Separate Cellular Localizations of Human T-Lymphotropic Virus 1 (HTLV-1) Env and Glucose Transporter Type 1 (GLUT1) Are Required for HTLV-1 Env-Mediated Fusion and Infection

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ABSTRACT

Interaction of the envelope glycoprotein (Env) of human T-lymphotropic virus 1 (HTLV-1) with the glucose transporter type 1 (GLUT1) expressed in target cells is essential for viral entry. This study found that the expression level of GLUT1 in virus-producing 293T cells was inversely correlated with HTLV-1 Env-mediated fusion activity and infectivity. Chimeric studies between GLUT1 and GLUT3 indicated that the extracellular loop 6 (ECL6) of GLUT1 is important for the inhibition of cell-cell fusion mediated by Env. When GLUT1 was translocated into the plasma membrane from intracellular storage sites by bafilomycin A1 (BFLA1) treatment in 293T cells, HTLV-1 Env-mediated cell fusion and infection also were inhibited without the overexpression of GLUT1, indicating that the localization of GLUT1 in intracellular compartments rather than in the plasma membrane is crucial for the fusion activity of HTLV-1 Env. Immunoprecipitation and laser scanning confocal microscopic analyses indicated that under normal conditions, HTLV-1 Env and GLUT1 do not colocalize or interact. BFLA1 treatment induced this colocalization and interaction, indicating that GLUT1 normally accumulates in intracellular compartments separate from that of Env. Western blot analyses of FLAG-tagged HTLV-1 Env in virus-producing cells and the incorporation of HTLV-1 Env in virus-like particles (VLPs) indicate that the processing of Env is inhibited by either overexpression of GLUT1 or BFLA1 treatment in virus-producing 293T cells. This inhibition probably is due to the interaction of the Env with GLUT1 in intracellular compartments. Taken together, separate intracellular localizations of GLUT1 and HTLV-1 Env are required for the fusion activity and infectivity of HTLV-1 Env.

IMPORTANCE

The deltaretrovirus HTLV-1 is a causative agent of adult T-cell leukemia (ATL) and HTLV-1–associated myelopathy/tropical spastic paraparesis (HAM/TSP). Although HTLV-1 is a complex retrovirus that has accessory genes, no HTLV-1 gene product has yet been shown to regulate its receptor GLUT1 in virus-producing cells. In this study, we found that a large amount of GLUT1 or translocation of GLUT1 to the plasma membrane from intracellular compartments in virus-producing cells enhances the colocalization and interaction of GLUT1 with HTLV-1 Env, leading to the inhibition of cell fusion activity and infectivity. The results of our study suggest that GLUT1 normally accumulates in separate intracellular compartments from Env, which is indeed required for the proper processing of Env.

Human T-lymphotropic virus 1 (HTLV-1) is a complex deltaretrovirus and a causative agent of adult T-cell leukemia (ATL) (62–64) and HTLV-1–associated myelopathy/tropical spastic paraparesis (HAM/TSP) (1, 2). The envelope glycoprotein (Env) of HTLV-1 is synthesized in virus-infected cells as a polyprotein precursor (gp62), which subsequently is cleaved by cellular protease(s) localized in the Golgi apparatus into two proteins, surface glycoprotein (gp46; SU) and transmembrane glycoprotein (gp21; TM). HTLV-1 entry is initiated by the specific interaction of SU with cellular receptors, resulting in TM-mediated fusion between viral and cellular membranes.

