Glycans, galectins, and HIV-1 infection

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During sexual transmission, HIV-1 must overcome physiological barriers to establish a founder cell population. Viral adhesion represents a bottleneck for HIV-1 propagation that the virus widens by exploiting some specific host factors. Recognition of oligomannosyl glycans of gp120 by C-type lectins is one such example. Recent works suggest that complex glycans of gp120 are recognized by another host lectin, galectin-1. This interaction results in rapid association of HIV-1 to susceptible cells and facilitates infection. The peculiar presentation of complex glycans on gp120 seems to impart specificity for galectin-1, as another member of the same family, galectin-3, is unable to bind gp120 or enhance HIV-1 infection. Other studies have shown that galectin-9 could also increase HIV-1 infectivity but via an indirect mechanism. Thus, current research suggests that galectins play various roles in HIV-1 pathogenesis. Drug discovery approaches targeting host lectins at early steps could benefit the current arsenal of antiretrovirals.

Keywords: glycobiology; HIV-1; lectin; galectin

AIDS and HIV-1 infection

In the summer of 1981, the world first became aware of an emerging disease that would later be named acquired immune deficiency syndrome (AIDS). Since then, AIDS has killed more than 25 million people.1,2 Human immunodeficiency virus type-1 (HIV-1), a lentivirus that belongs to the retrovirus family, is the causing agent of AIDS. The number of newly infected individuals has steadily, yet slowly, been declining since its peak of 3.2 million reached in 1997, down to 2.6 million in 2009, possibly due to restless public awareness and prevention campaigns.1,2 However, 33.3 million people are still living with HIV-1 today.1,2 Since this retrovirus mainly infects cells of the immune system—namely CD4+ T cells and macrophages—and cripples the adaptive immune system, HIV-1 infections have an enormous impact on the emergence and spread of other infectious diseases, especially in developing countries.3 First, in many of those pandemic areas access to medication is difficult and HIV-infected individuals rapidly develop AIDS; they thus become especially sensitive to infection by other pathogens—opportunistic or not—present in the environment. The ensuing immunocompromised status leads to high loads of coinfecting pathogens, a phenomenon that increases the chances of transmission to immunocompetent hosts. Immunosuppression of millions of people in a population can also reduce the effectiveness of immunization campaigns. In the worst possible scenario, one can hypothesize that bacteria, fungi, and protozoa in the environment, as well as zoonotic pathogens, could have a reservoir of ~30 million immunocompromised individuals in which to evolve and adapt to become human pathogens. It is thus paramount, not only for the most afflicted developing countries but also for industrialized countries, to control the HIV-1 pandemic in order to reduce the risk of widespread emergence of other pathogenic agents.3

HIV-1 replication cycle

The HIV-1 transmission process4 is initiated by the binding of the viral envelope glycoprotein complex
Figure 1. HIV-1 replication cycle.
Glycosylation). The Env precursor is also cleaved in these compartments by the furin host protease to generate a trimeric gp120/gp41 complex that is then transported to the plasma membrane. Assembly of Pr55Gag and Pr160Gag–Pol associated to viral genomic RNAs occurs at the inner surface of the plasma membrane and induces a characteristic curvature of the membrane that leads to budding of the virion (Fig. 1–7, Budding). During this budding process, HIV-1 also acquires membrane glycoproteins of cellular origin into the viral surface.7–9 Since HIV-1 carries very few Env trimers or spikes (as few as 14 ± 7) on its surface,10 the thick glycocalyx present on the virus surface is mainly composed of host membrane components. Host-derived glycans attached to membrane glycoproteins may thus also contribute to conceal antigenic surfaces.6 Once released, the viral particle achieves maturation through the action of the viral protease that cleaves the polyproteins Pr55Gag and Pr160Gag–Pol into structural and enzymatic components that assemble to form a mature HIV-1 particle (Fig. 1–8, Maturation).

**Glycosylation of gp120 of HIV-1**

Despite high genetic variability among different isolates and clades of HIV-1, the N-glycosylation sites of gp120 are spatially conserved.11 The glycans of the Env complex are synthesized by the host glycosylation machinery in the ER and Golgi complex. Like other host glycoproteins, two types of N-linked glycans are found on gp120, namely oligomannose-type glycans, which are rich in mannose residues (Man3–9GlcNAc2), and complex-type glycans, which carry 2–6 β-galactoside residues (lactosamine residue [GalGlcNAc; LacNAc]) on their trimannose core structure, Man3GlcNAc2 (Fig. 2A).12–18 Glycosylation of gp120 has at least two unique features that distinguish it from that of host membrane proteins.6 Those features are observed in both recombinant and viral gp120.12–18 Since N-linked glycan profiles of host glycoproteins found in HIV-1–infected cells are suggested to be quite similar to those of noninfected cells,17 it is unlikely that such unique patterns of gp120 glycosylation are entirely the result of viral modulation of the host glycosylation pathways. It has instead been proposed that the distinctive glycosylation patterns of gp120 compared to host glycoproteins may be due to the unusually dense arrangement of N-linked glycans of gp120, which limits the accessibility of the glycan processing enzymes.6,18

