Available online at www.sciencedirect.com

SCIENCE DIRECT®

Virology 324 (2004) 165–172

VIROLOGY

www.elsevier.com/locate/yviro

Envelope glycoproteins are not required for insertion of host ICAM-1 into human immunodeficiency virus type 1 and ICAM-1-bearing viruses are still infectious despite a suboptimal level of trimeric envelope proteins

Yannick Beauséjour and Michel J. Tremblay*

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

Received 18 December 2003; returned to author for revision 10 February 2004; accepted 10 March 2004

Abstract

Previous works have indicated that incorporation of surface glycoprotein into retroviruses such as the human immunodeficiency virus type 1 (HIV-1) is not a highly specific process because several cellular glycoproteins can be inserted within the mature viral particle. The mechanism(s) that govern the acquisition of such host constituents have remained so far elusive. In this study, we have investigated the role played by the viral envelope (Env) of HIV-1 in the acquisition of host intercellular adhesion molecule type I (ICAM-1). ICAM-1 proteins were still present on viruses carrying much lower levels of gp120/gp41 due to a mutation in the matrix (MA) domain or on Env-deficient viruses when produced in immortalized and primary human cell lines. Interestingly, infectivity of an HIV-1 MA mutant that carry a suboptimal amount of Env proteins was restored to a certain degree by the presence of ICAM-1 when infection was performed in cells expressing an activated form of its natural counter-ligand, LFA-1.

Keywords: ICAM-1; Envelope; gp120; LFA-1; HIV-1; Integrin; Adhesion; Infectivity

Introduction

It has been demonstrated in a large number of studies that retroviruses tolerate the incorporation of several foreign plasma membrane proteins. Studies aimed at identifying the nature of foreign constituents embedded within the human immunodeficiency virus type 1 (HIV-1) revealed that a high number of host plasma membrane proteins are associated with mature virions including significant amounts of HLA-DR and some adhesion molecules (e.g. CD44, ICAM-1, ICAM-2, ICAM-3, LFA-1, VCAM-1, VLA-4) (Arthur et al., 1992; Cantin et al., 1996, 2001; Guo and Hildreth, 1995; Liao et al., 2000; Orentas and Hildreth, 1993). The functional significance of the phenomenon of incorporation of such foreign membrane proteins is proven by the reported inhibition of HIV-1 replication, in both in vitro and in vivo studies, with the use of antibodies specific for host proteins (Arthur et al., 1992, 1995; Bounou et al., 2001; Chan et al., 1995; Fortin et al., 1997, 1998, 1999; Rizzuto and Sodroski, 1997).

The nature of factor(s) governing incorporation of foreign constituents in mature HIV-1 particles remains a matter of speculation. However, it seems clear that this event is influenced by numerous factors such as the virus itself, the nature of host cells, and the donor source (Cantin et al., 2001). Similarly, the exact molecular process responsible for the selective incorporation within HIV-1 of some defined host-encoded plasma membrane proteins is also obscure. It has been proposed that host membrane proteins that have interactions with some other cellular constituents may be excluded from mature HIV-1 virions due to a steric hindrance phenomenon or sequestration to some specific domains different from the viral assembly site (Henriksson et al., 1999). This concept is supported by the finding that the natural interaction between cell surface CD4 and the tyrosyl kinase $p56^{lck}$ prevents incorporation of CD4 into the HIV-1 envelope (Env) due to a phenomenon of steric inhibition (Henriks-

* Corresponding author. Laboratory of Human Immuno-Retrovirology, Research Center in Infectious Diseases, RC709, CHUL Research Center, 2705 Laurier Boulevard, Ste-Foy, Quebec, Canada G1V 4G2. Fax: +1-418-654-2212.

E-mail address: michel.j.tremblay@crchul.ulaval.ca (M.J. Tremblay).

0042-6822/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2004.03.029

son and Bosch, 1998). Furthermore, it has been proposed that HIV-1 Gag proteins are preferentially sorted out in lipid rafts, which are rich in cholesterol, sphingolipids, and GPI-linked proteins (Nguyen and Hildreth, 2000). Consequently, HIV-1 would have a propensity to incorporate host proteins located in such plasma membrane rafts (Nguyen and Hildreth, 2000). Recently, it has been proposed that a direct interaction between virus-encoded Env proteins and HLA-DR might be responsible for the selective uptake of this cell surface constituent by newly formed HIV-1 (Poon et al., 2000).

The intercellular adhesion molecule type I (ICAM-1) is a cell surface glycoprotein that contains five extracellular immunoglobulin-like domains, a hydrophobic transmembrane domain, and a short cytoplasmic domain. ICAM-1 is an inducible ligand for at least two members of the β2 family of leukocyte integrins, lymphocyte function-associated antigen-1 (LFA-1) (αLβ2) and Mac-1 (αMβ2) (reviewed in (Springer, 1990)). ICAM-1 is important for granulocyte extravasation, lymphocyte-mediated cytotoxicity, and the development of specific immunologic responses involving cell–cell interactions (reviewed in Plow et al., 2000). A previous study has revealed that ICAM-1 is acquired by clinical strains of HIV-1 expanded in primary human cells (Cantin et al, 1996). Moreover, incorporation of ICAM-1 into HIV-1 particles increased virus infectivity by several folds and diminished sensitivity to neutralization (Castilletti et al., 1995; Fortin et al., 1997, 2000; Rizzuto and Sodroski, 1997). Therefore, given that virion-bound host ICAM-1 does influence the replication cycle of HIV-1, it is important to understand the mechanism(s) governing the selective incorporation of ICAM-1 into mature HIV-1 particles.

The precise contribution of Env glycoproteins in ICAM-1 incorporation was tested through the use of a NL4-3-based matrix mutant (34VE), which leads to the production of HIV-1 carrying a reduced number of Env glycoproteins (Freed and Martin, 1996), and an Env-deficient HIV-1 vector. Here, we demonstrate that insertion of host ICAM-1 within HIV-1 produced in established and primary human cells does not necessitate the presence of Env. Moreover, we report for the first time that incorporation of host-derived ICAM-1 in viruses bearing a suboptimal level of Env glycoproteins restores HIV-1 infectivity in an ICAM-1/LFA-1-dependent fashion.

