Analysis of the molecules involved in human T-cell leukaemia virus type 1 entry by a vesicular stomatitis virus pseudotype bearing its envelope glycoproteins

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Introduction

Human T-cell leukaemia virus type 1 (HTLV-1) is a retrovirus that is aetiologically associated with adult T-cell leukaemia/lymphoma and chronic inflammatory disorders such as HTLV-1-associated myelopathy/tropical spastic paraparesis and uveitis (Hinuma et al., 1981; Osame et al., 1987; Mochizuki et al., 1992). HTLV-1 infection is endemic in southern Japan, southern USA, the Caribbean, Jamaica, South America and equatorial Africa (Poiesz et al., 1980; Hinuma et al., 1981). The main routes of HTLV-1 transmission are mother-to-child (especially via breast feeding), sexual and parenteral (Robert-Guroff et al., 1983; Hino et al., 1985).

HTLV-1 primarily infects CD4+ T-cells, but also infects many different types of cells, such as CD8+ T-cells, B-cells, monocyte/macrophages and endothelial cells. Experimentally, HTLV-1 infects various species, including monkeys, rabbits and rats (Poiesz et al., 1980; Krichbaum-Stenger et al., 1987; Fan et al., 1992). HTLV-1 envelope glycoproteins, which are synthesized as a precursor, gp61, consist of a cell surface (gp46) and a transmembrane (gp21) glycoprotein (Seiki et al., 1983; Pique et al., 1992). These glycoproteins are involved in virus entry into susceptible cells and the subsequent induction of syncytia: the formation of syncytia induced by the co-cultivation of HTLV-1-infected cells with uninfected cells is efficiently inhibited by monoclonal antibodies (MAbs) against envelope glycoproteins or several synthetic peptides of gp46 and gp21 (Baba et al., 1993; Tanaka et al., 1994; Sagara et al., 1996). It is thought that gp46 mediates virus attachment and that gp21 mediates membrane fusion through its N-terminal hydrophobic domain (Ragheb et al., 1995; Delamarre et al., 1997).

Identification of HTLV-1 receptor(s) has been attempted mainly by using MAbs that inhibit syncytium formation. Previous studies have shown that cell adhesion molecules, such
as intercellular adhesion molecule-1, lymphocyte function associated antigen-1 and vascular cell adhesion molecule-1, and members of the transmembrane 4 superfamily, such as CD82/C33/R2 and CD81/M38/TAPA-1, are required for HTLV-1-induced syncytium formation (Fukudome et al., 1992; Hildreth et al., 1997; Daenke et al., 1999). However, it was demonstrated recently that a large number of immunoglobulin (Ig) molecules (particularly MAbs against class II major histocompatibility complex molecules) bound to the surface of infected cells can nonspecifically block HTLV-1-induced syncytium formation by the protein crowding or steric effects. This suggests that the results obtained from studies using MAbs against the cellular molecules described above may need to be re-evaluated in the context of the cell surface expression level of the molecules (Hildreth, 1998). Thus, the cellular receptor(s) for HTLV-1 remain to be determined.

In the present study, we used vesicular stomatitis virus (VSV) and human immunodeficiency virus type 1 (HIV-1) pseudotypes containing the gene encoding the green fluorescent protein (GFP), which are complemented in trans with viral envelope glycoproteins in order to confer infectivity on these viruses. To analyse virus entry mediated by HTLV-1 envelope glycoproteins, we examined the infectivity of the pseudotypes both on various cell lines and on cells that were chemically modified by various reagents.

