Exclusive Decoration of Simian Immunodeficiency Virus Env with High-Mannose Type N-Glycans Is Not Compatible with Mucosal Transmission in Rhesus Macaques

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I nfectious disease, human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) envelope (Env) proteins are extensively decorated with N-glycans, predominantly of the high-mannose type. However, it is unclear how high-mannose N-glycans on Env impact viral spread. We show that exclusive modification of SIV Env with these N-glycans reduces viral infectivity and abrogates mucosal transmission, despite increasing viral capture by immune cell lectins. Thus, high-mannose N-glycans have opposing effects on SIV infectivity and lectin reactivity, and a balance might be required for efficient mucosal transmission.

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for capsid protein content revealed that production of HIV-1 NL4-3, SIVmac239, and SIVmac239/316 Env in GnTI−/H11002 cells diminished viral infectivity (Fig. 1). In contrast, HIV-1 JR-CSF from GnTI−/H11002 and control cells displayed similar infectivity (Fig. 1). Thus, generation of virions in cells that exclusively produce high-mannose type N-glycans can reduce HIV and SIV infectivity, but this effect depends on the molecular clone/strain studied.

SIVmac239/316 Env produced in GnTI−/H11002 cells harbors exclusively high-mannose type N-glycans. We focused our subsequent analyses on SIVmac239/316 Env because this virus showed the most profound reduction in infectivity when produced in GnTI− cells. First, we confirmed that virions generated in GnTI− cells (termed GnTI-SIV) and control cells (termed wt SIV) are differentially glycosylated. For this, viral particles were concentrated by centrifugation through a sucrose cushion and analyzed by Western blotting (Fig. 2A). Both virus preparations contained more gp160 than proteolytically processed gp120. This effect was not unexpected since earlier data demonstrated reduced cleavage efficiency when large amounts of Env are produced (29). Moreover, gp160/gp120 proteins of GnTI-SIV migrated faster than their counterparts of wt SIV (Fig. 2A), in keeping with incorporation of high-mannose type N-glycans, which exhibit a lower molecular weight than hybrid- or complex-type N-glycans. Indeed, digest with endo-β-N-acetylglucosaminidase H (endo H), which selectively removes high-mannose type N-glycans, and peptide-N-glycosidase F (PNGase F), which removes all N-linked glycans, confirmed that gp160/gp120 from GnTI-SIV but not wt SIV exclusively harbored high-mannose type N-glycans (Fig. 2A). The slightly faster migration of GnTI-SIV Env bands upon endo H relative to PNGase F treatment most likely reflects a more complete digest. Finally, multiplex capillary gel electrophoresis with laser-induced fluorescence detection (xCGE-LIF) confirmed that gp120 from GnTI-SIV was exclusively modified with high-mannose type N-glycans (not shown). Collectively, our results show that Env of GnTI-SIV is exclusively decorated with high-mannose type N-glycans.

Reduced incorporation of mature Env into GnTI-SIV virions does not account for reduced infectivity. A potential explanation for the low infectivity of GnTI-SIV could be altered incorporation of Env into the virion. The Western blot analysis described above was therefore repeated with virus preparations normalized for capsid protein content. The results showed that roughly 10-fold less gp120 was present in GnTI-SIV relative to wt SIV particles (Fig. 2B), raising the question whether the reduced virion incor-
poration of mature Env may account for the diminished viral infectivity of GnTI-SIV. To clarify this issue, we produced lentiviral pseudotypes in cells transfected to express rising amounts of Env and analyzed particle incorporation of Env (Fig. 2C) as well as infection of target cells (Fig. 2D). In general, pseudotypes generated in control cells were more infectious than their counterparts released from GnTI/H11002 cells. In particular, pseudotypes from control cells, which harbored roughly comparable amounts of gp120 relative to pseudotypes generated in GnTI/H11002 cells (Fig. 2C, compare lanes 4 and 12), were 23-fold more infectious (Fig. 2D), indicating that the reduced infectivity of GnTI-SIV relative to wt SIV was not due to reduced particle incorporation of Env.