Results

A previous study has shown that the process of HIV-1 Env packaging is necessary to achieve incorporation of host-encoded HLA-DR in HIV-1 (Poon et al., 2000). In an attempt to define whether the HIV-1 Env spike glycoproteins are also necessary to achieve incorporation of ICAM-1 proteins onto mature HIV-1 particles, wild type and mutant virions that are deficient in Env (Env−) were produced in ICAM-1-negative (i.e. parental 293T) and ICAM-1-positive 293T cells (i.e. Hi-ICAM-1). Such Hi-ICAM-1 cells stably express a moderate level of cell surface ICAM-1 (54.3% of positive cells with a mean fluorescence value of 12.6) that is not modulated following production of wild type and mutant viruses (data not shown). It should be noted that Hi-ICAM-1 cells express an ICAM-1 level comparable to what is detected on two cellular reservoirs of HIV-1, that is, activated $CD4^+$ T lymphocytes and macrophages (data not shown). Given that mutations in the HIV-1 matrix protein often result in a blocking of the incorporation of HIV-1 Env (gp120 and gp41) into assembling HIV-1 particles (Dorfman et al., 1994; Freed and Martin, 1995, 1996; Lee et al., 1997; Yu et al., 1992, 1993), a virus expression vector bearing a single-site mutation at position 34 (34VE) of the matrix (MA) domain was also used.

The presence of host ICAM-1 does not influence the level of incorporation of gp120 in mature HIV-1

First, we wanted to ascertain that production of the tested HIV-1 mutants in ICAM-1-expressing cells did not affect gp120 incorporation in viruses. We thus measured the levels of virus-associated gp120 that were present in our purified virus preparations using a homemade gp120 test (Paquette et al., 1998). Comparative analyses were made by calculating the molar ratio of Env to Gag proteins on the basis of known molecular masses, that is, 120 and 24 kDa for p24 and gp120, respectively. The MA 34VE mutant was found to express five times less Env than wild-type virions and, as expected, no gp120 could be detected in Env-deficient HIV-1 particles (Table 1). Results from this series of investigations also indicate that the presence of host-derived ICAM-1 glycoprotein into the mature virus particles does not affect the external envelope spike density on HIV-1.

Table 1
Virus-associated gp120 in wild type and mutated HIV-1 preparations

Virus stocks	gp120 (ng/ml)[a]	p24 (ng/ml)[a]	gp120/p24 molar ratios (10^{-2})	gp120/p24[b] (%)
ICAM-1/NEG				
WT	19 ± 0.9[c]	161 ± 8	2.4	100
34VE	31 ± 1.0	1202 ± 55	0.5	21
Env−	0	234 ± 12	NA	NA
ICAM-1/POS				
WT	12 ± 0.6	89 ± 4	2.7	100
34VE	6 ± 0.03	265 ± 13	0.5	19
Env−	0	129 ± 6	NA	NA

NA: not applicable.
[a] The amounts of viral gp120 and p24 were assessed by enzymatic assays.
[b] The percentage of gp120/p24 found in wild-type viruses was arbitrarily set at 100.
[c] Results shown are the mean ± SD of triplicate samples and are representative of three independent experiments.

Incorporation of host ICAM-1 in HIV-1 does not require Env

To dissect the exact contribution of HIV-1 Env to the process of ICAM-1 incorporation, the purified virus preparations produced by transient transfection of 293T and Hi-ICAM-1 cells were next subjected to a virus capture assay. Data from the virus precipitation test showed that the 34VE mutation in the MA domain of $Pr55^{Gag}$ has no effect on the capacity of such mutant viruses to acquire host ICAM-1 (Fig. 1A). A similar observation was made for HIV-1 particles that are completely Env-deficient (i.e. Env−). This suggests that, in contrast to what has been reported for HLA-DR (Poon et al., 2000), Env packaging is not essential to lead to the efficient insertion of host-derived ICAM-1 into mature HIV-1 viral entities. Western blot analyses were also performed on such virus particles to more quantitatively estimate virus-associated host ICAM-1. Data from this set of experiments confirm that ICAM-1 incorporation can still occur in the absence of Env.

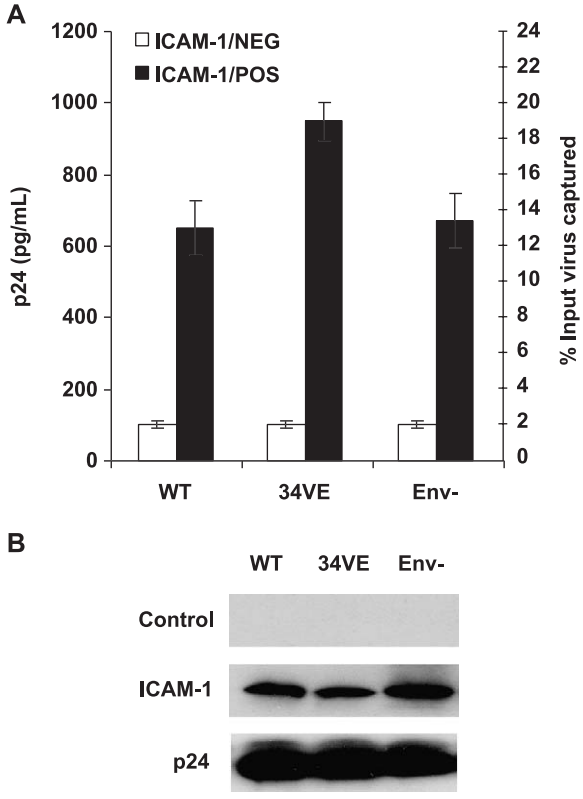

Fig. 1. ICAM-1 is acquired by MA mutant and Env-deficient viruses produced in established 293T cells. Wild type (WT), MA mutant (34VE), and Env-deficient (Env−) NL4-3 viruses either lacking (ICAM-1/NEG) or bearing on their surface host-derived ICAM-1 (ICAM-1/POS) were produced following transient transfection of 293T cells. (A) Virus preparations were next precipitated using magnetic beads coated with an anti-ICAM-1 antibody (i.e. R6.5). The amounts of captured HIV-1 were estimated by a quantitative p24 test (left *y* axis). The percentage of captured viruses from the initial virus input is shown on the right *y* axis. Data shown represent the means ± SD of triplicate samples and are representative of three independent experiments. (B) Lysed virus preparations were directly loaded on a 10% SDS-polyacrylamide gel (10 ng of p24 per lane), transferred to a membrane, and revealed by an anti-ICAM-1, anti-p24, or control antibody.

