Galectin-1 promotes HIV-1 infectivity in macrophages through stabilization of viral adsorption

Simon Mercier, Christian St-Pierre, Isabelle Pelletier, Michel Ouellet, Michel J. Tremblay ⁎, Sachiko Sato ⁎

Research Center for Infectious Diseases, CHUL Research Center, Quebec, Canada
Faculty of Medicine, Laval University, Quebec, Canada

Received 26 June 2007; returned to author for revision 23 July 2007; accepted 22 September 2007
Available online 29 October 2007

Abstract

Following primary infection with human immunodeficiency virus type-1 (HIV-1), macrophages are thought to play an important role, as they are one of the first target cells the virus encounters and can also sustain a significant production of viruses over extended periods of time. While the interaction between the primary cellular receptor CD4 and the virus-encoded external envelope glycoprotein gp120 initiates the infection process, it has been suggested that various host factors are exploited by HIV-1 to facilitate adsorption onto the cell surface. Macrophages and other cells found at the infection site can secrete a soluble mammalian lectin, galectin-1, which binds to β-galactoside residues through its carbohydrate recognition domain. Being a dimer, galectin-1 can cross-link ligands expressed on different constituents to mediate adhesion between cells or between cells and pathogens. We report here that galectin-1, but not galectin-3, increased HIV-1 infectivity in monocyte-derived macrophages (MDMs). This phenomenon was likely due to an enhancement of virus adsorption kinetics, which facilitates HIV-1 entry. The fusion inhibitors T-20 and TAK779 remained effective at reducing infection even in the presence of galectin-1, indicating that the galectin-1-mediated effect is occurring at a step prior to fusion. Together, our data suggest that galectin-1 can facilitate HIV-1 infection in MDMs by promoting early events of the virus replicative cycle (i.e. adsorption).

© 2007 Elsevier Inc. All rights reserved.

Keywords: Galectin-1; HIV-1; Macrophages

Introduction

It has been estimated that the human immunodeficiency virus type-1 (HIV-1) pandemic now affects more than 40 million people and has caused more than 20 million deaths worldwide. This retrovirus preferentially infects activated CD4+ T lymphocytes and macrophages through a pH-independent fusion process involving an association between the viral transmembrane and surface proteins gp41 and gp120 with a complex made of the cellular surface protein CD4 and a coreceptor of the chemokine receptor family, generally CCR5 or CXCR4. Following primary infection, continuous viral replication and its associated CD4+ T cell toxicity slowly but relentlessly cripple the host’s ability to mount an efficient immune response, leading to the appearance of a large variety of symptoms, better known as acquired immunodeficiency syndrome (AIDS).