**Increased binding of GnTI-SIV to high-mannose-specific lectins but absence of mucosal transmission.** Binding of Env to soluble lectins can block viral entry into target cells, while engagement of membrane-associated, mucosal lectins might augment mucosal transmission (11, 30). Therefore, we examined whether GnTI-SIV exhibits altered lectin reactivity compared to wt SIV. We found that GnTI-SIV was more sensitive toward inhibition by the soluble mannose-specific lectins cyanovirin-N (CV-N) and Galanthus nivalis agglutinin (GNA) than wt SIV (Fig. 3A). In contrast, infectivity of GnTI-SIV and wt SIV was slightly and comparably augmented by Ulex europaeus agglutinin (UEA), which recognizes fucose (Fig. 3A). Thus, GnTI-SIV is more readily inhibited by mannose-specific lectins than wt SIV, in line with the exclusive modification of GnTI-SIV with high-mannose type N-glycans.

The results obtained so far raised the question of whether the decreased viral infectivity for indicator cells or the increased capture by mucosal lectins would be predictive for the efficiency of mucosal transmission of GnTI-SIV. Therefore, we examined
FIG 3 GnTI-SIV is more sensitive to neutralization by soluble, mannose-specific lectins and is better transmitted by mucosal lectins than wt SIV. (A) Infectivity-normalized stocks of wt SIV, GnTI-SIV, HIV-1 NL4-3 (positive control), and HIV-1 NL4-3 pseudotyped with the G protein of vesicular stomatitis virus (VSV-G [negative control]) were incubated with phosphate-buffered saline (PBS) or lectins prior to infection of TZM-bl indicator cells. Input virus was removed at 5 h, and β-galactosidase activity in cell lysates was analyzed at 72 h postinfection. The results of a representative experiment performed with triplicate samples are shown. Infectivity in the absence of lectin was set as 100%, and error bars indicate SD. Similar results were obtained in at least one independent experiment. (B) Parental Raji B cells (control) or Raji cells engineered to express DC-SIGN (SIGN) or DC-SIGNR (SIGNR) were incubated with equal volumes of wt SIV or GnTI-SIV stocks normalized for infectivity. Unbound virus was removed by washing, the transmitter cell lines were cocultured with the CEMx174 R5 target cells, and the luciferase activity in the cell lysates was measured at 72 h post-cocultivation. The results of a single experiment performed with triplicate samples are shown and are representative of two separate experiments. Error bars indicate SD. *, P ≤ 0.05; **, P ≤ 0.01. c.p.s., counts per second; UEA, Ulex europaeus agglutinin; CV-N, cyanovirin-N; GNA, Galanthus nivalis agglutinin.
GnTI-SIV and wt SIV transmission to rhesus macaques upon repeated rectal inoculation. For this, three rhesus macaques per group were exposed to escalating doses of GnTI-SIV and wt SIV stocks, respectively. The stocks were normalized for equal capsid protein content, and animals were exposed to virus until they became SIV RNA positive or until the maximal virus dose applicable under the chosen conditions was reached. All animals exposed to wt SIV acquired infection (Fig. 4A) and showed peak viral loads of between $10^5$ and $10^8$ copies per ml (Fig. 4B), which matches previous findings obtained with intravenously inoculated SIVmac239/316 Env (33). In contrast, none of the animals inoculated with GnTI-SIV became SIV RNA positive or seroconverted (not shown), despite exposure to inocula containing up to 433 ng SIV capsid antigen. These findings suggest that GnTI-SIV is unable to overcome the mucosal barrier despite augmented interactions with mannose-specific lectins.

The impact of high-mannose type N-glycans on HIV/SIV infectivity was examined previously, and partially inconsistent results were obtained. A negative correlation between the amount of high-mannose glycans on Env and viral infectivity has been reported based on the analysis of host-cell-specific glycosylation differences (19–21). Moreover, removal of high-mannose type N-glycans from SIV Env was shown to increase viral infectivity (19), and blockade of glycan processing by a mannosidase I inhibitor was demonstrated to reduce HIV-1 YU-2 infectivity without diminishing virion incorporation of Env (23). In contrast to these findings, a separate study documented that HIV-1 LAI and JR-CSF from GnTI− and control cells were comparably infectious (25). The same authors showed that vectors bearing HIV and SIV Env proteins displayed reduced infectivity upon generation in GnTI− cells (24). However, it was concluded that the diminished infectivity was due to diminished particle incorporation of Env and not altered Env glycosylation (24).