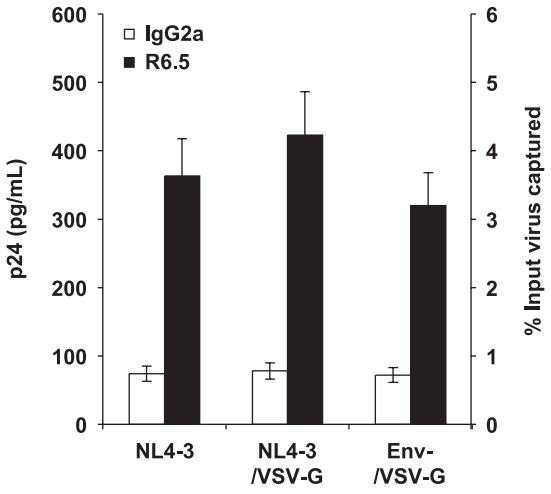

Fig. 2. ICAM-1 is incorporated in MA mutant and Env-deficient viruses produced in primary human cells. Wild type (NL4-3), pseudotyped wild type (NL4-3/VSV-G), and pseudotyped Env-deficient (Env-/VSV-G) HIV-1 particles were produced in 293T cells and were used to infect PBMCs. Progeny viruses were collected and subjected to the virus capture assay. An isotype-matched irrelevant antibody (IgG2a) was used to monitor nonspecific binding of HIV-1 to magnetic beads. The amounts of captured HIV-1 were estimated by a quantitative p24 test (left *y* axis). The percentage of captured viruses from the initial virus input is shown on the right *y* axis. Data shown represent the means ± SD of triplicate samples and are representative of three independent experiments.

Given that the virus producer cell might represent an important factor in the phenomenon of incorporation of host cell surface components in budding HIV-1, similar studies were also performed using virus preparations expanded in a more natural cellular reservoir, that is, PBMCs. Env-deficient viruses were pseudotyped with the envelope G proteins of the broad-host-range VSV. The pseudotyping strategy with VSV-G allows infection of target cells in a gp120-CD4-independent fashion. To control for any possible VSV-G-mediated modulatory effect on ICAM-1 incorporation, wild-type NL4-3 viruses were also pseudotyped using a similar strategy. Data from Fig. 2 indicate that the presence of Env is not mandatory to allow insertion of host ICAM-1 in HIV-1 particles produced by suspensions of PBMCs cultured in vitro, an observation that is in line with results obtained with virus preparations made in established cell lines.

ICAM-1/POS viruses carrying a suboptimal level of virion-associated gp120 are still partially infectious

The initial contact between HIV-1 and its host cell is generally thought to occur via an interaction between CD4 and gp120. Considering that the formation of a fusion pore requires the recruitment of a sufficient number of CD4 and

coreceptor molecules (reviewed in Clapham and McKnight, 2002) and that incorporation of ICAM-1 increases HIV-1 infectivity several folds (Fortin et al., 1997; Rizzuto and Sodroski, 1997), we next tested whether the acquisition of host ICAM-1 could restore to some extent infectivity of MA mutant viruses bearing a lower density of Env glycoproteins. As expected, the insertion of the ICAM-1 adhesion molecule within wild-type HIV-1 particles resulted in an enhancement of virus infectivity for LuSIV (11.5-fold increase; compare 15.0 and 172 RLU), an LFA-1-positive reporter cell line that is highly susceptible to HIV-1 infection (Fig. 3). No such increase in virus infectivity was detected when infection was performed with 34VE mutant viruses. Susceptibility of LFA-1-positive cells to infection with ICAM-1-bearing virions is significantly augmented by converting LFA-1 to a high avidity/affinity state for ICAM-1 (Fortin et al., 1998). LuSIV cells were thus pretreated with MEM-83, an anti-LFA-1 antibody known to enhance LFA-1-mediated T cell adhesion to ICAM-1 (Binnerts et al., 1994; Landis et al., 1993) before inoculation with wild type and MA mutated viruses either lacking or bearing host ICAM-1. Activation of LFA-1 rendered LuSIV cells even more susceptible to infection with wild-type ICAM-1/POS virus particles (20.2-fold increase; compare 17.9 and 361.0 RLU). Interestingly, a statistically significant 4-fold increase in reporter gene activity was observed when MEM-83-treated LuSIV cells were infected with ICAM-1-bearing 34VE viruses as compared to inoculation with isogenic ICAM-1/NEG HIV-1 particles.

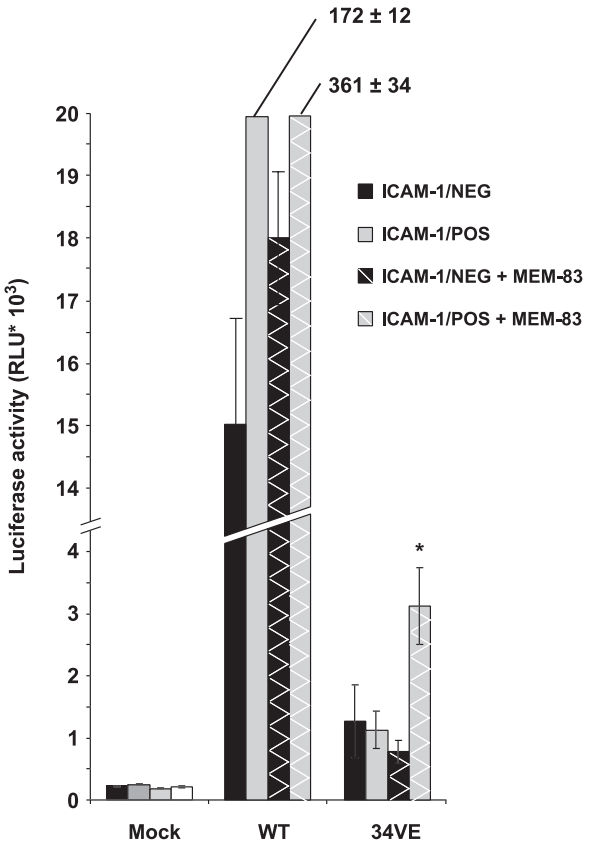

Fig. 3. Infectivity of HIV-1 MA mutant is partially restored upon interaction between virus-anchored ICAM-1 and activated LFA-1 on the target cell. The LFA-1-positive reporter LuSIV cell line was either left untreated or was treated with MEM-83, an activating anti-LFA-1 antibody. Cells were next inoculated with wild type and MA mutant NL4-3 viruses either lacking or bearing host ICAM-1. HIV-1 infection was allowed to proceed for 48 h before measuring luciferase activity (expressed in relative light units, or RLU). Results shown are the means ± SD of triplicate samples and are representative of three independent experiments. The asterisk indicates a significantly different value from that of MEM-83-treated LuSIV cells infected with ICAM-1/NEG 34VE virus ($P < 0.05$).