The majority of mammalian cell surface molecules, as well as those of enveloped virus, are heavily glycosylated. Specific sugar sequences presented by the glycans of these glycoproteins can be recognized by a variety of glycans-binding proteins called lectins. One of such lectin families, galectins, has been recently suggested to play functional roles in various immune response processes through binding to host surface glycoproteins (Almkvist et al., 2002; Barrionuevo et al., 2007; Baum et al., 1995; Correa et al., 2003; Gauthier et al., 2002; He and Baum, 2004; Perillo et al., 1995; Rabinovich et al., 1998, 2002a,b; Sato, 2002; Sato and Nieminen, 2004). For example, galectin-1 belongs to the galectin family, β-galactoside-binding proteins,
defined by conserved peptide sequence elements of carbohydrate recognition domain (CRD). Up to 14 galectins (galectin-1–14) have been found in mammals so far, which can be subdivided into three categories depending on the presentation of CRD domains: prototype, tandem-repeat and chimera (Hirabayashi and Kasai, 1993). Some galectins contain one CRD (prototype), and exist as monomers (galectin-5, 7, 10) or dimers (galectin-1, 2, 11, 13, 14), other galectins, such as galectin-4, 6, 8, 9 and 12, contain two CRD connected by a short linker region (tandem repeat) (Hirabayashi and Kasai, 1993). In contrast, galectin-3 uniquely occurs as a chimeric protein with one CRD and an additional non-CRD domain, which is involved in the oligomerization of galectin-3 (Hirabayashi and Kasai, 1993; Nieminen et al., 2007). Galectin-1 expression has been reported in thymus and lymphoid parenchymal epithelial cells, endothelial cells, trophoblasts, activated T cells, macrophages, activated B cells, follicular dendritic cells and CD4+CD25+ regulatory T cells, trophoblasts, activated T cells, macrophages, activated B cells, follicular dendritic cells and CD4+CD25+ regulatory T cells (Baum et al., 1995; Blaser et al., 1998; Dettin et al., 2003; Garin et al., 2007; Jeschke et al., 2004; Rabinovich et al., 1996; Stillman et al., 2006; Zuniga et al., 2001a). We recently found that human tonsil lymphoid tissues contain ∼16 μM of galectin-1 (Ouellet et al., 2005). Galectins are synthesized as cytosolic proteins, thereby the export of galectins into extracellular space is regulated (Cooper and Barondes, 1990; Hughes, 1999; Sato et al., 1993; Sato and Hughes, 1994), while their biological significance remains speculative (Hughes, 1999; Rabinovich et al., 2007; Sato, 2002; Sato and Nieminen, 2004). Indeed, galectin-1 is actively secreted by activated B cells, T cells and macrophages and certain epithelial cells (without compromising membrane integrity) through a “leaderless” secretory pathway (Baum et al., 1995; Blaser et al., 1998; Garin et al., 2007; Gauthier et al., 2002; Rabinovich et al., 1998; Zuniga et al., 2001b), which is also used by fibroblast growth factors and IL-1 (Hughes, 1999; Nickel, 2003, 2005; Oppenheim et al., 2007). Due to their multivalent nature, galectins can cross-link different constituents, acting as adhesion molecules (Baum et al., 1995; Gauthier et al., 2002; Hughes, 2001; Rabinovich et al., 2002a; Rabinovich and Gruppi, 2005; Sato, 2002; Sato and Nieminen, 2004). We have previously found that one member of the galectin family, galectin-1 (Gal-1), can potentiate infection of human tonsilar tissues and primary human peripheral CD4+ T cells by X4-tropic isolates of HIV-1 (Ouellet et al., 2005). Gal-1 can stabilize attachment of HIV-1 particles onto CD4+ T cells thereby facilitating the infection of such susceptible cells. Our previous study was the first demonstration of the impact of Gal-1 in the HIV-1 life cycle, but it mainly reflected the role of Gal-1 in the later stages of the infection, when the virus has reached the secondary lymphoid organs. However, there is still no informative data on the possible involvement of Gal-1 during primary infection. The value of such results resides in the possibility of developing strategies aimed at reducing the occurrence of initial infection.

Infection of macrophages by R5-tropic isolates of HIV-1 may play an important role during the early stages of infection (Noursadeghi et al., 2006). Indeed, macrophages found in the genital and intestinal tracts are mainly exposed to R5-tropic virions during primary infection (Brumme et al., 2005), possibly because the mucosal barrier restricts the transmission of X4 strains (Margolis and Shattock, 2006; Meng et al., 2002). Some studies also revealed that recently infected individuals predominantly harbor R5 isolates of HIV-1 (Koot et al., 1996; Zhang et al., 1998). Macrophages are also thought to play a role in later stages of infection because they are inherently resistant to the cytopathic effects of the virus, and are thus considered to be a stable and long-lived cellular reservoir (Verani et al., 2005). Moreover, their interplay with CD4+ T lymphocytes as professional antigen presenting cells may facilitate trans or cis infection of this cell type through their direct contact and activation (Verani et al., 2005). As macrophages are thought to be important actors in the pathogenesis of HIV-1 infection, and as they also express and secrete Gal-1 following their activation (Correa et al., 2003), it is essential to extend our initial observations to this particular cell type.

This study thus focuses on the role of Gal-1 in the infection process of monocyte-derived macrophages (MDMs) by R5-tropic variants of HIV-1. We report here that exogenous addition of Gal-1 to MDMs enhances HIV-1 infection possibly through an increase in viral binding kinetics. Interestingly, the antiviral potency of fusion inhibitors targeting gp41 (T-20) and CCR5 (TAK779) was not affected by Gal-1, thus indicating that this soluble factor is affecting the HIV-1 life cycle at a step prior to fusion. The possible implications of this set of results could prove to be important for the efficient use of the already available antiretroviral drugs.