Methods

■ Cells. HTLV-1-positive (MT-2, C91/PL) and -negative (MOLT-4, Jurkat, Raji, Daudi, 293T, HepG2, HeLa, Vero, COS, MDCK, LMTK− and NIH 3T3) cell lines have been maintained in our laboratory for a long period. The MAGIC-5A cell line (a transfectant of HeLa cells expressing CD4 and CCR5) was kindly provided by M. Tatsumi (National Institute of Infectious Diseases, Tokyo, Japan). All the adherent cell lines were cultured in complete Dulbecco’s Modified Eagle’s medium (DMEM, Nissui), supplemented with 10% foetal calf serum (FCS, Gibco), 50 µg/ml streptomycin (Gibco) and 50 units/ml penicillin G (Gibco). Cells were grown in 5% CO₂ at 37 °C. All the suspension cell lines were cultured in RPMI1640 (Gibco) containing the same supplements and grown in 5% CO₂ at 37 °C.

■ Plasmids. Mammalian expression plasmid vectors pCAGGS and pCXN2 contain the CAG promoter, which is composed of the cytomegalovirus immediate early enhancer and the chicken β-actin promoter (Niwa et al., 1991). A full-length cDNA of VSV (Indiana serotype) G protein was excised from pGL-1 (Rose & Bergmann, 1982) and subcloned into pCAGGS, resulting in pCAG-VSVG. The HTLV-1 env gene was excised from pHAproenv (Shida et al., 1987) and subcloned into pCAGGS, resulting in pCAGHTLV-I env. A cDNA clone of HIV-1 (HXB2 strain) envelope gp160 was inserted into pCXN2, resulting in pCXN2H and pCXN2F, respectively (Tanaka et al., 1999). pNL-E δNefδV3 was constructed from pNL4-3, containing a provirus of HIV-1 NL4-3 strain, by digesting the BglII site of the env gene (i.e. deletion of the V3 loop domain) and replacing part of the nef gene into the EGFP gene (pE GFP-1, Clontech). A cDNA clone of HIV-1 (HXB2 strain) envelope gp160 was inserted into pSV7d, which contains the SV40 promoter, resulting in HXB2-env.

■ Virus. VSVG*-G is the recombinant VSV which was generated by reverse genetics as described previously (Takada et al., 1997) and kindly provided by M. A. Whitt (University of Tennessee, TN, USA). The virus was derived from a full-length cDNA clone of the VSV genome (Indiana serotype) in which the coding region of the G protein was replaced by the coding region of a modified version of the GFP gene (ΔG*) and complemented with the VSV G protein. VSVG*-G was grown and harvested by infecting 293T cells that had been transfected previously with pCAG-VSVG.

■ Preparation of pseudotype viruses. To generate VSV pseudotypes, 0.75 × 10⁶ 293T cells per well were grown in a 6-well flat-bottom collagen I-coated microplate (IWAKI) and transfected with the expression plasmids using LipofectAmine reagent (Gibco), according to the manufacturer’s protocol. Briefly, 100 µl of Opti-MEM I reduced-serum medium (Gibco), 5 µl LipofectAMINE reagent and 1 µg pCAGHTLV-I env, 0.7 µg pCAG-VSVG or 1 µg pCAGGS were mixed and incubated at room temperature for 15 min. The mixture was then added to 293T cells that had been preincubated with 0.75 ml of Opti-MEM I medium. After a 3.5 h incubation at 37 °C, transfected cells were replenished with 1.5 ml of DMEM, supplemented with 10% FCS, and cultured for 24 h. Cells were then infected with VSVG*-G at an moi of 2 for 1 h at 37 °C. Virus-infected cells were washed with FCS-free DMEM seven times and 2 ml of complete medium was subsequently added. After 12–18 h of incubation at 37 °C in 5% CO₂ culture supernatants were collected and centrifuged to remove cell debris. VSVG*-Env, VSVG*-G and VSVG* were recovered from 293T cells transfected with pCAGHTLV-I env, pCAG-VSVG or pCAGGS, respectively. The VSV pseudotype complemented with MV (Edmonston strain) H and F proteins (VSGAG-Ed3F) was recovered from 293T cells transfected with both pCXN2H and pCXN2F (Tatsuo et al., 2000).