First, gp120 contains high levels of oligomannose-type glycans, which are considered as incomplete processing forms of glycans that are rarely found in the extracellular space.12–16 Indeed, 56–79% of the N-linked glycans of gp120 on HIV-1 are oligomannose-type glycans.17 Such levels of oligomannose-type glycans on HIV-1 gp120 have attracted a lot of attention and their biological significance has been extensively studied either as pathogen-associated molecular patterns (PAMPs) or as an antigen. Interestingly, recent studies also confirmed that intact virus-associated gp120 also contains steady levels of complex-type glycans (21–44% of N-glycans).16,17,19 In this case, however, the biological significance of complex-type glycans in HIV-1 pathology remains unexplored.

Second, oligomannose- and complex-type glycans are spatially distributed on the surface of gp120 and form distinct homogenous patches (Fig. S1). Oligomannose-type glycans are clustered in an area distal to the CD4 binding site and often associate with the immunologically silent face of gp120.11,20 Complex-type glycans patches are found proximal to the CD4 binding site (Fig. S1).20 It appears that high levels of glycan–glycan interactions occur between neighboring glycans, thus forming tight clusters. These clusters can mask the peptide backbone structure from antibodies.6,21 Since glycans attached to host glycoproteins normally exhibit considerable conformational flexibility because of their highly hydrophilic nature, such clustered arrangement of glycans on gp120 is considered unique and peculiar. Using the 2G12 neutralizing antibody that binds to the oligomannose-type glycans of gp120, it has been established that the high concentration and clustering of oligomannose-type glycans on the surface of gp120 leads to its recognition as nonself glycans, such as those found on many bacterial surfaces.11,22 Our recent study on the interaction between galectins and gp120 also suggests the presence of unusual clusters of complex-type glycans on gp120 (see later for more details).16 Densely packed glycan patches on gp120 may thus also exhibit non-self properties. Importantly, such unique glycan presentation depends on the tertiary structure of gp120 and requires it to be intact and properly folded.16,23,24
Galectins and HIV-1 infection

Since glycans found on host extracellular glycoproteins are mostly complex-type glycans, glycoproteins carrying high levels of oligomannose-type glycans can be recognized as PAMPs in some cases. HIV-1 is indeed recognized by dendritic cells (DCs) and Langerhans cells (LCs) via specific C-type lectins, such as DC-specific ICAM3-grabbing non-integrin (DC-SIGN), DC immunoreceptor (DCIR), and Langerin. DC-SIGN is highly expressed in monocyte-derived DCs and dermal and mucosal DCs but not in LCs. DC-SIGN binds to oligomannose-type glycans of gp120 and facilitates both direct infection of DCs (cis-infection) as well as DC-mediated HIV-1 transfer toward CD4+ T cells (trans-infection). DCIR is expressed in antigen-presenting cells, including myeloid and plasmacytoid DCs, as well as macrophages, but very few in LCs. This lectin has recently been suggested to be involved in both cis- and trans-infection by HIV-1 using monocyte-derived DCs. It remains elusive whether the oligomannose-type glycans of gp120 (Fig. 2A) are the primary HIV-1 ligands for DCIR or not. LCs express another type of C-type lectin, Langerin, which also binds the oligomannose-type glycans of gp120 (Fig. 2A). However, in contrast to DC-SIGN and DCIR, interaction of HIV-1 with Langerin leads to the rapid degradation of the virus following uptake. Thus, the clustered oligomannose-type glycans on gp120 have multifaceted roles in HIV-1 pathogenesis. Our recent work on clustered complex-type glycans of HIV-1 gp120 suggests that these complex-type patches can also be exploited by the virus and that they may favor the transmission or replication of HIV-1.

Figure 2. N-linked glycans of gp120 and description of galectins. (A) N-linked glycans of gp120 and the saccharide moieties that could be recognized by lectins are highlighted. (B) Galectin family. (C) Cross-linking of the ligands by galectins. (D) Expression of galectins in epithelia and gut-associated lymphoid tissue.
Host membrane proteins that assist HIV-1 infection