We found that HIV-1 NL4-3, SIVmac239, and SIVmac239/316 Env, but not HIV-1 JR-CSF, from GnTI− cells were less infectious than control viruses. The reduced infectivity of SIVmac239/316 Env (GnTI-SIV) relative to wt SIV was paralleled by a reduction of particle incorporation of gp120, but analysis of pseudotypes containing comparable amounts of gp120 revealed that modification with high-mannose type N-glycans and not reduced Env incorporation accounted for the diminished infectivity of GnTI-SIV. We cannot exclude that Env incorporation into HIV-1 NL4-3 and
SIVmac239 from GnTI− cells was altered relative to control viruses and contributed to the reduced infectivity. However, our findings and previous studies are most compatible with the concept that extensive decoration of HIV and SIV Env proteins with high-mannose type N-glycans can diminish viral infectivity in a viral strain/molecular clone-dependent fashion.

The lectin DC-SIGN binds to glycans on HIV Env and contributes to the ability of dendritic cells to capture and transmit infectious HIV to target T cells in vitro (31, 34), although the extent of the contribution is controversial (35–37), and it should be noted that DC-SIGN can also target HIV for degradation (9, 10). Moreover, DC-SIGN can facilitate the establishment of infectious synapses between dendritic cells and T cells (38) and can shape immune responses in a glycan-dependent fashion (8). Thus, DC-SIGN+ dendritic cells might play an important role in mucosal HIV/SIV transmission. DC-SIGN binds to mannose- and fucose-containing glycans (39, 40), and the increased DC-SIGN- and DC-SIGNR-dependent transfer of GnTI-SIV relative to wt SIV was therefore not unexpected. In fact, similar results were obtained by three separate studies (23, 25, 41), although, importantly, two noted that production of virus in GnTI− cells increased HIV-1 capture by DC-SIGN but not transmission (25, 41). In fact, HIV-1 NL4-3 from GnTI− cells was slightly less efficiently transmitted than control virus (not shown), suggesting that the impacts of high-mannose type N-glycans on viral transmission might differ between SIV and HIV and/or between viral isolates. In sum, the quantity and quality of the glycocalyx are believed to impact mucosal transmission of HIV and SIV, and the forced incorporation of high-mannose type N-glycans into Env increased SIV reactivity with DC-SIGN and potentially other mucosal lectins.

The repetitive rectal exposure of rhesus macaques to escalating doses of wt SIV established infection in all inoculated animals, although an unexpectedly high number of inoculations was required. In stark contrast, none of the GnTI-SIV-exposed animals became infected even after the viral dose was escalated to the maximum, which was applied two times (inoculations 20 and 21) and which contained 7.5-fold more infectious units than were minimally required to establish infection with wt SIV. These findings can be most easily reconciled with a scenario in which reduced SIV infectivity due to extensive decoration of Env with high-mannose type N-glycans might be incompatible with mucosal transmission, a process that encompasses penetration of the mucosa and establishment of a founder virus population (42), and cannot be rescued by augmented lectin reactivity. Our results await confirmation with other SIVs, and it needs to be considered that the effects of high-mannose type N-glycans on infectivity and transmission might differ between SIV and HIV and between viral isolates. Moreover, it cannot be excluded that the reduced Env incorporation into GnTI-SIV relative to wt SIV impacted the efficiency of mucosal transmission. Collectively, however, the results from our study suggest that incorporation of high-mannose type N-glycans into Env has opposed effects on SIV infectivity and lectin-mediated transfer, which may need to be in balance for efficient mucosal transmission. Tipping this balance toward optimal binding to mannose-recognizing lectins may not be compatible with penetrating the mucosal barrier and/or establishing a founder virus population.

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The authors declare they have no competing financial interests.

**REFERENCES**

1. UNAIDS. Global AIDS epidemic facts and figures. http://www.unaids.org/en/resources/documents/2014/20140716_FactSheet_en.pdf. Accessed 25 February 2015.