To prepare the HIV-1 pseudotype, 293T cells transfected with both pNLE δNefδV3 and HXB2-env were incubated with DMEM containing 10% FCS and 20 mM sodium butyrate for 24 h, and then in fresh medium for 24 h. The HIV-1 pseudotype complemented with HIV-1 (HXB2 strain) envelope glycoproteins (HIV-HXB2 env) was then recovered from the transfected 293T cells. Each virus stock was stored at −80 °C until use.

■ Immunoblot analysis. VSV pseudotypes were prepared, as described above. Viruses in the supernatants were filtered and partially purified through 20% sucrose by centrifugation at 284 000 g at 4 °C for 1 h. Purified virus pellets were dissolved in 40 µl of electrophoresis sample buffer (0.125 M Tris–HCl pH 6.8, 20% glycerol, 4% SDS, 2% β-mercaptoethanol, 0.012% bromophenol blue) and applied to SDS–PAGE (5–20% gradient). Separated proteins were electrophoretically transferred to a Sequi-Blot PVDF membrane (Bio-Rad). Transferred proteins were reacted with an anti-gp46 MAb, LAT-27 (rat IgG) (1:100 dilution), which was kindly provided by Y. Tanaka (Tanaka et al., 1994). Bound antibodies were then reacted with a 1:10³ dilution of horseshadish peroxidase-conjugated anti-rat IgG (heavy chain-specific) (Cappel, ICN) and were visualized by chemiluminescence and fluorography using an ECL Western immunoblotting kit (Amersham).

■ Titration of VSV pseudotypes in various cell lines. A 96-well collagen I-coated microplate was prepared with 5 × 10⁴ of adherent cells or 1 × 10⁶ of suspension cells per well. After aspirating off the media, 50 µl of each virus stock (serially diluted when necessary) was inoculated into each well. After incubation at 37 °C for 1 h, 100 µl of complete medium was added to each well. Cells were incubated at 37 °C for 12–18 h in a CO₂ incubator. Titres of each virus in the various cell lines were determined in infectious units (IU) by counting the number of GFP-expressing cells under a fluorescence microscope.
VSV pseudotype assay analysis of HTLV-1 entry

**Fig. 1.** (A) Incorporation of HTLV-1 envelope glycoproteins into VSV particles. Viral proteins of purified VSVΔG* (lane 1), VSVΔG*-G (lane 2) and VSVΔG*-Env (lane 3) particles were analysed by immunoblot analysis with LAT-27. A molecular mass ladder (kDa) is indicated on the left. (B) Expression of GFP upon infection with VSV pseudotypes. 293T cells were infected with VSVΔG*, VSVΔG*-G (1:10³ dilution) or VSVΔG*-Env and GFP expression was examined by fluorescence microscopy 12–18 h after infection. Magnification, × 200. (C) Effect of LAT-27 on VSVΔG*-Env infection. VSVΔG*-Env was preincubated with LAT-27 and then inoculated onto 293T and HepG2 cells. The effect of the antibody was examined 12–18 h after infection. An irrelevant MAb, rat IgG, was used as the negative control. Each datum point represents the percentage infectivity (%IU, mean ± SD) calculated from three microscopic fields compared with the infectivity of each virus on untreated cells.

**Inhibition of VSVΔG*-Env infection by neutralizing antibody.** A 50 µl aliquot of VSVΔG*-Env was preincubated with serially diluted LAT-27, which has HTLV-1-neutralizing activity, at 37 °C in a CO₂ incubator for 15 min and then the mixtures were inoculated onto 293T or HepG2 cells. After 1 h of incubation, the inocula were aspirated off and complete media were added, together with the diluted antibody, as described above. Cells expressing GFP were counted under a fluorescence microscope after incubation at 37 °C in 5% CO₂ for 12–18 h.