In addition to C-type lectins, two classes of host proteins, integrins and syndecans, are known to facilitate HIV-1 infection. Two integrin family members, α4β7 and α4β2, are listed as assisting HIV-1 entry into susceptible cells. CD4+ T cells express α4β2 (LFA-1), which promotes HIV-1 attachment by binding to ICAM-1, an adhesion molecule acquired by HIV-1 during the budding process.7,9,31 Activation of CD4+ T cells leads to a conformational switch of LFA-1 toward a high affinity state, which further improves the LFA-1–ICAM-1–mediated enhancement of HIV-1 infection. Additionally, the α4β7 gut-homing receptor is highly expressed in memory CD4+ T cells, and this integrin can interact directly with gp120. This interaction has also been shown to activate LFA-1 and to facilitate virus entry in gut-homing CD4+ T cells.32,33 Another family that promotes HIV-1 infection is syndecans.34 Syndecans belong to a class of proteoglycans that carry covalently linked, heparan sulfate glycosaminoglycans, to which gp120 directly binds.34,35 Interestingly, HIV-1 retains its infectivity for as long as a week when bound to cell-surface syndecans and can be readily transmitted upon contact with CD4+ T cells.34–36 In addition to these host membrane-bound proteins, the host soluble lectin galectin-1 also contributes to HIV-1 binding to CD4+ susceptible cells and promotes HIV-1 infection.16,19,37–39 The possible role played by galectin-1 in the pathogenesis of HIV-1 infection is described later.

Selective transmission of R5-tropic isolates of HIV-1

HIV-1 uses either CCR5 or CXCR4 as its coreceptor for entry. HIV-1 variants that use the CCR5 are called R5 variants, while those that use CXCR4 are referred to as X4 variants. Although both R5 and X4 HIV-1 variants are present in the body fluids (semen, blood, cervicovaginal secretion) of transmitting individuals, current studies suggest that in the majority of cases the transmitted/founder virus found in the GALT of recently infected individuals is an R5 variant. These data suggest highly selective mechanisms favoring R5 over X4 during transmission events and/or initial replication in the GALT.40–42 This selection appears to occur in multiple layers. First, HIV-1 infection occurs initially in GALT-associated CD4+ T cells, which express CCR5. Second, because gp120 from X4 variants has more exposed cationic charge than R5 variants, X4 HIV-1 may bind more strongly to polyanionic mucin and be subject to a preferential clearance.42–44

In addition to the selectivity in the transmission of HIV-1 R5 variants, several studies suggest that the gp120 molecules of transmitted/founder R5 HIV-1 contain reduced N-linked glycosylation sites in their V1/V2 regions.42,45–47 There thus seems to be another selection process among R5 variants. These hypoglycosylated variants do not display any direct replicative advantage using a HeLa cell-based reporter cell line expressing CD4 and CCR547 but instead display a unique sensitivity to antibody neutralization.45 Thus, there may be additional selective pressure for the HIV-1 R5 variant that carries a reduced number of glycosylation sites in spite of its increased susceptibility to neutralizing antibodies. One of such possibilities is related to the recognition of oligomannose-type glycans by host C-type lectins. Although HIV-1 replication is enhanced through trans- and cis-infection mediated by DC-SIGN and DCIR in DCs, recent studies suggest that early HIV-1 infection exclusively occurs in CD4+ T cells rather than macrophages or DCs, which reside in the subepithelium.42,48,49 In addition, a recent elegant work by the group of Sanders also demonstrated 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 (complex- and oligomannose-type).50 In addition, trans-infection of those HIV-1 with oligomannose-type rich gp120 is relatively inefficient.51 Finally, Langerin-expressing LCs residing in the genital mucosal epithelia are most probably the first DC subset to encounter HIV-1 and could contribute to the rapid destruction of HIV-1 to provide a barrier to transmission.26 Thus, it is possible that at the initial stage of the infection process, HIV-1 with reduced oligomannose-type glycans has some advantage for the establishment of an infection by escaping its uptake by antigen-presenting cells in the genital epithelia. An alternative hypothesis was recently proposed by the group lead by Fauci.33 His group argues that due to fewer glycosylation sites, gp120 molecules located on transmitted/founder virus could bind
more efficiently to $\alpha_4\beta_7$ integrin, resulting in the activation of LFA-1 and enhancement of infection via stronger ICAM-1/LFA-1 interactions.\textsuperscript{33,51} Thus, despite the fact that the presence of highly constrained oligomannose-type glycans on gp120 renders HIV-1 significantly resistant to neutralizing immunity,\textsuperscript{6} HIV-1 does not fully utilize its glycan shield during a sexual transmission event. Instead, transmitted/founder virus selectively lowers this oligomannose-type glycan shield in order to both evade the innate mucosal defense system and facilitate its interaction with its target cells, activated CD4$^+$ T cells. Importantly, those HIV-1 particles that penetrate across genital epithelia have to interact rapidly with susceptible cells or with cells expressing alternative receptors (such as syndecans), since cell-free HIV-1 virion becomes inactive in a relatively short period of time.