Results

Galectin-1, but not galectin-3, promotes HIV-1 infection in MDMs

MDMs were initially infected with recombinant luciferase-encoding reporter viruses that were pseudotyped with the R5-tropic JR-FL envelope. This experimental setup allows a rapid, accurate and quantitative evaluation of single-cycle infection events through measurement of cell-associated luciferase activity following infection. As shown in Fig. 1A, the presence of Gal-1 significantly increased virus-induced luciferase activity. This Gal-1-mediated augmentation in virus replication was dose-dependent up to a 4 μM final concentration of Gal-1 (data not shown). In order to assess the specificity of Gal-1 action, further infection experiments were performed in the presence of another member of the galectin family, namely galectin-3 (Gal-3). Interestingly, infection of MDMs by the reporter virus remained unaffected when it was performed in the presence of Gal-3 (Fig. 1A). These results suggest that the Gal-1-mediated enhancing effect on HIV-1 infection is specific and is not a general phenomenon that can take place with all members of the galectin family.

Although the initial series of investigations is informative, it is important to emphasize that such experiments have been performed with pseudotyped, replication-incompetent, reporter viruses produced in a human cell line, which is not considered as a natural cellular reservoir for HIV-1 (i.e. 293T cells). Moreover, it is well established that HIV-1 acquires a vast array of
host-derived surface molecules in its bilayer lipid envelope (Tremblay et al., 1998). Since the majority of surface proteins on mammalian cells are glycosylated to increase their stability and lock-in their conformation, viruses budding from these cells will carry such host-derived glycosylated proteins on their surface. While the glycosylation patterns of surface proteins are protein-specific, their patterns can vary, to some extent, depending on the cell-type and cell status (e.g. resting versus activated, apoptotic, etc.) (Ohtsubo and Marth, 2006). Thus, viruses produced in different cell types might acquire different glycosylation patterns that could, in turn, have an impact on the ability of Gal-1 to affect viral replication. Acknowledging this possibility, the experimental setup described above was repeated, but this time using replication-competent R5-tropic virions (i.e. NL4-3/BaL) harvested from acutely infected MDMs. Data shown in Fig. 1B demonstrate that replication of fully competent virus was augmented by the presence of Gal-1. Together, the data suggest that Gal-1 facilitates infection of MDMs by R5-tropic viruses. Additional studies revealed that Gal-1 was unable to induce transcription from the HIV-1 regulatory elements (i.e. long terminal region/LTR) (Fig. 1C). Therefore it can be concluded that the Gal-1-dependent effect on HIV-1 replication is not due to signal transduction events that can in turn promote HIV-1 LTR-driven activity.