Discussion

In this study, we have performed analyses to gain novel information on the molecular basis that is responsible for the incorporation of host-encoded ICAM-1 adhesion molecule into HIV-1. More specifically, we have addressed the question of whether the packaging of virus-encoded gp120/gp41 Env proteins is required for efficient incorporation of cell surface ICAM-1 glycoprotein in HIV-1. This scenario was tested using Env-deficient HIV-1 as well as viruses bearing a mutation in the MA protein (34VE) that have been shown to significantly reduced Env incorporation into assembling virus particles (Freed and Martin, 1995). A more physiological significance to our findings was provided by monitoring acquisition of ICAM-1 in pseudotyped viruses (Env-deficient and MA mutant) produced in primary human cells. We established that the incorporation process of ICAM-1 was not affected by Env packaging. This observation is in sharp contrast with a previous study which indicated that there is a specific interaction between HLA-DR and the gp41 transmembrane envelope protein (Poon et al., 2000). In this study, a 43-amino-acid sequence located between amino acids 708 and 750 in the gp41 cytoplasmic tail was found to be necessary for insertion of host-derived HLA-DR into mature HIV-1.

Although we could not demonstrate a direct link between HIV-1 Env and the process of ICAM-1 incorporation, we were interested in addressing the possible physiological significance of the presence of host ICAM-1 on the surface of HIV-1 bearing a suboptimal amount of gp120/gp41. For this purpose, we inoculated an LFA-1-expressing reporter cell line with isogenic ICAM-1/NEG and ICAM-1/POS viruses. Infectivity was not restored by the acquisition of virion-anchored host ICAM-1 when target cells were expressing LFA-1 under an inactive state. However, the presence of an activated form of LFA-1 on target cells resulted in a low but detectable HIV-1 infection. This last observation might reveal some relevance for the pathogenesis of HIV-1 infection considering that virus attachment to host cells is generally seen as a limiting step under in vivo situations. Indeed, the initial binding of HIV-1 to a target is considered to take place via high-affinity binding between

multiple HIV-1 gp120 oligomers and clusters of CD4 molecules (reviewed in (Ugolini et al., 1999)). This event is thought to allow a rapid docking of the mature viral particle that has to circulate in flowing fluids such as peripheral blood. However, in persons infected with HIV-1, the reported shedding of gp120 (reviewed in (Wyatt et al., 1998)) and the covering of the viral entity with neutralizing antibodies that are directed predominantly toward the surface envelope glycoprotein gp120 (Dimmock, 1993) will reduce the overall number of multivalent interactions between virion-associated gp120 and cellular CD4 possibly below the threshold necessary to allow infection to proceed. The attachment of HIV-1 would be even more problematic for cells expressing low levels of surface CD4 such as macrophages, microglia, and dendritic cells (Dick et al., 1997; Sonza et al., 1995). It can thus be proposed that the presence of cellular ICAM-1 proteins within a virus membrane possessing a limited number of Env proteins will enhance the probability of a successful initial contact between HIV-1 and a target cell expressing LFA-1 under an active form.

We are currently exploring several putative mechanisms that could permit to shed light on factors involved in insertion of ICAM-1 into mature HIV-1. For example, we are focusing on the role played by the cytoskeleton in this process. This is based on the idea that envelope viruses are known to interact with the cytoskeleton during the processes of assembly and budding (Damsky et al., 1977). Moreover, data from numerous studies have led to propose that some specific retroviral proteins seem to interact with actin filaments (Edbauer and Naso, 1984; Fais et al., 1995; Karczewski and Strebel, 1996; Kim et al., 1998; Liu et al., 1999; Luftig and Lupo, 1994; Ott et al., 1996; Pearce-Pratt et al., 1994; Rey et al., 1996; Sasaki et al., 1995; Tang et al., 1999; Tremblay et al., 1998; Wilk et al., 1999). More relevant to our study, ICAM-1 associates with moesin and ezrin, two cytoplasmic constituents that are acting as linkers between plasma membrane components and the actin-containing cytoskeleton (Barreiro et al., 2002; Heiska et al., 1998). It is of interest to note that several cellular proteins found associated with HIV-1 are connected to the cytoskeleton (e.g. ezrin, moesin, and cofilin) and actin filaments are incorporated into the virion (Ott et al., 1996, 2000).

In conclusion, the information gained from the current analyses provides new insight on the processes governing incorporation of host proteins into mature HIV-1 particles and on the importance of virion-bound foreign molecules for the virus life cycle. Future studies on the phenomenon of ICAM-1 incorporation should provide a clearer picture of the molecular event(s) responsible for the acquisition of this host molecule by HIV-1. A better characterization of this event and its consequence for the virus life cycle might shed light on viral pathogenesis and might also have relevance for the development of new therapeutic and vaccine strategies.

Materials and methods

Cell lines

The human embryonic kidney cell line 293T was used to produce virus particles that do not carry foreign ICAM-1 on their surface (ICAM-1/NEG) whereas a stable transfectant of 293T cells expressing a high surface level of ICAM-1 (Hi-ICAM-1) (Paquette et al., 1998) was used to generate ICAM-1-bearing virions (ICAM-1/POS). Both cell lines were maintained in Dulbecco's modified Eagle medium (DMEM; Gibco-BRL, Burlington, ON) supplemented with 10% fetal bovine serum (FBS) (Altanta Biologicals, Norcross, GA), glutamine (2 mM), penicillin G (100 U/ml), and streptomycin (100 mg/ml). The CEMx174-derived LuSIV cell line was maintained in RPMI-1640 (Gibco-BRL) supplemented with 10% FBS, glutamine (2 mM), penicillin G (100 U/ml), streptomycin (100 mg/ml), and hygromycin B (300 μg/ml) (Boehringer Mannheim, Indianapolis, IN). LuSIV cells were kindly supplied by J.E. Clements (Johns Hopkins University School of Medicine, Baltimore, MD) (Roos et al., 2000). Primary peripheral blood mononuclear cells (PBMCs) from healthy donors were isolated by Ficoll-Hypaque density gradient centrifugation and cultured in the presence of 3 μg/ml phytohemagglutinin-P (PHA-P; Sigma, St. Louis, USA) and 30 U/ml of recombinant human interleukin-2 for 3 days at 37 °C in a 5% CO_2 atmosphere before HIV-1 infection.

