-mannose glycans was captured more efficiently by DC-SIGN and shuttled towards a degradation pathway, which augmented the MHCII-antigen presentation but impeded trans-infection [30]. The process of decorating surface of Env with glycans adds a layer of diversity to the already extremely high level of Env variation found among circulating HIV-1 isolates. At the genetic level, N-linked glycosylation is dictated by the N-X-S/T motif, where X is any amino acid except P. The Env proteins have varying number of these potential N-glycosylation sites (PNGSs), ranging from 23 to 34 per gp160 protomer; the majority of these are in the gp120 surface subunit, whereas only four to eight are in the external domain of the gp41 subunit [4]. Most PNGSs are not conserved. In fact, only six to eight PNGSs found in Env of clades A, B, C, G, and CRF_01.AE are >90% conserved, and for several PNGSs, conservation is <20% **(Figure 2)** [4,43]. The PNGSs on HIV-1 Env are not fully occupied [44,45]. The level of PNGS occupancy is dictated by site accessibility for a series of enzymes participating in the glycan maturation process [46]. The glycosylation pathway is initiated by the addition of $Glc_3Man_9GlcNAc_2$en bloc onto a nascent protein in the ER. As the protein is transported across the ER and the Golgi apparatus, the high-mannose structure is trimmed and subsequently elaborated with hybrid- and complex-type glycans [19]. In contrast to cellular glycoproteins, which are usually adorned with mature complex-type glycans, virus Env carries all three glycan types, including early and intermediate high-mannose, intermediate hybrid, and mature complex glycans [47-49]. In fact, various glycan types and glycoforms are found in proportions that vary depending on Env strain and host cell type [4,23,44,45,50]. On soluble and membrane-anchored Env mimics, the high-mannose-type glycans range from 60–70%, with the most prominent being the least processed Man9GlcNAc2 glycans at 20–40% [19,47,48]. Analysis of soluble, uncleaved, prefusion-optimized BG505 Env gp140 trimers produced in 293F cells, similarly has shown that 56% are high-mannose type composed of Man5 (6%), Man6 (3%), Man7 (6%), Man8 (15%), and Man9 (26%) [51] hen the same proteins were produced in ExpiCHO cells, the total oligomannose content increased to 64%, with observable changes in glycoform proportions. Site-specific analysis further revealed that each PNGS on gp120 incorporated multiple glycoforms of only the oligomannose type or a mix of oligomannose and complex type, whereas the gp41 PNGSs had mainly complex type glycoforms [46,51].

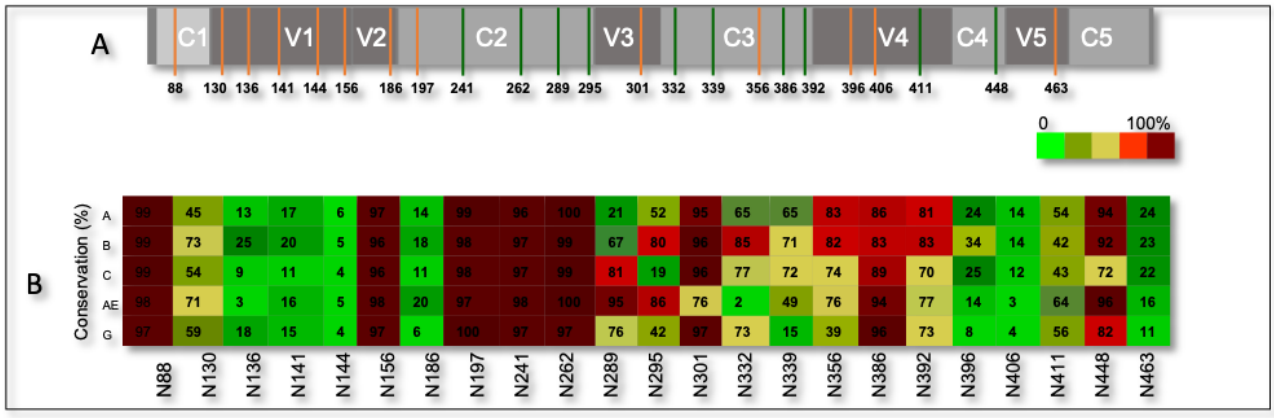

Figure 2. Schematic representation of glycan distribution, occupancy and conservation on HIV-1 gp120. (A) The distribution of potential N-linked glycosylation sites (PNGSs) encoded by N-X-S/T (X#P) of $gp120_{Bal}$. The various PNGSs are represented as putative complex type (orange) or putative oligomannose-type (green) based on previous findings [54-56]. (B) Percent conservation and occupancy of the various PNGSs across different clades calculated from Env sequences available from LANL database. (Adapted from Pritchard et al. 2015) [43].

The abundance of high-mannose glycans on HIV-1 Env and predominant binding of high-mannose glycans by DC-SIGN has been implicated in viral capture upon exposure and viral dissemination within the host. The interaction between HIV Env and DC-SIGN is influenced to a great extent by the glycan composition of virus [30,33,52]. Our recent findings also provide supporting evidence that virions carrying gp120 with higher numbers of oligomannose-type glycans are more efficiently endocytosed through DC-SIGN and more proficiently processed for antigen presentation than HIV-1 containing gp120 with heterogeneous glycans [34]. The transmission of oligomannose-enriched HIV-1 was relatively inefficient. Thus, the expression of oligomannose by HIV-1 enhances capture of DC-SIGN and transmission, but presence of heterogenous-glycansnegatively affect transmission by enhancing viral degradation **(Figure 1)**. The mechanisms by which hybrid and complex glycans help HIV-1 evade degradation are not well understood yet. DC-

SIGN has been shown to shuttle HIV-1 bearing high-mannose glycans to the degradative pathway, similar to other pathogens with such glycans [30]; this was indicated by an increased association with vesicles containing early and late endosomal markers and by more efficient antigen processing and presentation to MHCII-restricted CD4 T cells [30,34,53]. This study demonstrates that HIV-1 evades this degradative pathway by signals that prevent virus transport to endolysosomal compartments and preserve infectious virions on dendrites to promote their transfer to T cells [34].

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