Given the reported capacity of Gal-1 to cross-link ligands expressed on different constituents (Baum et al., 1995; Gauthier et al., 2002; Hughes, 2001; Rabinovich et al., 2002a; Rabinovich and Gruppi, 2005; Sato, 2002; Sato and Nieminen, 2004) and its inability to drive HIV-1 LTR-driven gene expression, it can be proposed that Gal-1 favors the first steps in the HIV-1 replicative cycle by promoting the initial interactions between the virus and the surface of target cells, thereby facilitating viral entry. Adsorption of HIV-1 particles on MDMs was thus assessed by measuring the cell-associated p24 levels following incubation for 1 h at 37 °C in the presence or absence of 2 μM of Gal-1 or Gal-3 as described in Materials and methods. As our experiments are performed at 37 °C, we refer to it as an adsorption experiment. Adsorption includes virus that bind and enter the cell through fusion or endocytosis. We chose to proceed this way to better approximate the initial steps of HIV-1 infection in vivo. As shown in Fig. 2A, virus adsorption was significantly enhanced upon addition of Gal-1 (i.e. 9-fold increase) but not Gal-3 (data not shown). The Gal-1-mediated enhancement of HIV-1 adsorption was totally abolished by lactose, which competes for the CRD of Gal-1 and acts as an antagonist of this soluble β-galactoside-binding protein. When the incubation period was extended to 6 h, cell-associated p24 levels in the absence of Gal-1 became comparable to those in the presence of Gal-1 (Fig. 2B). This observation suggests that Gal-1 accelerates the kinetics of the initial steps of HIV-1 replication. The Gal-1-mediated enhancement of HIV-1 adsorption was also observed when experiments were conducted with X4-using virus (i.e. NL4-3 strain) (data not shown). This result suggests that the increased adsorption observed is independent of specific co-receptors. Given that our observations led us to propose that virus adsorption occurred with faster kinetics in the presence of Gal-1, we next investigated the level of viral internalization in these cells in the presence or absence of Gal-1. MDMs were incubated with NL4-3/BaL and increasing concentrations of Gal-1 (0 to 4 μM) for 1 h at 37 °C, in the presence or absence of 50 mM of lactose. Unbound virus particles and those that bound but did not enter the cells were removed by a trypsin treatment as published previously (Tardif and Tremblay, 2005a,b). Results depicted in Fig. 2C demonstrate that virus internalization was promoted in a dose-dependent manner by Gal-1 (i.e. 2.5-fold increase in presence of 4 μM Gal-1; 1070±75 versus 440±34 pg/ml). As seen with adsorption assays, this...
Gal-1-dependent increase is lost upon addition of lactose. In order to confirm these results using a different experimental strategy to remove bound but uninternalized viruses, we also performed acid washes instead of trypsin treatment as described previously (Vidricaire and Tremblay, 2005). Similar observations were made when using this method (data not shown), which confirm that Gal-1 is affecting the earliest step in the HIV-1 replicative cycle (i.e. adsorption).

The Gal-1-mediated increase in virus internalization could be due to an enhancement in virus-mediated fusion and/or an augmentation in HIV-1 entry through an endocytic process such as macropinocytosis. We thus performed additional entry assays, but this time in the presence of the fusion inhibitors T-20 and TAK779, which are targeting different actors of the infection process. T-20 prevents fusion between virus and cell membranes by binding to the viral fusion peptide of gp41, whereas TAK779 is a chemical antagonist of CCR5 that inhibits its interaction with the V3-loop of gp120 after it has bound to CD4. These two inhibitors act directly on the essential interactions required for the fusion of viral and cellular membranes. Results illustrated in Fig. 2D indicate that, at the doses which gave maximal inhibition of HIV-1 entry in CD4+ T cells (Tardif and Tremblay, 2005a), HIV-1 internalization within MDMs, regardless of the presence or the absence of Gal-1, was not affected by either T-20 or TAK779. These data suggest that, at least under these experimental conditions, the gp41-mediated membrane fusion events do not contribute significantly to the process of HIV-1 internalization into MDMs.

Galectin-1 does not reduce the overall sensitivity of HIV-1 to fusion inhibitors

Our previous report in CD4+ T lymphocytes indicates that T-20 efficiently inhibits Gal-1-promoted viral replication, suggesting that Gal-1 facilitates initial adsorption of X4-tropic HIV-1 to CD4+ T lymphocytes, but not the following membrane fusion step (Ouellet et al., 2005). In the case of MDMs, the data presented above suggest that the fusion inhibitors T-20 and TAK779 exhibit a limited capacity to reduce viral internalization, which was significantly increased by Gal-1, possibly through an increase of adsorption kinetics of the virus. Therefore, we next studied whether these fusion inhibitors can effectively limit infection of MDMs by HIV-1. In the presence of Gal-1, HIV-1 infection of MDMs was still detectable even at high doses of T-20 or TAK779 due to the Gal-1-mediated increase in HIV-1 infection (Figs. 3A and B). However, the
percentages of inhibition of virus infection in MDMs achieved by T-20 were comparable whether the cells were treated or not with 2 μM of Gal-1 (Fig. 3C). Similar observations were made when experiments were carried out in the presence of TAK779 (Fig. 3D). Indeed, the calculated IC50 are 37 and 41 ng/ml for T-20 and 0.028 and 0.030 nM for TAK779, in the presence and absence of 2 μM of Gal-1. Together, the data suggest that although Gal-1 significantly increased viral internalization and infection in MDMs, the relative efficiency of the tested fusion inhibitors was unaffected by Gal-1, even if they fail to limit viral entry.