**Chemical modification of cells.** To treat cell surface proteins, 293T and MAGIC-5A cells were preincubated with pronase (Sigma) or trypsin (Gibco) in FCS-free DMEM at 37 °C in 5% CO₂ for 20 min. To
treat carbohydrates, 293T cells were preincubated with sodium periodate (NaIO₄) (Sigma), heparinase or heparitinase (Flavobacterium heparrinum) (Seikagaku) in FCS-free DMEM at 37 °C in 5% CO₂ for 1 h. Also, to treat cell surface phospholipids, 293T cells were preincubated with either phospholipase A₂ (porcine pancreas) (Sigma) or phospholipase C (Bacillus cereus) (Boehringer Mannheim) in FCS-free DMEM at 37 °C for 30 min. After incubation, an equal volume of complete medium was added to each well to stop the reaction of each agent. Cells were subsequently infected with approximately 1–5 × 10³ IU of VSV∆G*-G, VSV∆G*-EdHF, HIV-HXB2 env (only when MAGIC-5A cells were treated) or VSV∆G*-Env. Infected cells were counted under a fluorescence microscope.

Inhibition of VSV∆G*-Env infection by purified lipids.
Phosphatidic acid (PA), phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and sphingomyelin (SP) were obtained from Sigma. Chloroform solutions of these purified lipids were dried and solubilized in PBS containing 50 mM n-octyl-β-D-glucopyranoside (OG) (Sigma) at a concentration of 10 mM. Lipids were stored at 4 °C. Aliquots of 100 µl per well of VSV∆G*-Env, VSV∆G*-EdHF or VSV∆G*-G (1.0 × 10³ IU) were preincubated with 100 µM of each purified lipid at room temperature for 1 h, inoculated onto 5 × 10⁴ 293T cells and incubated at 37 °C for 1 h. Inocula were aspirated after incubation and cells were then incubated with 200 µl DMEM per well containing 10% FCS. After 12–18 h of incubation, infectivity of the viruses was evaluated under a fluorescence microscope.

### Results

#### Development of an assay system with a novel VSV pseudotype bearing HTLV-1 envelope glycoproteins

Recombinant VSV containing the GFP gene instead of the G protein gene was either uncomplemented (VSV∆G*) or complemented with either VSV G protein (VSV∆G*-G) or HTLV-1 envelope glycoproteins (VSV∆G*-Env). Viruses were purified by centrifugation and analysed by immunoblot for the incorporation of HTLV-1 envelope glycoproteins (Fig. 1A). Using MAb LAT-27, HTLV-1 gp46 protein was observed only in VSV∆G*-Env (Fig. 1A, lane 3). To test whether HTLV-1 envelope glycoproteins could rescue the infectivity of VSV∆G* lacking the G protein, we infected 293T cells with VSV∆G*, VSV∆G*-G or VSV∆G*-Env (Fig. 1B). A large number of cells expressed GFP after VSV∆G*-G or VSV∆G*-Env infection, but few cells expressed GFP when infected with VSV∆G*. The background infectivity of VSV∆G* may be attributable to the remaining VSV∆G*-G used when the VSV pseudotypes were prepared. Because the background infectivity was negligible (compared with the infectivity of VSV∆G*-Env in 293T cells), VSV∆G*-Env was not treated with anti-VSV antibody throughout this study. To ascertain...
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Fig. 3. Infectivity of pseudotype viruses on cells whose surface proteins were chemically modified. 293T (A) and MAGIC-5A (B) cells were preincubated with indicated concentrations of pronase or trypsin and infectivity of VSVΔG*-G (VSV), VSVΔG*-EdHF (MV), HIV-HXB2 env (HIV-1) (only on MAGIC-5A cells) and VSVΔG*-Env (HTLV-1) was examined. The titre of each virus was 1.5 × 10^7 IU/ml, 3.4 × 10^4 IU/ml, 1.7 × 10^4 IU/ml (on MAGIC-5A cells) and 5.3 × 10^4 IU/ml on 293T cells, respectively. The viruses were diluted when necessary. The same viruses were also used in the experiments described in Figs 4 and 5. Each bar represents the percentage infectivity (%IU, mean ± SD) calculated from three microscopic fields compared with the infectivity of each virus on untreated cells.