**Biological processes involved in the transmission and initial replication of HIV-1**

The frequency of HIV-1 transmission following an unprotected sexual intercourse is quite low (0.05–0.1\%) when compared to other sexually transmitted viruses, such as the hepatitis B virus.\textsuperscript{52–57} Few virus particles penetrate across genital epithelial layers to reach the mucosa-associated lymphoid tissues (MALT) and especially the gut-associated lymphoid tissues (GALT) that are rich in HIV-1–susceptible CD4$^+$ T cells.\textsuperscript{49,58–61} Indeed, recent works have established that more than 75\% of HIV-1 infection cases are initiated by a single founder virus.\textsuperscript{53,42,49,62}

Further, unlike other enveloped viruses like influenza, HIV-1 virions carry as low as 14 ± 7 Env molecules that are required for viral entry via CD4.\textsuperscript{60} Viral attachment to CD4 thus displays an intrinsically weak avidity, especially in vivo where it occurs under suboptimal conditions.\textsuperscript{7–9,34,63} Notably, during this initial replication period, HIV-1 susceptible cells in the GALT express low levels of CD4 and CCR5 and are less competent than fully activated T cells.\textsuperscript{64–66} In addition, GALT-associated LCs can capture cell-free HIV-1 and degrade it.\textsuperscript{26} Despite this relatively unfavorable environment, when HIV-1 transmission occurs, HIV-1 infect CD4$^+$ T cells to create a replicative focus that will expand to establish a self-propagating infection within a day post infection.\textsuperscript{61,64–66} More than 90\% of memory CD4$^+$ T cells, which reside in the GALT, are depleted by local viral replication within one month of infection.\textsuperscript{58–61}

Thus, contrary to the traditional view of HIV-1 infection characterized by the slow decline of CD4$^+$ T cells, recent findings of this rapid and extensive removal of the major portion of the immunological memory initiated by only a few viral particles reemphasizes the need to control viral replication as early as possible and ideally to prevent transmission altogether. Since HIV-1 establishes its infection within a day under those restrictive conditions, it is possible that the virus exploits additional host factors to achieve rapid viral attachment to susceptible cells, but such possibility has remained more or less unexplored until recently. As mentioned later, galectin-1, a soluble host $\beta$-galactoside–binding lectin, strongly increases the association of HIV-1 viral particles to susceptible cells and this activity is observed within minutes.\textsuperscript{16,19,37,39} Another member of the same family, galectin-3 could not display the same activity while a recent work suggests that galectin-9 also promotes HIV-1 infection.\textsuperscript{68}

**Galectins**

Galectins form one host soluble lectin family defined by an ability to bind $\beta$-galactoside–containing glycans and a conserved amino-acid sequence in their carbohydrate recognition domain (CRD; Fig. 2B).\textsuperscript{69} Extracellular functions of galectins rely on the multivalency of glycan binding.\textsuperscript{69–71} Galectin-1 is dimer, and galectin-3 forms oligomer through its N-terminal domain. Galectin-9 contains two CRDs connected by a linker.\textsuperscript{72–74} Therefore, galectin-1, 3, and 9 can crosslink specific ligands. When those galectins bind to ligands from different entities, they can mediate cell/cell or cell/pathogen interactions (Fig. 2C).\textsuperscript{74–76} Studies from different laboratories including ours suggest that galectin-1, but not galectin-3, promotes HIV-1 binding to susceptible CD4$^+$-expressing human cells and directly enhances virus infection.\textsuperscript{16,19,37–39,68} Since the roles of galectins in host–pathogen interactions and immune responses are extensively reviewed in this issue and elsewhere,\textsuperscript{72,77–79} only the aspects of galectins that are related to HIV-1 infection will be introduced and discussed here.

**Galectin expression**

Galectin-1 is expressed in the thymus and by lymphoid parenchymal epithelial cells, endothelial cells, trophoblasts, activated T and B cells, macrophages, follicular DCs, and CD4$^+$CD25$^+$ regulatory T cells.\textsuperscript{80–86} Among subtypes of CD4$^+$ T cells,
Galectin-1 is highly expressed and secreted by Th1 cells. Significantly accumulation of galectin-1 is found in the lamina muscularis mucosae, just beneath the epithelium and the lamina propria, where HIV-1 susceptible CD4+ T cells can be found. While galectin-9 is expressed in endothelial cells, macrophages, and microglia, and their activation enhances its expression level. Galectin-3 is also highly expressed and secreted at the apical side (luminal side) of the mucosal epithelium while galectin-9 is expressed by T cells, eosinophils, endothelial cells, DCs, and macrophages, as well as by the epithelium of the gastrointestinal tract.