Discussion

Several lines of evidence imply that galectins participate in the immune response, both as immunomodulators and molecules that facilitate pathogen-host cell interactions (Kohatsu et al., 2006; Mey et al., 1996; Rabinovich and Gruppi, 2005; Sato, 2002; Sato and Nieminen, 2004). In addition, recent works suggest that galectins could facilitate pathogen internalization in phagocytic cells, such as macrophages (Pelletier et al., 2003; van den Berg et al., 2004). However, there is very few, if any, information concerning the roles of galectins in viral infection, even though many enveloped virus express glycoproteins on their surface. We have recently reported that Gal-1 can increase adsorption of X4-using isolates of HIV-1 onto CD4+ T lymphocytes, thus enhancing the overall infection process (Ouellet et al., 2005). This observation is in line with previous studies showing that some galectins can act as adhesion molecules by cross-linking surface ligands expressed on pathogens and their target cells (Ouellet et al., 2005; Pelletier et al., 2003; Rabinovich and Gruppi, 2005; Sato, 2002). Results depicted in the present work therefore represent additional evidence of the specific cross-linking capacity of galectins and suggest that Gal-1 can modulate sexual transmission of HIV-1 through enhancement of viral adsorption kinetics on diverse target cells’ surface. Interestingly, another type of galectin, Gal-3, displayed no effect on HIV-1 adsorption, entry and infection in a similar context, even though both Gal-1 and Gal-3 bind efficiently to macrophages (Camby et al., 2006; Dumic et al., 2006). This is in contrast to its ability to facilitate the interaction between parasites and macrophages (Pelletier et al., 2003; van den Berg et al., 2004). Likely due to structural differences between Gal-1 and Gal-3, both in their CRDs and in its presentation, some (but not all) of the ligands of macrophages preferentially bind to galectin-1. In addition, it is possible that the affinities of those galectins for HIV-1 are different. Such ligand specificity might be responsible for this differential effect (Rabinovich et al., 2002a; Rabinovich and Gruppi, 2005).
An increase in HIV-1 adsorption mediated by Gal-1 might bear some significant relevance with respect to primary infection, considering that intracellular Gal-1 could be released by sheared fibroblasts and epithelial cells following sex-related trauma such as micro-abrasions (Akimoto et al., 1995; Baum et al., 1995). In the context of sexual transmission of HIV-1, the presence of Gal-1 in the ejaculate could also be an important factor since Gal-1 has been shown to be present on the heads and tails of late spermatids in rat testes (Dettin et al., 2003). In addition, Gal-1 is actively secreted by activated macrophages, Langerhans cells, dendritic cells, and activated CD4+ and CD8+ T lymphocytes (Blaser et al., 1998; Rabinovich et al., 1996, 1998, 2002b; Reynolds et al., 2007; Zuniga et al., 2001a). Fibroblasts present at the infection site could also be another source of Gal-1. Thus, mucosal macrophages could be exposed to both Gal-1 and R5-tropic HIV-1 particles (Verani et al., 2005), likely creating a situation that might facilitate and accelerate primary infection. Concerning our infection model, even though Gal-1 is secreted by macrophages, tissular concentrations of Gal-1 could never be reached simply by maintaining MDMs in culture due to the frequent addition of fresh culture medium and the fact that the differentiation process does not lead to the activation of MDMs so that they do not secrete Gal-1 themselves. Moreover, as mentioned earlier, other sources (cells or sperm) of Gal-1 are normally present in vivo but are absent in the context of cell culture. Therefore, addition of exogenous Gal-1 to our infection model is physiologically relevant, in order to partially reconstitute the initial mucosal infection site where the presence of extracellular Gal-1 is expected.

