





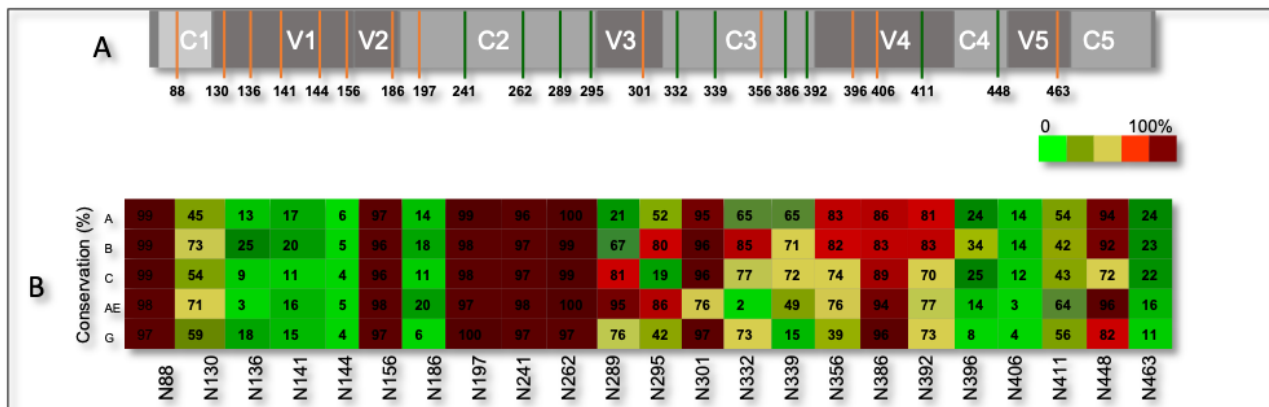
transmission to CD4 T cells [15,30,34,37,38]. DC-SIGN interaction with HIV-1 is thus implicated in the initial stages of virus acquisition and spread from the mucosal site of virus entry [39]. However, the relative importance of these CLR expressing cells particularly DC-SIGN in HIV-1 transmission remains 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<sub>5-9</sub>GlcNAc<sub>2</sub> 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 CLR. 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<sub>3</sub>Man<sub>9</sub>GlcNAc<sub>2</sub>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 Man<sub>9</sub>GlcNAc<sub>2</sub> 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 Man<sub>5</sub> (6%), Man<sub>6</sub> (3%), Man<sub>7</sub> (6%), Man<sub>8</sub> (15%), and Man<sub>9</sub> (26%) [51] when 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<sub>Bal</sub>. 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 heterogeneous-glycans negatively 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].

## REFERENCES

- Lee JH, Ozorowski G, Ward AB (2016) Cryo-EM structure of a native, fully glycosylated, cleaved HIV-1 envelope trimer. *Science* 351: 1043-1048.
- Rudd PM, Dwek RA (1997) Glycosylation: Heterogeneity and the 3D structure of proteins. *Crit Rev Biochem Mol Biol* 32: 1-100.
- Jones GBS, Soto C, Lemmin T, Chuang GY, Druz A, et al. (2016) Trimeric HIV-1-Env Structures Define Glycan Shields from Clades A, B, and G. *Cell* 165: 813-826.
- Go EP, Ding H, Zhang S, Ringe RP, Nicely N, et al. (2017) Glycosylation benchmark profile for HIV-1 envelope glycoprotein production based on eleven env trimers. *J Virol* 91.
- Binley JM, Ban YE, Crooks ET, Eggink D, Osawa K, et al. (2010) Role of complex carbohydrates in human immunodeficiency virus type 1 infection and resistance to antibody neutralization. *J Virol* 84: 5637-5655.
- Crooks ET, Tong T, Chakrabarti B, Narayan K, Georgiev IS, et al. (2015) Vaccine-Elicited Tier 2 HIV-1 neutralizing antibodies bind to quaternary epitopes involving glycan-deficient patches proximal to the cd4 binding site. *PLoS Pathog* 11: e1004932.
- Kumar R, Tuen M, Liu J, Nadas A, Pan R, et al. (2013) Elicitation of broadly reactive antibodies against glycan-modulated neutralizing V3 epitopes of HIV-1 by immune complex vaccines. *Vaccine* 31: 5413-5421.