the specificity of HTLV-1 envelope glycoprotein-mediated infection, we examined the effect of MAb LAT-27, which inhibits HTLV-1-induced syncytium formation (Fig. 1C). VSVΔG*-Env that had been preincubated with LAT-27 at various concentrations was inoculated onto 293T and HepG2 cells. LAT-27, at a low concentration, inhibited the infection of both cell lines by VSVΔG*-Env, whereas an irrelevant rat IgG, even at high concentrations, did not inhibit infection. These results indicated that the HTLV-1 envelope glycoproteins incorporated into VSV particles specifically induced virus entry by interacting with the putative cellular receptor(s) of susceptible cells.

Susceptibility of various cell lines to VSV pseudotypes

To analyse the presence of putative HTLV-1 receptor(s), we examined the infectivity of VSV pseudotypes (as determined by counting the number of GFP-expressing cells) in various cell lines derived from several species (Fig. 2). All tested cells showed a high susceptibility to VSVΔG*-G, as reported previously (Takada et al., 1997). All suspension cells, except for HTLV-1-infected cells, exhibited susceptibility to VSVΔG*-Env, as the infectivity titre of VSVΔG*-Env was at least 1 log greater than that of VSVΔG* (Fig. 2A). As HTLV-1-infected MT-2 and C91/PL cells showed similar titres with VSVΔG* and VSVΔG*-Env, virus entry dependent on HTLV-1 envelope glycoproteins could not be observed on these cells. All adherent cell lines examined also exhibited susceptibility to VSVΔG*-Env (Fig. 2B) and showed titres between 1 and 4 logs greater than the titres seen with VSVΔG*. These results suggested that cell-free HTLV-1 could infect various cell types derived from several species.

Effects of chemical modifications of cells on the infectivity of pseudotype viruses

To characterize the biochemical nature of the molecule(s) involved in HTLV-1 entry, we examined the infectivity of VSVΔG*-Env on 293T cells that were chemically modified by various reagents (Figs 3 and 4).
Cellular receptors for VSV and MV, Edmonston strain, have been reported to be phospholipids (Schlegel et al., 1983) and glycoprotein CD46 (Naniche et al., 1993), respectively. VSV pseudotypes bearing their envelope proteins (VSVΔG*-G and VSVΔG*-EdHF) were used as controls.

First, in order to determine the role of cell surface proteins in virus entry, 293T cells were preincubated with pronase or trypsin and then infected with VSVΔG*-G, VSVΔG*-EdHF or VSVΔG*-Env (Fig. 3A). Pronase treatment of cells markedly reduced VSVΔG*-EdHF infectivity and also affected VSVΔG*-G infectivity. In contrast, VSVΔG*-Env infectivity remained almost the same even after the treatment with 500 µg/ml pronase. Trypsin treatment of cells reduced the infectivity of all three pseudotype viruses to a similar extent.
Among the various purified lipids tested, PC significantly reduced (and PG slightly reduced) the number of GFP-expressing cells produced by VSV\textsuperscript{AG*-EdHF} infection, but not that produced by either VSV\textsuperscript{AG*-EdHF} or VSV\textsuperscript{AG*-G} infection. These findings suggest that HTLV-1 envelope glycoproteins interact with some carbohydrates and phospholipids on the cell surface during virus entry.