This study suggests that Gal-1 increases viral adsorption kinetics on MDMs, thereby shortening the time required to establish an infection. During primary infection, the number of HIV-1 particles is limited. In addition, complement, natural antibodies and other soluble factors at the primary infection sites could reduce the infectious potential of the virus (Neil et al., 2005). Thus, shortening the exposure time required for the virus to establish an infection would significantly increase the possibility of a successful HIV-1 infection. Moreover an increase in viral adsorption and entry kinetics reduces the time required to establish infection and significantly shortens the crucial period in which post-exposure HAART treatment could prevent it.

Even in the presence of Gal-1, fusion inhibitors retain their antiviral potency and display similar IC₅₀ values. These results confirm that Gal-1 acts mainly at the initial adsorption step, and not at the fusion-dependent process that would allow HIV-1 to gain access to the cytoplasm. While fusion inhibitors were potent inhibitors of HIV-1 infection in MDMs, these drugs could not efficiently inhibit the inherent ability of MDMs to internalize large amount of viruses. It has been suggested that following internalization of HIV-1 within MDMs, HIV-1 could still gain access to the cytoplasm through fusion of its membrane with the endosomal membrane. This step remained dependent on gp120 (Marechal et al., 2001) and sensitive to fusion inhibitors such as T-20. Our data also support the notion that MDMs mainly internalize HIV-1 through endocytosis, a step that is insensitive to fusion inhibitors, and are then infected by HIV-1 when it accesses the cytosol from endosomes in a fusion-dependent manner. Importantly, despite of the presence of fusion inhibitors, Gal-1 was still efficient at increasing the amount of internalized HIV-1 by enhancing initial viral adsorption onto MDMs. In a situation where the virus is unable to perform fusion (T-20 treated individual), Gal-1 could thus act as a soluble scavenger receptor and enhance the uptake of the virus by macrophages. As those accumulated virions in endosomes are likely to be degraded and presented in the MHC-II context, the corollary of these results is that early treatment of patients with fusion inhibitors could lead to the development of a stronger immune response towards HIV-1 while limiting viral replication and spread.

Further investigations are necessary to define whether Gal-1 might act as DC-SIGN, a C-type lectin involved in the endocytosis of HIV-1 that is known to facilitate both HIV-1 antigen-presentation and viral transmission to CD4+ T lymphocytes (Geijtenbeek et al., 2000, 2004; Turville et al., 2002, 2004). It could otherwise be possible that, in the presence of fusion inhibitors, Gal-1 could act as Langerin, another C-type lectin found on Langerhans cells, which promotes endocytosis and degradation of HIV-1, thereby preventing its spread (de Witte et al., 2007).

Materials and methods

Cell lines

Peripheral blood mononuclear cells (PBMCs) were purified from blood of healthy donors by Ficoll centrifugation (Lymphocyte Separation Medium; Wisent Inc.; St-Bruno, QC). MDMs were produced by incubating PBMCs at a concentration of 1.25 × 10⁶ cells in a T-75 flask for 2 h at 37 °C, thus allowing adhesion of monocytes to the bottom of the flask. Non-adherent cells were then removed and monocytes were washed twice with RPMI 1640 containing 5% autologous heat-inactivated plasma and cultured for 1 week in medium supplemented with 100 ng/ml macrophage-colony stimulating factor (M-CSF) to allow differentiation into MDMs. Next, MDMs were detached using Accutase® (Sigma-Aldrich Inc.; St-Louis, MO) as recommended by the manufacturer and gently scraped with a cell scraper. MDMs were then dispensed in a 48-well plate at a final concentration of 5 × 10⁴ cells per well. Isolation, differentiation and assays described below were all performed in RPMI 1640 medium containing 5% autologous heat-inactivated plasma.