- Sanders RW, van Anken E, Nabatov AA, Liscaljet IM, Bontjer I, et al. (2008) The carbohydrate at asparagine 386 on HIV-1 gp120 is not essential for protein folding and function but is involved in immune evasion. *Retrovirology*. 5: 10.
- Townsley S, Mohamed Z, Guo W, McKenna J, Cleveland B, et al. (2016) Induction of heterologous tier 2 HIV-1-Neutralizing and cross-reactive v1/v2-specific antibodies in rabbits by prime-boost immunization. *J Virol* 90: 8644-8660.
- Wei X, Decker JM, Wang S, Hui H, Kappes JC, et al. (2003) Antibody neutralization and escape by HIV-1. *Nature* 422: 307-312.
- Reitter JN, Means RE, Desrosiers RC (1998) A role for carbohydrates in immune evasion in AIDS. *Nat Med* 4: 679-684.
- Lavine CL, Lao S, Montefiori DC, Haynes BF, Sodroski JG, et al. (2012) High-mannose glycan-dependent epitopes are frequently targeted in broad neutralizing antibody responses during human immunodeficiency virus type 1 infection. *J Virol* 86: 2153-2164.
- Walker LM, Huber M, Doores KJ, Falkowska E, Pejchal R, et al. (2011) Broad neutralization coverage of HIV by multiple highly potent antibodies. *Nature* 477: 466-470.
- Garcia-Vallejo JJ, van Kooyk Y (2013) The physiological role of DC-SIGN: A tale of mice and men. *Trends Immunol* 34: 482-486.
- Geijtenbeek TB, Torensma R, van Vliet SJ, van Duijnhoven GC, Adema GJ, et al. (2000) Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses. *Cell* 100: 575-585.
- .Useros NI, Lorizate M, Puertas MC, Plata MTR, Zangger N, et al. (2012) Siglec-1 is a novel dendritic cell receptor that mediates HIV-1 trans-infection through recognition of viral membrane gangliosides. *PLoS Biol* 10: e1001448.
- Lambert AA, Gilbert C, Richard M, Beaulieu AD, Tremblay MJ (2008) The C-type lectin surface receptor DCIR acts as a new attachment factor for HIV-1 in dendritic cells and contributes to trans- and cis-infection pathways. *Blood* 112: 1299-1307.
- .Zolt DP, Perez JP, Erkizia I, Benet S, Pino M, et al. (2019) Dendritic Cells From the Cervical Mucosa Capture and Transfer HIV-1 via Siglec-1. *Front Immunol* 10: 825.
- Doores KJ, Bonomelli C, Harvey DJ, Vasiljevic S, Dwek RA, et al. (2010) Envelope glycans of immunodeficiency virions are almost entirely oligomannose antigens. *Proc Natl Acad Sci USA* 107: 13800-13805.
- Platt EJ, Wehrly K, Kuhmann SE, Chesebro B, Kabat D, et al. (1998) Effects of CCR5 and CD4 cell surface concentrations on infections by macrophagetropic isolates of human immunodeficiency virus type 1. *J Virol* 72: 2855-2864.
- Li Y, Svehla K, Mathy NL, Voss G, Mascola JR, et al. (2006) Characterization of antibody responses elicited by human immunodeficiency virus type 1 primary

- isolate trimeric and monomeric envelope glycoproteins in selected adjuvants. *J Virol* 80: 1414-1426.
22. Li M, Gao F, Mascola JR, Stamatatos L, Polonis VR, et al. (2005) Human immunodeficiency virus type 1 env clones from acute and early subtype B infections for standardized assessments of vaccine-elicited neutralizing antibodies. *J Virol* 79: 10108-10125.
  23. Bonomelli C, Doores KJ, Dunlop DC, Thaney V, Dwek RA, et al. (2011) The glycan shield of HIV is predominantly oligomannose independently of production system or viral clade. *PLoS One* 6: e23521.