2. Wilen CB, Tilton JC, Doms RW. 2012. HIV: cell binding and entry. Cold Spring Harb Perspect Med 2:a006866. http://dx.doi.org/10.1101/cshperspect.a006866.

3. Haqian AA, Tilton JC. 2013. Entry inhibitors and their use in the treatment of HIV-1 infection. Antiviral Res 98:158–170. http://dx.doi.org/10.1016/j.antiviral.2013.03.017.

4. Hallenberg S, Bosch V, Angliker H, Shaw E, Klenk HD, Garten W. 1992. Inhibition of furin-mediated cleavage activation of HIV-1 glycoprotein gp160. Nature 360:358–361. http://dx.doi.org/10.1038/360358a0.

5. Scanlan CN, Offer J, Zitzmann N, Dwek RA. 2007. Exploiting the defensive sugars of HIV-1 for drug and vaccine design. Nature 446:1038–1045. http://dx.doi.org/10.1038/nature05818.

6. Wei X, Decker JM, Wang S, Hui H, Kappes JC, Wu X, Salazar-Gonzalez JF, Salazar MG, Kilby JM, Saag MS, Komarova NL, Nowak MA, Hahn BH, Kwong PD, Shaw GM. 2003. Antibody neutralization and escape by HIV-1. Nature 422:307–312. http://dx.doi.org/10.1038/nature01470.

7. Tsegaye TS, Pohlmann S. 2010. The multiple facets of HIV attachment to dendritic cell lectins. Cell Microbiol 12:1553–1561. http://dx.doi.org/10.1111/j.1462-5822.2010.01519.x.

8. Gringhuis SI, den Dunnen J, Litiens M, van der Vlist M, Geijtenbeek TB. 2009. Carbohydrate-specific signaling through the DC-SIGN signalingosome tailors immunity to Mycobacterium tuberculosis, HIV-1 and Helicobacter pylori. Nat Immunol 10:1081–1088. http://dx.doi.org/10.1038/ni.1778.

9. Moris A, Nobile C, Buseyne F, Porrot F, Abastado JP, Schwartz O. 2004. DC-SIGN promotes exogenous MHC-I-restricted HIV-1 antigen presentation. Blood 103:2648–2654. http://dx.doi.org/10.1182/blood-2003-07-2532.

10. Turville SG, Santos JJ, Frank I, Cameron PU, Wilkinson J, Miranda-Saksena M, Dable J, Stossel H, Romani N, Piatak M, Jr, Lifson JD, Pope M, Cunningham AL. 2004. Immunodeficiency virus uptake, turnover, and 2-phase transfer in human dendritic cells. Blood 103:2170–2179. http://dx.doi.org/10.1182/blood-2003-09-3129.

11. Van Breedam W, Pohlmann S, Favreole HW, de Groot RJ, Nauwynck HJ. 2014. Bitter-sweet symphony: glycan-lectin interactions in virus biology. FEMS Microbiol Rev 38:598–632. http://dx.doi.org/10.1111/1574-6976.12052.

12. Go EP, Chang Q, Liao HW, Sutherland LL, Alam SM, Haynes BF, Desaire H. 2009. Glycosylation site-specific analysis of clade C HIV-1 envelope proteins. J Proteome Res 8:4231–4242. http://dx.doi.org/10.1021/pr9002728.

13. Go EP, Hewawasam G, Liao HW, Chen H, Ping LH, Anderson JA, Hua DC, Haynes BF, Desaire H. 2011. Characterization of glycosylation profiles of HIV-1 transmitted/founder envelopes by mass spectrometry. J Virol 85:8270–8284. http://dx.doi.org/10.1128/JVI.05053-11.

14. Zhu X, Borchers C, Bienstock RJ, Tomer KB. 2000. Mass spectrometric characterization of the glycosylation pattern of HIV-gp120 expressed in CHO cells. Biochemistry 39:11194–11204. http://dx.doi.org/10.1021/bi000432m.

15. Bonomelli C, Doores KJ, Dunlop DC, Thaney V, Dwek RA, Burton DR, Crispin M, Scanlan CN. 2011. The glycan shield of HIV is predominantly
oligomannose independently of production system or viral clade. PLoS One 6:e23521. http://dx.doi.org/10.1371/journal.pone.0023521.