Viral preparations

Virus particles (NL4-3/BaL) were produced by transiently transfecting human embryonic kidney 293T cells with the R5-tropic infectious molecular clone NL4.3BaLenv (kindly supplied by Dr. R.J. Pomerantz, Thomas Jefferson University, Philadelphia, PA) (Dormadula et al., 1999). The infectious molecular clone pNL4-3 (X4-tropic) was also used in some experiments. Recombinant luciferase-encoding reporter R5-tropic viruses were generated using pNL4-3.3Luc+Env− (AIDS Repository Reagent Program, Germantown, MD) and a vector
coding for JR-FL \textit{env} (kind gift by Dr. N.R. Landau, The Salk Institute, La Jolla, CA) or VSV-G \textit{env}. Briefly, 2 × 10^6 cells were seeded in a T-75 flask for 24 h before transient transfection, which was carried out by adding a total of 30 \(\mu\)g of vectors (pNL4-3/BaL or pNL4-3-Luc + Envelope and a vector coding for JR-FL \textit{env}) to the cells as a calcium phosphate precipitate. At 2 days post-transfection, the virus-containing supernatant was filtered and frozen at \(-85\,^\circ\text{C}\) until used. Infectious virus particles were also prepared from the culture supernatant of MDMs infected for 3 to 4 weeks using NL4-3/BaL. Titers of virus particles were normalized by their content of the capsid protein p24 as determined by a sandwich ELISA or by TCID\(_{50}\) as determined by testing virus dilution performed on the TZM-BL reporter cell line (Derdyn et al., 2000; Platt et al., 1998; Wei et al., 2002) (obtained through the NIH AIDS Repository Reagent Program; TZM-bl from Dr. John C. Kappes, Dr. Xiaoyun Wu and Tranzyme, Inc.).

\textbf{Recombinant galectins}

Recombinant galectins were produced and purified as previously described (Nieminen et al., 2007, 2005; Ouellet et al., 2005; Pelletier et al., 2003) with the following modifications of the protocol: lactose was first removed either by using Hiprep 26/10 desalting column (GE Healthcare, Fairfield, Connecticut) or by extensive dialysis against PBS and the purified galectins were then passed through Aacticlean ETOX endotoxin removing gels (Sterogene, Carlsbad, California). The purified galectin preparations were then sterilized by filtration through a 0.22 \(\mu\)m filter. Mock preparations were also prepared using \textit{E. coli} JM109 that does not express Gal-1 using the same purification protocol. The bioactivities of galectins were estimated by using a hemagglutinin assay as described previously (Giguere et al., 2006a,b).

\textbf{Infection assays}

MDMs were infected for 1 h with HIV-1 (1 \times 10^4 TCID\(_{50}\) of NL4-3-Luc + Env− pseudotyped with JR-FL \textit{env}) in the presence of various concentrations of Gal-1 (ranging from 0 to 4 \(\mu\)M). Cells were then washed twice with cell culture medium and incubated for 5 days at 37 \(^\circ\text{C}\) prior to lysis with 5\% Triton X-100 and 50\% glycerol) before measuring luciferase activity as described previously (Ouellet et al., 1999). Integrated LTR will respond to external stimuli and lead to a dose-dependent increase in luciferase gene transcription and, therefore, activity. TNF-\(\alpha\) is used as a positive control. It is important to note that time course experiments were performed since gene integration requires an, as yet, undetermined amount of time.

\textbf{Acknowledgments}

We thank Ms. Sylvie Méthot and Julie Nieminen for editorial assistance. We also want to thank Dr. Jun Hirabayashi from the Research Centre for Glycoscience (National Institute of Advanced Industrial Science and Technology, Tsukuba, Ibaraki, Japan) for giving us the Gal-1-encoding plasmid. This study was made possible by an operating grant to M.J.T. and S.S. from the Canadian Institutes of Health Research (CIHR) HIV/AIDS Research Program (grant #HOP-75351). This work was performed by S.M. in partial fulfillment of a Ph.D. degree from the Microbiology-Immunology Program, Faculty of Medicine, Laval University. S.M. and C.S.-P. each hold a CIHR Doctoral Award. M.J.T. is the recipient of a Tier 1 Canada Research Chair in Human Immuno-Retrovirology and S.S. holds a Scholarship Award (senior level) from the Fonds de la Recherche en Santé du Québec.

\textbf{References}


Dornadula, G., Zhang, H., Shetty, S., Pomerantz, R.J., 1999. HIV-1 virions produced from replicating peripheral blood lymphocytes are more infectious than those from nonproliferating macrophages due to higher levels of intravirion reverse transcripts: implications for pathogenesis and transmission. Virology 253 (1), 10–16.