  24. Doores KJ (2015) The HIV glycan shield as a target for broadly neutralizing antibodies. *FEBS J* 282(24): 4679-4691.
  25. Feinberg H, Castelli R, Drickamer K, Seeberger PH, Weis WI, et al. (2007) Multiple modes of binding enhance the affinity of DC-SIGN for high mannose N-linked glycans found on viral glycoproteins. *J Biol Chem* 282: 4202-4209.
  26. van Liempt E, Bank CM, Mehta P, Vallejo JJG, Kawar ZS, et al. (2006) Specificity of DC-SIGN for mannose- and fucose-containing glycans. *FEBS Lett* 580: 6123-6131.
  27. Vallejo JJG, van Liempt E, da Costa Martins P, Beckers C, van het Hof B, et al. (2008) DC-SIGN mediates adhesion and rolling of dendritic cells on primary human umbilical vein endothelial cells through LewisY antigen expressed on ICAM-2. *Mol Immunol* 45: 2359-2369.
  28. Lekkerkerker AN, van Kooyk Y, Geijtenbeek TB (2006) Viral piracy: HIV-1 targets dendritic cells for transmission. *Curr HIV Res* 4: 169-176.
  29. Yokota YT, Muhsen M (2013) Development of human dendritic cells and their role in HIV infection: antiviral immunity versus HIV transmission. *Front Microbiol* 4: 178.
  30. van Montfort T, Eggink D, Boot M, Tuen M, Hioe CE, et al. (2011) HIV-1 N-glycan composition governs a balance between dendritic cell-mediated viral transmission and antigen presentation. *J Immunol* 187: 4676-4685.
  31. van Montfort T, Nabatov AA, Geijtenbeek TB, Pollakis G, Paxton WA, et al. (2007) Efficient capture of antibody neutralized HIV-1 by cells expressing DC-SIGN and transfer to CD4+ T lymphocytes. *J Immunol* 178: 3177-3185.
  32. Jan M, Upadhyay C, Pertejo JA, Hioe CE, Arora SK, et al. (2018) Heterogeneity in glycan composition on the surface of HIV-1 envelope determines virus sensitivity to lectins. *PLoS One* 13: e0194498.
  33. Jan M, Upadhyay C, Hioe CE (2019) HIV-1 envelope glycan composition as a key determinant of efficient virus transmission via DC-SIGN and resistance to inhibitory lectins. *iScience* 21: 413-427.
  34. Jan M, Arora SK (2017) Innate Sensing of HIV-1 by dendritic cell-specific ICAM-3 grabbing nonintegrin on dendritic cells: Degradation and presentation versus transmission of virus to T cells is determined by glycan composition of viral envelope. *AIDS Res Hum Retroviruses* 33: 765-767.
  35. Mitchell DA, Fadden AJ, Drickamer K (2001) A novel mechanism of carbohydrate recognition by the C-type lectins DC-SIGN and DC-SIGNR. Subunit organization and binding to multivalent ligands. *J Biol Chem* 276: 28939-28945.
  36. Geijtenbeek TB, Krooshoop DJ, Bleijs DA, van Vliet SJ, van Duijnhoven GC, et al. (2000) DC-SIGN-ICAM-2 interaction mediates dendritic cell trafficking. *Nat Immunol* 1: 353-357.
  37. Baribaud F, Doms RW, Pohlmann S (2002) The role of DC-SIGN and DC-SIGNR in HIV and Ebola virus infection: Can potential therapeutics block virus transmission and dissemination? *Expert Opin Ther Targets* 6: 423-431.
  38. Manel N, Hogstad B, Wang Y, Levy DE, Unutmaz D, et al. (2010) A cryptic sensor for HIV-1 activates antiviral innate immunity in dendritic cells. *Nature* 467: 214-217.
  39. Geijtenbeek TB, van Kooyk Y (2003) DC-SIGN: A novel HIV receptor on DCs that mediates HIV-1 transmission. *Curr Top Microbiol Immunol* 276: 31-54.
  40. Burleigh L, Lozach PY, Schiffer C, Staropoli I, Pezo V, et al. (2006) Infection of dendritic cells (DCs), not DC-SIGN-mediated internalization of human immunodeficiency virus, is required for long-term transfer of virus to T cells. *J Virol* 80: 2949-2957.