Antibodies and enzymatic assays

The anti-ICAM-1 R6.5 antibody was kindly provided by R. Rothlein (Boehringer Ingelheim, Ridgefield, CN) and the anti-LFA-1 activating MEM-83 antibody was obtained from V. Horejsi (Institute of Molecular Genetics, Prague, Czech Republic) (Bazil et al., 1990). For the purposes of the virus capture assay, biotinylation of the anti-ICAM-1 antibody was performed using EZ-Link Sulfo-NHS-LC-Biotin (Pierce). The measurement of virus-encoded gp120 protein was determined by an in-house enzymatic assay (Paquette et al., 1998). In this test, F105 is used as the coating antibody and biotinylated sheep anti-HIV-1 gp120 are used as secondary antibodies. F105 is specific for a conformational epitope on HIV-1 gp120 mapping to the CD4 binding site (provided by M. Posner through the AIDS Repository Reagent Program) (Posner et al., 1993). Western blotting membranes were revealed with anti-ICAM-1 (G-5 from Santa Cruz Biotechnology) and anti-p24 antibody (183-H12-5C from the NIH AIDS Repository Reagent Program). An isotype-matched irrelevant antibody was used as a negative control for western blot analysis.

Molecular constructs

pNL4-3 is a full-length infectious molecular clone of HIV-1 (Adachi et al., 1986). In this work, we have also used

an Env-deficient NL4-3 plasmid (Env−) and a vector coding for HIV-1 bearing a mutation into the matrix protein (i.e. 34VE) (supplied by E. O. Freed, National Institute of Allergy and Infectious Diseases, Besthesda, MD) (Freed and Martin, 1995, 1996). The pHCMV-G molecular construct, under the control of the human cytomegalovirus promoter, codes for the broad-host-range vesicular stomatitis virus envelope glycoprotein G (VSV-G) (Yee et al., 1994).

Preparation of virus stocks

Virus stocks were produced by transient transfection of 293T and Hi-ICAM-1 cells with NL4-3-based vectors using a technique established in the laboratory (Cantin et al., 1997; Fortin et al., 1997). For production of pseudotyped virus, 293T cells were transfected with either pNL4-3 or pNL4-3 Env− and both were complemented by adding pHCMV-G. Briefly, cells were plated 24 h before transfection to obtain a 50−80% confluent monolayer and calcium phosphate transfection was performed as described in the manufacturer's protocol (BD Biosciences Clontech, Palo Alto, CA). At 16 h after transfection, the cells were washed twice with phosphate-buffered saline (PBS) and were next incubated for another 24-h time period. The virus-containing supernatants were filtered through a 0.45 μM pore size cellulose acetate membrane (Millipore, Bedford, MA) before being aliquoted in 1 ml fractions that were finally frozen at −85 °C for future use. Virus stocks were normalized for virion content using an in-house sensitive double antibody sandwich ELISA specific for the major core viral p24 protein (Bounou et al., 2002). For virus capture test and determination of gp120/p24 ratio, the virus-containing supernatants were centrifuged at 16 000 × *g* for 90 min at 4 °C through a 20% sucrose cushion before storage at −85 °C.

Flow cytometry

293T and Hi-ICAM-1 cells (10^5 cells) were transiently transfected with wild type and mutated viruses, washed twice with PBS, and centrifuged at 1000 × *g* for 5 min at 4 °C. Pelleted cells were incubated for 30 min on ice with a saturated concentration of the biotinylated R6.5 antibody (1 μg/10^6 cells). The cells were washed twice in PBS and incubated for 30 min on ice with a saturated concentration of *R*-phycoerythrin-conjugated goat anti-mouse IgG. Finally, cells were washed twice in PBS and resuspended in 500 μl of PBS containing 1% paraformaldehyde before flow cytometry analysis (EPICS XL; Coulter Corporation, Miami, FL).

Virus amplification in PBMCs

Wild type and pseudotyped viruses (i.e. NL4-3, NL4-3/VSV-G, and Env-/VSV-G) were used to inoculate PHA-/IL-2-treated PBMCs (100 ng of p24 per 10^6 target cells). At the peak of HIV-1 production, cell-free culture supernatants were passed through a 0.45-μm filter. Viruses were next concentrated using CentriconPlus-20 Biomax-100 filter devices (Millipore Corporation). Finally, virus stocks were aliquoted before storage at −85 °C.

Virus capture assay

The physical presence of host-derived ICAM-1 was monitored by capturing viruses with immunomagnetic beads using a recently reported procedure with slight modifications (Cantin et al., 2001). In brief, magnetic beads (6.25×10^5) (Streptavidin-coated BioMag; PerSeptive Diagnostics Inc., Cambridge, MA) were washed four times with PBS + 0.1% bovine serum albumin (binding medium) using a vertical magnetic plate. The beads were incubated with the biotinylated R6.5 antibody for 1 h at room temperature and then washed again with PBS before use. The immunomagnetic beads were next incubated for 16 h at 4 °C with ultracentrifuged virus preparations (1 ng of p24) under a gentle agitation. The beads were washed five times with 180 μl of binding medium. Finally, the amount of immunocaptured HIV-1 particles was defined by measuring the viral p24 protein content found associated with such immunomagnetic beads. Beads coated with a biotinylated irrelevant isotype-matched antibody (i.e. IgG2a) were used as a negative control.