**Galectin-1 promotes HIV-1 infection**

We previously established that galectin-1, but not galectin-3, accelerates (as high as 40-fold) the binding kinetics of HIV-1 to susceptible cells, and facilitates robust HIV-1 replication. These activities of galectin-1 in HIV-1 infection are completely inhibited by lactose, a β-galactoside-containing saccharide, but not mannose, confirming that galectin-1 recognizes β-galactoside residues expressed on HIV-1. Importantly, even in this condition, HIV-1 entry strictly relies on the interaction between gp120 and CD4/coreceptor, suggesting that galectin-1 only assists the initial virus binding event without affecting the rest of the HIV-1 entry process. An incubation of less than two minutes is sufficient to detect an increase in HIV-1 binding to susceptible cells by galectin-1 (Ref. 19). Further, in the presence of galectin-1, more than 30% of the initial virus input is associated with peripheral blood mononuclear cells (PBMCs) after 30 min, while less than 1% of the virus is bound in the absence of galectin-1. This activity can be observed at 4 °C, suggesting that increased binding kinetics is due to direct cross-linking of HIV-1 to cells, rather than galectin-1–induced signal transduction. As shown in Table 1, galectin-1 can promote the infection of HIV-1 X4, X4R5, and R5 variants in various susceptible cells. Our recent work suggests that galectin-1 directly binds to HIV-1 virus particles. This interaction is sensitive to lactose but not mannose, confirming that galectin-1 utilizes its β-galactoside-binding activity to recognize HIV-1. In contrast to our studies, the group led by Mahal reports that galectin-1 binds to mannose residues on HIV-1, based on the results obtained by a lectin microarray system where galectin-1 is densely plotted on the array. This report contradicts the previously established glycan-binding specificity of galectin-1. In addition, galectin-1 is sensitive to oxidation and is liable to become oxidized in dry conditions such as those required for array spotting. Indeed, caution on the quality of galectin-1 in assays has been recently raised by several groups. Thus, further investigation under strict quality control is necessary to verify whether galectin-1 may aberrantly change its specificity from β-galactoside to mannose upon immobilization on microarray plates (see discussion in Ref. 39). Nonetheless, densely clustered immobilized galectin-1 would simply not be expected in a physiological environment, especially in the context of HIV-1 infection. In a physiological setting, galectin-1 preferentially binds to β-galactoside residues, such as those presented on the surface of HIV-1.

As listed in Table 2, galectin-1 can directly interact with several HIV-1 variants produced in both HEK 293T cells or primary PBMCs. Importantly, pseudovirus that lacks gp120 cannot be recognized by galectin-1, and purified gp120 interacts with galectin-1 but not galectin-3. Thus, gp120 is the specific viral ligand for galectin-1 (see later for detailed glycan-binding specificity). It is expected that differences in cell surface protein glycosylation are present in different susceptible cells and virus-producing cells. Despite this fact, galectin-1 can cross-link HIV-1 to all susceptible cells tested and promote their infection, suggesting that susceptible cells and HIV-1 both carry N-linked glycans that have affinity for galectin-1. Indeed, recent structural studies of gp120 glycans purified from HIV-1 produced in 293T cells as well as PBMCs indicate that HIV-1 gp120 carries significant levels (as high as 44%) of N-linked complex-type glycans. Thus, while further studies are warranted, galectin-1 can be listed as one of the main targets of HIV-1.

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Michel Ouellet, Michael Imbeault, and Michel J. Tremblay, unpublished observations.
Galectins and HIV-1 infection

Table 1. Cells in which galectin-1 promotes HIV-1 infection

<table>
<thead>
<tr>
<th>Infected cells</th>
<th>Virus strain</th>
<th>Virus producer cells</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>LuSIV cells</td>
<td>NL4-3 (X4)</td>
<td>293T</td>
<td>17</td>
</tr>
<tr>
<td>1G5 cells</td>
<td>NL4-3 (X4)</td>
<td>PBMCs</td>
<td>17</td>
</tr>
<tr>
<td>PBMCs</td>
<td>NL4-3 (X4)</td>
<td>293T</td>
<td>17</td>
</tr>
<tr>
<td>CD4+ T cells</td>
<td>93US151 (R5×4)</td>
<td>PBMCs</td>
<td>17</td>
</tr>
<tr>
<td>CD4+ T cells</td>
<td>NL4-3 (X4)</td>
<td>293T</td>
<td>16</td>
</tr>
<tr>
<td>CD4+ T cells</td>
<td>NL4-3Bal (R5)</td>
<td>293T</td>
<td>16</td>
</tr>
<tr>
<td>Macrophages</td>
<td>NL4-3-Bal (R5)</td>
<td>Macrophages</td>
<td>35</td>
</tr>
<tr>
<td>Tonsillar tissues (cultured ex vivo)</td>
<td>Virus pseudotyped with JR-FL env (R5)</td>
<td>293T</td>
<td>35</td>
</tr>
</tbody>
</table>

potential host factor that could directly contribute to HIV-1 transmission or to its early expansion in the GALT.