16. Doores KJ, Bonomelli C, Harvey DJ, Vasilevic S, Dwek RA, Burton DR, Crispin M, Scanlan GN. 2010. Envelope glycans of immunodeficiency virions are almost entirely oligomannose antigens. Proc Natl Acad Sci USA 107:13800–13805. http://dx.doi.org/10.1073/pnas.1006498107.

17. Pritchard LK, Harvey DJ, Bonomelli C, Crispin M, Doores KJ. 2015. Cell- and protein-directed glycosylation of native cleaved HIV-1 envelope. J Virol 89:8932–8944. http://dx.doi.org/10.1128/JVI.01190-15.

18. Pritchard LK, Vasilevic S, Ozorowski G, Seabright GE, Cupo A, Ringe R, Kim HJ, Sanders RW, Doores KJ, Burton DR, Wilson IA, Ward AB, Moore JP, Crispin M. 2015. Structural constraints determine the glycosylation of HIV-1 envelope trimers. Cell Rep 11:1604–1613. http://dx.doi.org/10.1016/j.celrep.2015.05.017.

19. Gaskell PJ, Zandonatti M, Gilmartin T, Head SR, Fox HS. 2008. Macrophage-derived simian immunodeficiency virus exhibits enhanced infectivity by comparison with T-cell-derived virus. J Virol 82:1615–1621. http://dx.doi.org/10.1128/JVI.01757-07.

20. Lin G, Simmons G, Polhamn S, Baribaud F, Ni H, Leslie GJ, Haggarty BS, Bates P, Weissman D, Hoxie JA, Doms RW. 2003. Differential N-linked glycosylation of human immunodeficiency virus and Ebola virus envelope glycoproteins modulates interactions with DC-SIGN and DC-SIGNR. J Virol 77:1337–1346. http://dx.doi.org/10.1128/JVI.77.2.1337-1346.2003.

21. Willey RL, Shibata R, Freed EO, Cho MW, Martin MA. 1996. Differential glycosylation, virion incorporation, and sensitivity to neutralizing antibodies of human immunodeficiency virus type 1 envelope produced from infected primary T-lymphocyte and macrophage cultures. J Virol 70:6431–6436.

22. Raska M, Takahashi K, Czernekova L, Zachova K, Hall S, Moldoveanu Z, Elliott MC, Wilson I, Brown R, Jancova D, Barnes S, Vrbkova J, Tomana M, Smith PD, Mestecky J, Renfrow MB, Novak J, Zangger N, Lorizate M, Osawa K, Schief WR, Sanders RW. 2010. Role of complex carbohydrates in human immunodeficiency virus type 1 infection and resistance to antibody neutralization. J Virol 84:5637–5655. http://dx.doi.org/10.1128/JVI.00105-10.

23. Eggink D, Melchers C, Wuhrer M, van Montfort T, Dey AK, Niajikens BA, David KB, Le Douce V, Deelder AM, Kang K, Olson WC, Berkhour B, Hokke CH, Moores JP, Sanders RW. 2010. Lack of complex N-glycans on HIV-1 envelope glycoproteins preserves protein conformation and entry functionality. Virology 401:236–247. http://dx.doi.org/10.1016/j.virol.2010.02.019.

24. Reeves PJ, Callewaert N, Contreras R, Khorana HG. 2002. Structure and function in rhodopsin: high-level expression of rhodopsin with restricted and homogeneous N-glycosylation by a tetracycline-inducible N-acetylgalactosaminyltransferase 1-negative HER2935 stable mammalian cell line. Proc Natl Acad Sci USA 99:13419–13424. http://dx.doi.org/10.1073/pnas.212519299.

25. Mori K, Ringler DJ, Kodama T, Desrosiers RC. 1992. Complex determinants of macrophage tropism in env of simian immunodeficiency virus. J Virol 66:2067–2075.

26. Mori K, Ringler DJ, Desrosiers RC. 1993. Restricted replication of simian immunodeficiency virus strain 239 in macrophages is determined by env but is not due to restricted entry. J Virol 67:2807–2814.