Virus infection and luciferase assay

The kinetics of infection of LuSIV cells with wild type and mutated HIV-1_{NL4-3} particles that lack or carry foreign ICAM-1 were determined as described below. LuSIV cells (10^5) were either left untreated or were treated for 30 min at 37 °C with MEM-83 (3.0 μg/ml) before inoculation with similar amounts of isogenic ICAM-1/NEG and ICAM-1/POS virions (i.e. 10 ng of p24 in a final volume of 200 μl). Infection was allowed to proceed for 48 h at 37 °C and 100 μl were withdrawn from each well before the addition of a cell culture lysis buffer (25 mM Tris phosphate, pH 7.8, 2.0 mM DTT, 1% Triton X-100, 10% glycerol). Cells were next incubated under a gentle agitation for 30 min at room temperature. Finally, a fraction of 20 μl was mixed with 100 μl of luciferase assay buffer [20 mM Tricine, 1.07 mM $(MgCO_3)_4{\cdot}Mg(OH)_2{\cdot}5H_2O$, 2.67 mM $MgSO_4$, 0.1 mM EDTA, 270 μM coenzyme A, 470 μM luciferin, 530 μM ATP, 33.3 mM DTT]. The photon emission produced by the reaction was measured with a microplate luminometer device (MLX, Dynex Technologies, Chantilly, USA).

Western blot analysis

Virus-containing supernatants from transiently transfected cells were first centrifuged and next passed through a 0.45-μm filter to eliminate cellular debris. Viruses were concentrated using CentriconPlus-20 Biomax-100 filter

devices (Millipore Corporation). Virus stocks were lysed in a loading buffer [125 mM Tris–HCl (pH 6.8), 4% SDS, 10% β-mercaptoethanol, 17.5% glycerol, and 0.0025% bromophenol blue]. Samples were then loaded (10 ng of p24 per lane) on 10% sodium dodecyl sulfate polyacrylamide gels. Proteins were transferred onto Immobilon-P membranes by a standard blotting technique (Millipore) and probed with an anti-ICAM-1 (G-5), anti-p24 (183-H12-5C), or isotype-matched irrelevant antibody (i.e. IgG2a). The blots were developed using the ECL detection system (Pierce).

Statistical analysis

Results presented are expressed as means ± SD of triplicate samples and are representative of three independent experiments. Statistically significant differences between groups were computed by analysis of variance. *P* value calculations were done with the Student *t* test in Microsoft Excel and $P < 0.05$ were considered statistically significant.

Acknowledgments

We thank Dr. M. Dufour for technical assistance in flow cytometry studies and Ms. S. Methot for critical reading of the manuscript. This work was performed by Y.B. in partial fulfillment of his PhD degree in the Program of Microbiology-Immunology, Faculty of Medicine, Laval University. This study was supported by a grant to M.J.T. from the Canadian Institutes of Health Research (CIHR) HIV/AIDS Research Program (Grant # HOP-14438). Y.B. is the recipient of a CIHR Doctoral Research Award from the HIV/AIDS Research Program and M.J.T. holds a Tier 1 Canada Research Chair in Human Immuno-Retrovirology.

References

Adachi, A., Gendelman, H.E., Koenig, S., Folks, T., Willey, R., Rabson, A., Martin, M.A., 1986. Production of acquired immunodeficiency syndrome-associated retrovirus in human and nonhuman cells transfected with an infectious molecular clone. J. Virol. 59 (2), 284–291.

Arthur, L.O., Bess Jr., J.W., Sowder II, R.C., Benveniste, R.E., Mann, D.L., Chermann, J.C., Henderson, L.E., 1992. Cellular proteins bound to immunodeficiency viruses: implications for pathogenesis and vaccines. Science 258 (5090), 1935–1938.

Arthur, L.O., Bess Jr., J.W., Urban, R.G., Strominger, J.L., Morton, W.R., Mann, D.L., Henderson, L.E., Benveniste, R.E., 1995. Macaques immunized with HLA-DR are protected from challenge with simian immunodeficiency virus. J. Virol. 69 (5), 3117–3124.

Barreiro, O., Yanez-Mo, M., Serrador, J.M., Montoya, M.C., Vicente-Manzanares, M., Tejedor, R., Furthmayr, H., Sanchez-Madrid, F., 2002. Dynamic interaction of VCAM-1 and ICAM-1 with moesin and ezrin in a novel endothelial docking structure for adherent leukocytes. J. Cell Biol. 157 (7), 1233–1245.

Bazil, V., Stefanova, I., Hilgert, I., Kristofova, H., Vanek, S., Horejsi, V., 1990. Monoclonal antibodies against human leucocyte antigens: IV. Antibodies against subunits of the LFA-1 (CD11a/CD18) leucocyte-adhesion glycoprotein. Folia Biol. 36 (1), 41–50.

Binnerts, M.E., van Kooyk, Y., Simmons, D.L., Figdor, C.G., 1994. Distinct binding of T lymphocytes to ICAM-1, -2 or -3 upon activation of LFA-1. Eur. J. Immunol. 24 (9), 2155–2160.

Bounou, S., Dumais, N., Tremblay, M.J., 2001. Attachment of human immunodeficiency virus-1 (HIV-1) particles bearing host-encoded B7-2 proteins leads to nuclear factor-kappa B- and nuclear factor of activated T cells-dependent activation of HIV-1 long terminal repeat transcription. J. Biol. Chem. 276 (9), 6359–6369.

Bounou, S., Leclerc, J.E., Tremblay, M.J., 2002. Presence of host ICAM-1 in laboratory and clinical strains of human immunodeficiency virus type 1 increases virus infectivity and CD4(+)-T-cell depletion in human lymphoid tissue, a major site of replication in vivo. J. Virol. 76 (3), 1004–1014.

Cantin, R., Fortin, J.F., Tremblay, M., 1996. The amount of host HLA-DR proteins acquired by HIV-1 is virus strain- and cell type-specific. Virology 218 (2), 372–381.

Cantin, R., Fortin, J.F., Lamontagne, G., Tremblay, M., 1997. The presence of host-derived HLA-DR1 on human immunodeficiency virus type 1 increases viral infectivity. J. Virol. 71 (3), 1922–1930.

Cantin, R., Martin, G., Tremblay, M.J., 2001. A novel virus capture assay reveals a differential acquisition of host HLA-DR by clinical isolates of human immunodeficiency virus type 1 expanded in primary human cells depending on the nature of producing cells and the donor source. J. Gen. Virol. 82 (Pt. 12), 2979–2987.