  41. Cambi A, de Lange F, van Maarseveen NM, Nijhuis M, Joosten B, et al. (2004) Microdomains of the C-type lectin DC-SIGN are portals for virus entry into dendritic cells. *J Cell Biol* 164: 145-155.
  42. Arrighi JF, Pion M, Garcia E, Escola JM, van Kooyk Y, et al. (2004) DC-SIGN-mediated infectious synapse formation enhances X4 HIV-1 transmission from dendritic cells to T cells. *J Exp Med* 200: 1279-1288.
  43. Pritchard LK, Spencer DI, Royle L, Bonomelli C, Seabright GE, et al. (2015) Glycan clustering stabilizes the mannose patch of HIV-1 and preserves vulnerability to broadly neutralizing antibodies. *Nat Commun* 6: 7479.
  44. Cao L, Pauthner M, Andrabi R, Rantalainen K, Berndsen Z, et al. (2018) Differential processing of HIV

- envelope glycans on the virus and soluble recombinant trimer. *Nat Commun* 9: 3693.
45. Struwe WB, Chertova E, Allen JD, Seabright GE, Watanabe Y, et al. (2018) Site-Specific Glycosylation of Virion-Derived HIV-1 Env Is Mimicked by a Soluble Trimeric Immunogen. *Cell Rep* 24: 1958-1966 e5.
46. Cao L, Diedrich JK, Kulp DW, Pauthner M, He L, et al. (2017) Global site-specific N-glycosylation analysis of HIV envelope glycoprotein. *Nat Commun* 8: 14954.
47. Behrens AJ, Vasiljevic S, Pritchard LK, Harvey DJ, Andev RS, et al. (2016) Composition and Antigenic Effects of Individual Glycan Sites of a Trimeric HIV-1 Envelope Glycoprotein. *Cell Rep* 14: 2695-2706.
48. Go EP, Herschhorn A, Gu C, Menendez LC, Zhang S, et al. (2015) Comparative analysis of the glycosylation profiles of membrane-anchored hiv-1 envelope glycoprotein trimers and soluble gp140. *J Virol* 89: 8245-8257.
49. Kong L, Sheppard NC, Jones GBES, Robson CL, Chen H, et al. (2010) Expression-system-dependent modulation of HIV-1 envelope glycoprotein antigenicity and immunogenicity. *J Mol Biol* 403: 131-147.
50. Torrents de la Pena A, Rantalainen K, Cottrell CA, Allen JD, van Gils MJ, et al. (2019) Similarities and differences between native HIV-1 envelope glycoprotein trimers and stabilized soluble trimer mimetics. *PLoS Pathog* 15: e1007920.
51. He L, Kumar S, Allen JD, Huang D, Lin X, et al. (2018) HIV-1 vaccine design through minimizing envelope metastability. *Sci Adv* 4: eaau6769.
52. Guo Y, Feinberg H, Conroy E, Mitchell DA, Alvarez R, et al. (2004) Structural basis for distinct ligand-binding and targeting properties of the receptors DC-SIGN and DC-SIGNR. *Nat Struct Mol Biol* 11: 591-598.
53. Gringhuis SI, den Dunnen J, Litjens M, van der Vlist M, Geijtenbeek TB, et al. (2009) Carbohydrate-specific signaling through the DC-SIGN signalosome tailors immunity to *Mycobacterium tuberculosis*, HIV-1 and *Helicobacter pylori*. *Nat Immunol* 10: 1081-1088.
54. Cutalo JM, Deterding LJ, Tomer KB (2004) Characterization of glycopeptides from HIV-1(SF2) gp120 by liquid chromatography mass spectrometry. *J Am Soc Mass Spectrom* 15: 1545-1555.
55. Go EP, Chang Q, Liao HX, Sutherland LL, Alam SM, et al. (2009) Glycosylation site-specific analysis of clade C HIV-1 envelope proteins. *J Proteome Res* 8: 4231-4242.
56. Pabst M, Chang M, Stadlmann J, Altmann F (2012) Glycan profiles of the 27 N-glycosylation sites of the HIV envelope protein CN54gp140. *Biol Chem* 393: 719-730.