Complex-type glycan clusters of gp120 are found on a domain close to the CD4 binding site (Fig. S1). It has been recently proposed that one of the host ligands of galectin-1 is the CD4 glycoprotein. Indeed, a significant increase in gp120 binding to CD4 is observed upon adding galectin-1 to a column of immobilized CD4 through which soluble recombinant gp120 is run. This suggests that galectin-1 can directly cross-link gp120 to CD4. Furthermore, using CD4-expressing and CD4-deficient cell lines, we have shown that galectin-1 cannot promote HIV-1 binding to cells that do not express CD4. Together, these results suggest that CD4 is one of the host ligands of galectin-1, and galectin-1 facilitates HIV-1 infection through direct cross-linking of gp120 and CD4 (Fig. 3).

Recently, the group lead by Baum reported that galectin-9 also potentiates HIV-1 infection. In the case of galectin-9, however, this assistance requires a longer exposure than galectin-1. Interestingly, they found that galectin-9 binds to host surface protein disulfide isomerase (PDI), leading to an increased retention of PDI on the cell surface. Since surface PDI promotes HIV-1 infection in T cells through alteration of the redox state surrounding the cell, galectin-9–induced changes in the membrane dynamics of PDI creates a favorable environment for HIV-1 entry. Thus, at least two galectins found in the genital tract or GALT can facilitate HIV-1 infection.

**Glycan-binding specificity of galectins**

In order to better delineate the distinctive activity of galectin-1 toward HIV-1 binding to CD4+ susceptible cells, we would first like to briefly introduce the general glycans-binding specificity of galectins. The minimal binding unit recognized by galectins is a β-galactoside (galactose residue linked to a glycan through β linkage), such as N-acetyllactosamine (LacNAc). While β-galactoside-containing glycans are often found in complex-type glycans attached to proteins (Fig. 2A), each galectin binds to a relatively limited set of ligands. This is likely due to a distinctive presentation of their CRDs, as well as to the structural differences found in the CRD of each galectin. Modifications of galactose residues found on β-galactosides drastically alter their affinity for galectins. For example, an additional β-linked galactose residue on β-galactosides increases their affinity for galectin-3, but abolishes their affinity for galectin-1. In contrast, α2–6 (but not α2–3) sialic acid modification dramatically reduces the affinity for galectin-1, galectin-3, and galectin-9. Due to this difference in affinity, galectins can bind to Th1 and Th17 but not Th2 polarized cells (in both mice and humans) since glycans of Th1 and Th17 carry α2–3 sialyl modifications while those of Th2 cells are α2–6. This is one good example of seemingly minor peripheral differences in glycan structures that regulate important immune functions and alter the biological activity of lectins. Interestingly, HIV-1 infection reduces sialylation of surface glycoproteins, thereby likely increasing galectin-1 binding. We previously reported that bacterial and influenza-derived sialidases (neuraminidases) can significantly increase HIV-1 infection. Individuals who are chronically infected with HIV-1 are susceptible to various infections with pathogens that carry sialidase. Thus, the level of
sialylation of both HIV-1 virions and host susceptible cells in lymph nodes might be significantly reduced and thus more susceptible to be recognized by galectins.

**Differential recognition of CD4, the main HIV-1 receptor, by galectin-1 and galectin-3**

Although both galectin-1 and galectin-3 bind to LacNAc residues, significant difference in their binding preferences have been reported. We previously reported that galectin-3 binds to human amniotic fluid fibronectin but fails to bind to plasma fibronectin. Glycan profile analysis shows that amniotic fluid fibronectin carry tri- and tetra-antennary complex-type glycans (i.e., they carry three or four LacNAc per glycan) while serum fibronectin instead contains bi-antennary complex-type glycans (i.e., they carry only two LacNAc per glycan). Therefore, those data suggest that galectin-3 cannot steadily bind to bi-antennary complex-type glycans attached on a protein despite the presence of LacNAc. Recently, by using glycan microassays, Cummings and his colleagues reported a more sustained binding of galectin-1 to bi-antennary complex-type glycans compared to galectin-3 at low lectin concentrations. While this preferential binding is less clear at higher lectin concentrations, our unpublished results using affinity chromatography analysis also confirm such preference that galectin-1 but not galectin-3 is better retained by a column of immobilized asialotransferrin that carries only bi-antennary complex-type glycans. In contrast, both galectin-1 and galectin-3 are retained similarly by a column of immobilized asialofetuin that carries tri-antennary complex-type glycans.

More relevant to HIV-1 infection, recombinant CD4 contains only two bi-antennary complex-type glycans, like plasma fibronectin and transferrin. Indeed, we recently reported that galectin-1, but not galectin-3, strongly binds to recombinant CD4, the main host receptor for HIV-1. Further, galectin-1 increases HIV-1 binding to CD4-expressing cells. Thus, the selective recognition of CD4 by galectin-1 but not galectin-3 is likely due to the difference in their binding preferences for bi-antennary complex-type glycans presented on the surface of CD4. In contrast to those results based on biological relevant assays, binding affinity of galectin-1 for bi-antennary glycans is consistently lower than galectin-3, underlining some difficulty in the usage of affinity constants or dissociation rates obtained by kinetics assays to access or predict the interaction between natural galectin ligands and galectins.