Castilletti, C., Capobianchi, M.R., Fais, S., Abbate, I., Ficociello, B., Ameglio, F., Cordiali Fei, P., Santini, S.M., Dianzani, F., 1995. HIV type 1 grown on interferon gamma-treated U937 cells shows selective increase in virion-associated intercellular adhesion molecule 1 and HLA-DR and enhanced infectivity for CD4-negative cells. AIDS Res. Hum. Retroviruses 11 (5), 547–553.

Chan, W.L., Rodgers, A., Grief, C., Almond, N., Ellis, S., Flanagan, B., Silvera, P., Bootman, J., Stott, J., Kent, K., et al., 1995. Immunization with class I human histocompatibility leukocyte antigen can protect macaques against challenge infection with SIVmac-32H. AIDS 9 (3), 223–228.

Clapham, P.R., McKnight, A., 2002. Cell surface receptors, virus entry and tropism of primate lentiviruses. J. Gen. Virol. 83 (Pt. 8), 1809–1829.

Damsky, C.H., Sheffield, J.B., Tuszynski, G.P., Warren, L., 1977. Is there a role for actin in virus budding? J. Cell Biol. 75 (2 Pt 1), 593–605.

Dick, A.D., Pell, M., Brew, B.J., Foulcher, E., Sedgwick, J.D., 1997. Direct ex vivo flow cytometric analysis of human microglial cell CD4 expression: examination of central nervous system biopsy specimens from HIV-seropositive patients and patients with other neurological disease. Aids 11 (14), 1699–1708.

Dimmock, N.J., 1993. Neutralization of animal viruses. Curr. Top Microbiol. Immunol. 183, 1–149.

Dorfman, T., Mammano, F., Haseltine, W.A., Gottlinger, H.G., 1994. Role of the matrix protein in the virion association of the human immunodeficiency virus type 1 envelope glycoprotein. J. Virol. 68 (3), 1689–1696.

Edbauer, C.A., Naso, R.B., 1984. Cytoskeleton-associated Pr65gag and assembly of retrovirus temperature- sensitive mutants in chronically infected cells. Virology 134 (2), 389–397.

Fais, S., Capobianchi, M.R., Abbate, I., Castilletti, C., Gentile, M., Cordiali Fei, P., Ameglio, F., Dianzani, F., 1995. Unidirectional budding of HIV-1 at the site of cell-to-cell contact is associated with co-polarization of intercellular adhesion molecules and HIV-1 viral matrix protein. AIDS 9 (4), 329–335.

Fortin, J.F., Cantin, R., Lamontagne, G., Tremblay, M., 1997. Host-derived ICAM-1 glycoproteins incorporated on human immunodeficiency virus type 1 are biologically active and enhance viral infectivity. J. Virol. 71 (5), 3588–3596.

Fortin, J.F., Cantin, R., Tremblay, M.J., 1998. T cells expressing activated LFA-1 are more susceptible to infection with human immunodeficiency

virus type 1 particles bearing host-encoded ICAM-1. J. Virol. 72 (3), 2105–2112.

Fortin, J.F., Barbeau, B., Hedman, H., Lundgren, E., Tremblay, M.J., 1999. Role of the leukocyte function antigen-1 conformational state in the process of human immunodeficiency virus type 1-mediated syncytium formation and virus infection. Virology 257 (1), 228–238.

Fortin, J.F., Cantin, R., Bergeron, M.G., Tremblay, M.J., 2000. Interaction between virion-bound host intercellular adhesion molecule-1 and the high-affinity state of lymphocyte function-associated antigen-1 on target cells renders R5 and X4 isolates of human immunodeficiency virus type 1 more refractory to neutralization. Virology 268 (2), 493–503.

Freed, E.O., Martin, M.A., 1995. Virion incorporation of envelope glycoproteins with long but not short cytoplasmic tails is blocked by specific, single amino acid substitutions in the human immunodeficiency virus type 1 matrix. J. Virol. 69 (3), 1984–1989.

Freed, E.O., Martin, M.A., 1996. Domains of the human immunodeficiency virus type 1 matrix and gp41 cytoplasmic tail required for envelope incorporation into virions. J. Virol. 70 (1), 341–351.

Guo, M.M., Hildreth, J.E., 1995. HIV acquires functional adhesion receptors from host cells. AIDS Res. Hum. Retroviruses 11 (9), 1007–1013.

Heiska, L., Alfthan, K., Gronholm, M., Vilja, P., Vaheri, A., Carpen, O., 1998. Association of ezrin with intercellular adhesion molecule-1 and -2 (ICAM-1 and ICAM-2). Regulation by phosphatidylinositol 4, 5-bisphosphate. J. Biol. Chem. 273 (34), 21893–21900.

Henriksson, P., Bosch, V., 1998. Inhibition of cellular glycoprotein incorporation into human immunodeficiency virus-like particles by coexpression of additional cellular interaction partner. Virology 251 (1), 16–21.

Henriksson, P., Pfeiffer, T., Zentgraf, H., Alke, A., Bosch, V., 1999. Incorporation of wild-type and C-terminally truncated human epidermal growth factor receptor into human immunodeficiency virus-like particles: insight into the processes governing glycoprotein incorporation into retroviral particles. J. Virol. 73 (11), 9294–9302.

Karczewski, M.K., Strebel, K., 1996. Cytoskeleton association and virion incorporation of the human immunodeficiency virus type 1 Vif protein. J. Virol. 70 (1), 494–507.

Kim, W., Tang, Y., Okada, Y., Torrey, T.A., Chattopadhyay, S.K., Pfleiderer, M., Falkner, F.G., Dorner, F., Choi, W., Hirokawa, N., Morse III, H.C., 1998. Binding of murine leukemia virus Gag polyproteins to KIF4, a microtubule-based motor protein. J. Virol. 72 (8), 6898–6901.

Landis, R.C., Bennett, R.I., Hogg, N., 1993. A novel LFA-1 activation epitope maps to the I domain. J. Cell Biol. 120 (6), 1519–1527.

Lee, Y.M., Tang, X.B., Cimakasky, L.M., Hildreth, J.E., Yu, X.F., 1997. Mutations in the matrix protein of human immunodeficiency virus type 1 inhibit surface expression and virion incorporation of viral envelope glycoproteins in CD4+ T lymphocytes. J. Virol. 71 (2), 1443–1452.