**Discussion**

HTLV-1 appears to infect cells more efficiently by cell-to-cell transmission, which may lead to syncytium formation, than by cell-free transmission (Fan et al., 1992; Delamarre et al., 1997). However, susceptible cells do not necessarily produce syncytia when co-cultivated with HTLV-1-infected cells (Somerfelt et al., 1988; Delamarre et al., 1997). Thus, it is difficult to analyse the expression of HTLV-1 receptor(s) and identify cells lacking them by using an assay based on syncytium formation. Recently, quantitative and sensitive assay systems for analysing envelope glycoprotein-mediated cell fusions were developed for HIV-1, HTLV-1 and HTLV-2 (Nussbaum et al., 1994; Poon & Chen, 1998; Okuma et al., 1999). Although analysis of HIV-1-induced cell fusion by this assay system resulted in the identification of a HIV-1 co-receptor, fusin/CXCR4 (Feng et al., 1996), similar studies have failed to determine the HTLV-1 receptor(s).

It has been difficult to quantitatively detect cell-free HTLV-1 infection because of the inefficient infectivity of cell-free HTLV-1. The reason for its low infectivity is not clear, but it may be due to a low fusion activity of the HTLV-1 envelope glycoproteins, resulting from their relatively small size, hydrophobicity, fragility and low affinity (Delamarre et al., 1996; Hildreth, 1998). To date, cell-free HTLV-1 infection has been analysed using pseudotype viruses carrying the genome of another virus and HTLV-1 envelope glycoproteins. Clapham et al. (1984) developed a plaque assay using a VSV pseudotype bearing both HTLV-1 envelope glycoproteins and VSV G protein, obtained by infecting HTLV-1-producing cells with VSV. The infectious titre of virus in susceptible cells ranged from $10^3$ to $10^4$ IU/ml, depending upon the cell types.
However, the plaque assay using this VSV pseudotype may be affected by factors that interfere with the plating of VSV (Hoshino et al., 1985) and requires anti-VSV antibody with/without DEAE-dextran or -polybrene. The pseudotype viruses possessing Moloney murine leukaemia virus (Mo-MuLV) core and HTLV-1 envelope glycoproteins were also generated with a selectable marker gene (Wilson et al., 1989; Vile et al., 1991). Though HTLV-1 envelope glycoproteins were incorporated into Mo-MuLV particles, the titre of the viruses was so low (less than 200 c.f.u./ml) that they could not efficiently infect susceptible cells. It was thought that these results may reflect low expression levels of HTLV-1 envelope glycoproteins, toxicity of expression plasmids to transfected cells, inefficiency of virus assembly, lability of pseudotype virions and/or inefficient uptake of pseudotype virions by susceptible cells. A pseudotype system using a defective HIV-1 genome in combination with HTLV-1 envelope glycoproteins in 293T producer cells has been developed recently (Sutton & Littman, 1996). Introduction of additional copies of the rev gene and treatment of cells with sodium butyrate resulted in cell-free virus titres of greater than 10^4 IU/ml in HOS cells. However, the cell surface molecules on susceptible cells that interact with HTLV-1 gp46 or gp21 have not yet been identified definitively and the molecular mechanisms underlying HTLV-1 entry/membrane fusion and HTLV-1-induced syncytium formation remain to be elucidated.

In this study, we developed a novel quantitative assay system in which a recombinant VSV possessing the G protein gene instead of the G protein gene was complemented with HTLV-1 envelope glycoproteins provided in trans to recover infectivity. Immunoblot analysis showed the incorporation of HTLV-1 envelope glycoproteins into VSV particles (Fig. 1A). Since the incorporation of foreign envelope proteins into VSV particles appears to require oligomerization of the proteins (Owens & Rose, 1993), HTLV-1 envelope glycoproteins may form oligomers. In fact, by analysis of a chimera with maltose-binding protein, it was reported recently that HTLV-1 gp21 forms trimers (Kobe et al., 1999).

Infection by recombinant VSV complemented with HTLV-1 envelope glycoproteins (VSVG*-Env) was markedly inhibited by the anti-gp46 neutralizing MAb LAT-27 (Fig. 1C). Since LAT-27 recognizes aa 191–196 of gp46 (Tanaka et al., 1994), this region appears to be a critical domain for the interaction with the putative HTLV-1 receptor(s) on susceptible cells in this system.