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**Table 2. Interaction between galectins and HIV-1**

<table>
<thead>
<tr>
<th>Producer cells</th>
<th>Galectin binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1 virus (NL4-3, X4)</td>
<td>293T galectin-1 &gt; galectin-3</td>
</tr>
<tr>
<td>HIV-1 virus (89.6, R5×X4)</td>
<td>293T galectin-1 &gt; galectin-3</td>
</tr>
<tr>
<td>HIV-1 virus (NL4-3Bal, R5)</td>
<td>293T galectin-1 &gt;&gt; galectin-3</td>
</tr>
<tr>
<td>Envelope-deficient HIV-1 virus (NL4-3, X4)</td>
<td>293T Galectin-1 binding is low and galectin-3 binding remains similar to the virus with Env</td>
</tr>
<tr>
<td>Detergent lysates (Triton X100-soluble) of HIV-1 virus (NL4-3, X4)</td>
<td>293T galectin-1 &gt; galectin-3</td>
</tr>
<tr>
<td>Recombinant gp120 (96ZM651, X4)</td>
<td>293 galectin-1 &gt; galectin-3</td>
</tr>
<tr>
<td>Recombinant gp120 (NL4-3Bal, R5)</td>
<td>293 galectin-1 &gt; galectin-3</td>
</tr>
<tr>
<td>DTT-treated recombinant gp120 (96ZM651, X4)</td>
<td>293 galectin-1 = galectin-3</td>
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<tr>
<td>DTT-treated recombinant gp120 (NL4-3Bal, R5)</td>
<td>293 galectin-1 = galectin-3</td>
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<tr>
<td>N-linked glycans of recombinant gp120 (96ZM651, X4)</td>
<td>293 galectin-1 = galectin-3</td>
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<td>293 galectin-1 = galectin-3</td>
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*b* Christian St-Pierre and Sachiko Sato, unpublished observations.

*c* Christian St-Pierre and Sachiko Sato, unpublished observations.

*d* Michel Ouellet, Michel J. Tremblay, and Sachiko Sato, unpublished observations.
Differential gp120 recognition by galectin-1 and galectin-3

Being lactosamine-binding proteins, it would be expected that all galectins exhibit higher affinity for glycans that contain a higher number of lactosamine residues (like polylactosamine or tri-/tetra-antennary complex type). Indeed, this polylactosamine effect is significant for galectin-3 and galectin-9, but not for galectin-1. Thus, galectin-1 exhibits constant $K_d$ for lactosamine-containing glycans regardless of the number of lactosamine residues. In contrast, the affinity of galectin-3 for glycans increases with good correlation upon increasing their number of lactosamine residues. Importantly, since galectin-3 recognizes both internal and external lactosamine residues, this lectin can bind to glycan as long as the glycan contains a couple of internal lactosamine residues. However, it remains elusive which lactosamine residues, internal or external, are more critical to form persistent interaction of the glycan with galectin-3. In contrast, it is likely that galectin-1 preferentially binds to peripheral Gal residues presented by lactosamine.