Liao, Z., Roos, J.W., Hildreth, J.E., 2000. Increased infectivity of HIV type 1 particles bound to cell surface and solid-phase ICAM-1 and VCAM-1 through acquired adhesion molecules LFA-1 and VLA-4. AIDS Res. Hum. Retroviruses 16 (4), 355–366.

Liu, B., Dai, R., Tian, C.J., Dawson, L., Gorelick, R., Yu, X.F., 1999. Interaction of the human immunodeficiency virus type 1 nucleocapsid with actin. J. Virol. 73 (4), 2901–2908.

Luftig, R.B., Lupo, L.D., 1994. Viral interactions with the host-cell cytoskeleton: the role of retroviral proteases. Trends Microbiol. 2 (5), 178–182.

Nguyen, D.H., Hildreth, J.E., 2000. Evidence for budding of human immunodeficiency virus type 1 selectively from glycolipid-enriched membrane lipid rafts. J. Virol. 74 (7), 3264–3272.

Orentas, R.J., Hildreth, J.E., 1993. Association of host cell surface adhesion receptors and other membrane proteins with HIV and SIV. AIDS Res. Hum. Retroviruses 9 (11), 1157–1165.

Ott, D.E., Coren, L.V., Kane, B.P., Busch, L.K., Johnson, D.G., Sowder II, R.C., Chertova, E.N., Arthur, L.O., Henderson, L.E., 1996. Cytoskeletal proteins inside human immunodeficiency virus type 1 virions. J. Virol. 70 (11), 7734–7743.

Ott, D.E., Coren, L.V., Johnson, D.G., Kane, B.P., Sowder II, R.C., Kim, Y.D., Fisher, R.J., Zhou, X.Z., Lu, K.P., Henderson, L.E., 2000. Actin-binding cellular proteins inside human immunodeficiency virus type 1. Virology 266 (1), 42–51.

Paquette, J.S., Fortin, J.F., Blanchard, L., Tremblay, M.J., 1998. Level of ICAM-1 surface expression on virus producer cells influences both the amount of virion-bound host ICAM-1 and human immunodeficiency virus type 1 infectivity. J. Virol. 72 (11), 9329–9336.

Pearce-Pratt, R., Malamud, D., Phillips, D.M., 1994. Role of the cytoskeleton in cell-to-cell transmission of human immunodeficiency virus. J. Virol. 68 (5), 2898–2905.

Plow, E.F., Haas, T.A., Zhang, L., Loftus, J., Smith, J.W., 2000. Ligand binding to integrins. J. Biol. Chem. 275 (29), 21785–21788.

Poon, D.T., Coren, L.V., Ott, D.E., 2000. Efficient incorporation of HLA class II onto human immunodeficiency virus type 1 requires envelope glycoprotein packaging. J. Virol. 74 (8), 3918–3923.

Posner, M.R., Cavacini, L.A., Emes, C.L., Power, J., Byrn, R., 1993. Neutralization of HIV-1 by F105, a human monoclonal antibody to the CD4 binding site of gp120. J. Acquired Immune Defic. Syndr. 6 (1), 7–14.

Rey, O., Canon, J., Krogstad, P., 1996. HIV-1 Gag protein associates with F-actin present in microfilaments. Virology 220 (2), 530–534.

Rizzuto, C.D., Sodroski, J.G., 1997. Contribution of virion ICAM-1 to human immunodeficiency virus infectivity and sensitivity to neutralization. J. Virol. 71 (6), 4847–4851.

Roos, J.W., Maughan, M.F., Liao, Z., Hildreth, J.E., Clements, J.E., 2000. LuSIV cells: a reporter cell line for the detection and quantitation of a single cycle of HIV and SIV replication. Virology 273 (2), 307–315.

Sasaki, H., Nakamura, M., Ohno, T., Matsuda, Y., Yuda, Y., Nonomura, Y., 1995. Myosin–actin interaction plays an important role in human immunodeficiency virus type 1 release from host cells. Proc. Natl. Acad. Sci. U.S.A. 92 (6), 2026–2030.

Sonza, S., Maerz, A., Uren, S., Violo, A., Hunter, S., Boyle, W., Crowe, S., 1995. Susceptibility of human monocytes to HIV type 1 infection in vitro is not dependent on their level of CD4 expression. AIDS Res. Hum. Retroviruses 11 (7), 769–776.

Springer, T.A., 1990. Adhesion receptors of the immune system. Nature 346 (6283), 425–434.

Tang, Y., Winkler, U., Freed, E.O., Torrey, T.A., Kim, W., Li, H., Goff, S.P., Morse III, H.C., 1999. Cellular motor protein KIF-4 associates with retroviral Gag. J. Virol. 73 (12), 10508–10513.

Tremblay, M.J., Fortin, J.F., Cantin, R., 1998. The acquisition of host-encoded proteins by nascent HIV-1. Immunol. Today 19 (8), 346–351.

Ugolini, S., Mondor, I., Sattentau, Q.J., 1999. HIV-1 attachment: another look. Trends Microbiol. 7 (4), 144–149.

Wilk, T., Gowen, B., Fuller, S.D., 1999. Actin associates with the nucleocapsid domain of the human immunodeficiency virus Gag polyprotein. J. Virol. 73 (3), 1931–1940.

Wyatt, R., Kwong, P.D., Desjardins, E., Sweet, R.W., Robinson, J., Hendrickson, W.A., Sodroski, J.G., 1998. The antigenic structure of the HIV gp120 envelope glycoprotein. Nature 393 (6686), 705–711.

Yee, J.K., Miyanohara, A., LaPorte, P., Bouic, K., Burns, J.C., Friedmann, T., 1994. A general method for the generation of high-titer, pantropic retroviral vectors: highly efficient infection of primary hepatocytes. Proc. Natl. Acad. Sci. U.S.A. 91 (20), 9564–9568.

Yu, X., Yuan, X., Matsuda, Z., Lee, T.H., Essex, M., 1992. The matrix protein of human immunodeficiency virus type 1 is required for incorporation of viral envelope protein into mature virions. J. Virol. 66 (8), 4966–4971.

Yu, X., Yuan, X., McLane, M.F., Lee, T.H., Essex, M., 1993. Mutations in the cytoplasmic domain of human immunodeficiency virus type 1 transmembrane protein impair the incorporation of Env proteins into mature virions. J. Virol. 67 (1), 213–221.