The present VSV pseudotype assay system yielded from 10^8 to 10^9 IU/ml on 293T cells, demonstrating that this assay system might be more useful than systems already available for cell-free HTLV-1 infection. Various cell lines tested showed susceptibility to VSVG*-Env in different degrees (Fig. 2). Since VSVG*-Env could infect HepG2, 293T, Vero and COS cells more efficiently than the other cell lines tested, then these susceptible cell lines may express more putative HTLV-1 receptor(s) and/or virus entry-associated molecule(s). The inefficient infection of HTLV-1-infected MT-2 and C91/PL cells by VSVG*-Env may reflect down-regulation of HTLV-1 receptor(s) on these cells.

Chemical modification of 293T and MAGIC-5A cells by various reagents influenced the infectivity of VSVG*-Env on these cells (Figs 3 and 4). These results indicated the importance of some cell surface proteins, phospholipids and carbohydrates, such as glycosaminoglycans (GAGs), in HTLV-1 envelope glycoprotein-mediated entry. The high susceptibility of HepG2, 293T, Vero and COS cells to VSVG*-Env (Fig. 2) might result from high cell surface expression of these molecules on these cells. Involvement of GAGs in HTLV-1 entry is consistent with previous reports that heparin and dextran sulfate inhibit HTLV-1-induced cell fusion (Ida et al., 1994; Okuma et al., 1999) and that a plant lectin (wheat-germ agglutinin) inhibits adsorption of HTLV-1 (Yang et al., 1994). Treatment of 293T cells with either phospholipase A2 or C inhibited the infectivity of VSVG*-Env and purified lipids, such as PC, also blocked infection (Figs 4 and 5). These data suggested that cell surface phospholipids, including unknown lipids, that are reported to bind to HTLV-1 gp21 and inhibit HTLV-1-induced syncytium formation (Sagara et al., 1997), might be involved not only in cell–cell fusion, but also in cell-free HTLV-1 entry through interaction with gp21. Although treatment of 293T cells with either phospholipase A2 or C inhibited the infectivity of VSVG*-G, the various purified lipids tested did not (Figs 4 and 5). PS was reported to markedly inhibit VSV plaque formation (Schlegel et al., 1983) and it is not clear why PS did not reduce the infectivity of VSVG*-G in our study. Another phospholipid, which was not tested in the present study, may be involved in VSV entry. Furthermore, the infectivity of VSVG*-Env was significantly inhibited only on the MAGIC-5A cells treated with 500 µg/ml pronase, whereas the infectivity of VSVG*-EdHF and HIV-HXB2 env could be inhibited at the lower concentrations of pronase. The differences of the effects of pronase treatment on virus infectivity might reflect the difference in pronase sensitivity among the respective virus receptors. By treating human peripheral blood mononuclear cells with a high concentration (500 µg/ml) of trypsin, Trejo & Ratner (2000) demonstrated recently that the HTLV receptor is a protein. Together with our data, these data may also indicate that the HTLV-1 receptor is relatively resistant to treatment with proteases. On the other hand, the infectivity of VSVG*-G was reduced to some extent by treatment with pronase and trypsin (Fig. 3). This effect may reflect excessive cell damage, since the receptor for VSV has been reported to be a phospholipid.

Thus, this novel and quantitative assay system using VSV pseudotypes revealed that cell surface proteins, phospholipids and GAGs are involved in HTLV-1 infection. Therefore, HTLV-1 appears to utilize multiple ubiquitous molecules on the surface of susceptible cells as the cellular receptor/co-receptor(s), resulting in the wide distribution of the receptor(s)
and the broad host range of HTLV-1 (Sutton & Littman, 1996; Okuma et al., 1999). Because it was demonstrated that HTLV-1 Tax most efficiently enhances virus transcription in CD4+ T-cells (Newbound et al., 1996), it is possible that post-entry events rather than virus attachment and virus–cell fusion are critical in determining the tropism of HTLV-1.

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References


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