27. Provine NM, Puryear WB, Wu X, Overbaugh J, Haigwood NL. 2009. The infectious molecular clone and pseudotyped virus models of human immunodeficiency virus type 1 exhibit significant differences in virion composition with only moderate differences in infectivity and inhibition sensitivity. J Virol 83:9002–9007. http://dx.doi.org/10.1128/JVI.00423-09.

28. Balzarini J. 2007. Targeting the glycans of glycoproteins: a novel paradigm for antiviral therapy. Nat Rev Microbiol 5:583–597. http://dx.doi.org/10.1038/nrmicro1707.

29. Geijtenbeek TB, Kwon DS, Torenスマラ, van Vliet SJ, van Duijnhoven GC, Middel J, Cornelissen I, Nottet HS, KewalRamani VN, Littman DR, Figdor CG, van Kooyk Y. 2000. DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances trans-infection of T cells. Cell 100:587–597. http://dx.doi.org/10.1016/S0092-8674(00)00869-4.

30. Polhamn S, Baribaud F, Lee B, Leslie GJ, Sanchez MD, Hiebenthal MJ, Munch J, Kirchhoff H, Doms RW. 2001. DC-SIGN interactions with human immunodeficiency virus type 1 and 2 and simian immunodeficiency virus. J Virol 75:4664–4672. http://dx.doi.org/10.1128/JVI.75.10.4664-4672.2001.

31. Johnson PR, Schnepp BC, Zhang J, Connell MJ, Greene SM, Yuste E, Desrosiers RC, Clark KR. 2009. Vector-mediated gene transfer engenders long-lived neutralizing activity and protection against SIV infection in monkeys. Nat Med 15:901–906. http://dx.doi.org/10.1038/nm.1967.

32. Kwoe AT, Gregorio B, Bottin N, Hendrickson WA, Littman DR. 2002. DC-SIGN-mediated internalization of HIV is required for trans-infection of T cell. Immunity 16:135–144. http://dx.doi.org/10.1016/S1076-7866(02)00259-5.

33. Boggiano C, ManeL N, Littman DR. 2007. Dendritic cell-mediated trans-infection of human immunodeficiency virus type 1 infectivity is independent of DC-SIGN. J Virol 81:2519–2523. http://dx.doi.org/10.1128/JVI.01787-07.

34. Izquierdo-Useros N, Lorizate M, Puertas MC, Rodriguez-Plata MT, Zangger N, Erikson E, Pino M, Erkizia I, Glass B, Clotet B, Keppler OT, Tenenti A, Krausslich HG, Martinez-Picado J. 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. http://dx.doi.org/10.1371/journal.pbio.1001448.

35. Arrighi JF, Pion M, Garcia E, Escala JM, van Kooyk Y, Geijtenbeek TB, Piquet V. 2004. DC-SIGN-mediated infectious synapse formation enhances X4 HIV-1 transmission from dendritic cells to T cells. J Exp Med 200:1279–1288. http://dx.doi.org/10.1084/jem.20041356.

36. Feinberg H, Mitchell DA, Drickamer K, Weis WI. 2001. Structural basis for selective recognition of oligosaccharides by DC-SIGN and DC-SIGNR. Science 294:2163–2166. http://dx.doi.org/10.1126/science.1066371.

37. Guo Y, Feinberg H, Conroy E, Mitchell DA, Alvarez R, Blixt O, Taylor ME, Weis WI, Drickamer K. 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. http://dx.doi.org/10.1038/nsmb784.

38. van Montfort T, Eggink D, Boot M, Tuen M, Hioe CE, Berkhour B, Sanders RW. 2011. HIV-1 N-glycan composition governs a balance between dendritic cell-mediated viral transmission and antigen presentation. J Immunol 187:4676–4685. http://dx.doi.org/10.4049/jimmunol.1101876.

39. Arrighi JF, Pion M, Kelly CL, boyer A, Tuen M, Hioe CE, Berkhour B, Sanders RW. 2011. Early events in sexual transmission of HIV and SIV and opportunities for interventions. Annu Rev Med 62:127–139. http://dx.doi.org/10.1146/annurev-med-080709-124939.