Complex-type glycans of HIV-1 gp120 contain bi-, tri-, and tetra-antennary chains. Depending on clinical HIV-1 quasispecies or variants,
virus-producing cells, and gp120-producing cells, some differences in the composition of complex-type glycans have been reported.\textsuperscript{12–17} Our recent work using recombinant gp120 derived from R5 and X4 variants reveals that galectin-1 firmly binds to each of these gp120 variants, while galectin-3 fails to form a tight interaction with any of them. Since this interaction is disrupted by lactose but not mannose, the binding of galectin-1 to gp120 depends on the presence of β-galactoside residues. Curiously, compositions (percentage of total N-linked glycans) of complex-type glycans of gp120 X4 and R5 carrying bi-antennary and tri-/tetra-antennary, both of which exhibit high affinity for galectin-1 and/or galectin-3, are 16:10 and 9:18, respectively.\textsuperscript{16} Thus, the obtained binding data are not reconciled with the established glycan specificities based on biochemical kinetic analysis. Indeed this observed preference of galectin-1 for gp120 compared to that of galectin-3, is not likely due to a \textit{de facto} preference of a galectin toward specific glycans displayed by gp120. Importantly, when complex-type glycans released from gp120 are subject to galectin binding analysis, both galectin-1 and galectin-3 bind similarly to those protein-free glycans (Table 2),\textsuperscript{16} fitting well with their previously established binding specificity. Similarly, when disulfide bonds of recombinant gp120 are partially denatured by Dithiothreitol, the above distinct galectin-1 specific recognition of gp120 is also lost and both galectin-1 and galectin-3 equally interact with the partially denatured gp120 (Table 2). Together, it seems to be the spatial organization of complex-type glycans on gp120 that significantly contributes to the repulsion of galectin-3 and the firm binding to galectin-1 (Fig. 4). Such peculiar presentation of complex-type glycans is likely to be supported by the local tertiary structure of the peptide backbone. As such, studies of antibodies suggest that N-linked glycans are constrained within tight clusters on gp120, where unusual sugar–sugar interactions are formed between neighboring glycans as mentioned above.\textsuperscript{6,11,20,21,23} Thus, like oligomannose-type glycans, complex-type glycans appear to be also tightly constrained, forming a cluster where only peripheral or external β-galactoside residues are available for lectin recognition.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{Glycan density-dependent gp120 recognition by galectin-1.}
\end{figure}
binding. In this context, binding by galectin-1 is clearly favored (Fig. 4). The failure of galectin-3 to strongly bind to gp120 also seems to suggest that internal lactosamine residues of gp120 complex-type glycans are sequestered within the glycan patch or via glycan–protein interactions. This interpretation is also strengthened by results showing that the presence of galectin-3 neither interferes nor promotes galectin-1–enhanced HIV-1 infectivity. Galectin-3 was previously considered to interact with both external and internal β-galactoside residues similarly. Unexpectedly, the failure of galectin-3 to bind to intact gp120 suggests that, at least in the case of gp120, the presence of external β-galactoside residues is not sufficient per se to ensure a strong binding by galectin-3. Additional studies are warranted to understand if such binding requirement of galectin-3 to have access to internal β-galactosides is a common phenomenon or not.

As mentioned above, clusters of common host glycans could display nonself properties to the innate immune system. In the case of HIV-1 gp120, clustering of complex-type glycan prevents it from being bound by galectin-3, which is abundantly expressed at the apical surface of the mucosal membranes, including in the genital epithelia, the entry site of HIV-1. Since galectin-3 is believed to be an active participant of the mucosal clearance system, such exclusion is exploited to avoid binding of gp120 by galectin-3, which would lead to a biological dead-end for the virus.

**Conclusion and future directions**

Unlike the traditional view of HIV-1 infection characterized by a slow decline of CD4+ T cells from circulation, recent advance indicates that extensive infection and removal of local CD4+ T cells in GALT occurs within the first month of infection. Since only a few virus particles successfully penetrate the genital epithelia, it thus becomes critical to understand the basic mechanisms underlying the rapid establishment of HIV-1 in the GALT. As HIV-1 is known to carry relatively few Env spikes that are essential for its binding and entry into host cells, it appears important to study how it exploits host factors that may enhance its association with its initial target cells and/or successfully evade the innate immune system. This could open unexplored and interesting avenues of therapeutic intervention targeting transmission and/or the early stage of infection where HIV-1 is more vulnerable to host natural defenses. Recent studies suggest that in addition to C-type lectins of DCs, galectins expressed in the GALT may facilitate HIV-1 transmission and early infection. Galectin-1 greatly increases the association of HIV-1 to susceptible cells through cross-linking of gp120 and CD4 (Fig. 3). The roles of galectin-1 as an HIV-1 assisting molecule should be further investigated to gain deeper insights in the context of viral transmission or early replication stages using different *in vitro* and *ex vivo* systems. In addition, the relevance of these findings in the pathobiology of the virus would need to be further verified *in vivo*, with a model of rhesus macaques infected by the simian immunodeficiency virus, a close relative of HIV-1. Recent advances in synthetic glycochemistry now makes it possible to develop highly specific galectin-1 antagonists that do not interfere with other galectins such as galectin-3.

The use of specific inhibitors or antagonists of galectin-1 thus becomes an attractive therapeutic strategy to further reduce the transmission rate or to limit early replication in the GALT following sexual transmission. However, before such chemical intervention can be accepted and used, it is crucial to better understand the more subtle aspects of galectin-1–mediated facilitation of HIV-1 infection in conjunction with the many other immunological activities that galectin-1 may have in the GALT (see extensive reviews by Rabinovich and others in Refs. 72, 77–79).

**Acknowledgments**

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Supporting information

Additional supporting information may be found in the online version of this article:

Figure S1. The glycan clustering on the surface of gp120 (trimerized form). The glycans are clustered roughly in three domains, oligomannose-type glycan (pink), which is away from CD4 binding sites (*) and complex-type glycans (blue) are relatively close to CD4 binding pockets. Remaining surface of the peptide are marked in red. (Modified from Fig. 3 of Ref. 43 and Fig. 2 of Ref. 6).

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Conflicts of interest

The authors declare no conflicts of interest.

References

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