Three distinct molecules have been shown to be involved in efficient entry of HTLV-1: glucose transporter 1 (GLUT1) (3), heparin sulfate proteoglycans (HSPGs) (4), and neuropilin-1 (NRP-1) (5). It should be noted that transmission of HTLV-1 from virus-infected cells to target cells is mediated mainly by cell-to-cell contact (cell-to-cell infection) (6–8) via virological synapse (9) or biofilm-like extracellular assemblies (10), not by cell-free virus, except in the case of transmission to dendritic cells (11). Although GLUT1 is ubiquitously distributed, HTLV-1 mainly infects human CD4+ T cells (12–15) and immortalizes them (16). In general, the expression of the receptor molecules in target cells is essential for enveloped virus entry. However, surface expression of the receptor molecules in virus-infected cells may interfere with the incorporation of Env or the release of virions because of the association of Env and the receptors. This effect is commonly avoided by simple trapping of the Env-receptor complex in the endoplasmic reticulum (ER) in most viruses. In contrast, another human retrovirus, HIV-1, downregulates or degrades its receptor, CD4, from the plasma membrane of the infected cells by HIV-1
accessory proteins, such as Nef (17–19) and Vpu (20–22), to protect infected cells from superinfection or to maintain the infectivity of HIV-1. However, it remains to be determined how the receptors for HTLV-1, such as GLUT1, are regulated in HTLV-1-infected cells. To address this issue, we overexpressed GLUT1 in virus-producing cells with HTLV-1 Env and checked the cell fusion activity and infectivity. We found that increased expression of GLUT1 in the virus-producing cells inhibited the Env function. Further analyses revealed that GLUT1 is localized in different cellular compartments from Env, resulting in the efficient processing and surface expression of Env in virus-producing cells.

MATERIALS AND METHODS

Cells and culture conditions. The 293T and HeLa cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS; Gibco BRL, Carlsbad, CA, USA). A human CD4-expressing glioma cell line (NP-2/CD4) (23) and its derivatives (24) were maintained in Eagle’s minimum essential medium (MEM; Sigma-Aldrich) supplemented with 10% FBS. The TZM-bl cell line was provided through the AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, and maintained in DMEM supplemented with 10% FBS. The Jurkat cell line was purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA) and maintained in RPMI 1640 (Invitrogen, Carlsbad, CA, USA) supplemented with 10% FBS.

Plasmids. An HTLV-1 clone, pMT-2 (25), was provided by M. Matsuoka (Kyoto University). Reporter and packaging plasmids of HTLV-1 and HIV-1 for cell-to-cell infection (6) were kindly provided by D. Derse and G. Heidecker (National Cancer Institute, USA). The plasmids, pNLA-3, pcMV-rev, and pH pl-N/A, were obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases. The HIV-1 NL-Luc plasmid pNLLucΔBglII was kindly provided by I. S. Chen (UCSF). A plasmid-enhancing translation initiation, pAdvantage, was purchased from Promega (Madison, WI, USA).

Construction of expression vectors. An expression vector for HTLV-1 Env was constructed from pMT-2 (25). Briefly, the SphI-PstI fragment of pMT-2 carrying HTLV-1 env (1.6 kb) was blunt ended and ligated with an EcoRI linker. The env fragment digested with EcoRI was further ligated into pcDNA3.1 (−) to give pcDNA-1E. The BglIII-HindIII fragment carrying the Rev-responsive element (RRE) of pNL4-3 was further ligated into pcDNA3.1(−) to give pcDNA-1E-RRE. An HTLV-1 Env expression vector, pcDNA-1E-FLAG-RRE, was constructed from pMT-2 (25). Briefly, the SphI-PstI fragment carrying epitope tag was further removed by replacing the StuI-EcoRI fragment to give pcDNA-1E-FLAG. The genes for GLUT1-FLAG, pcDNA-1E-RRE, pNL43

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Transfection and HTLV-1 Env-mediated cell fusion and infection assay. For the expression of HTLV-1 Env and the production of virus-like particles (VLPs), 293T cells in six-well plates were transfected with 3.5 μg of pNL43ΔBglII, 1.5 μg of pcDNA-1E-RE, 0.5 μg of pcCMV-rev, and 0.5 μg of pAdvantage (Promega) using a Pection kit (Promega) or Lipofectamine 2000 (Invitrogen) according to the manufacturer’s recommendations. The expression of GLUT1 in 293T cells was decreased in the indicated experiments by using short interfering RNA (siRNA) against GLUT1 (Sigma) transfected with Lipofectamine 2000 (Invitrogen). The siRNA universal negative control (Sigma) also was used as a negative control. For cell-cell fusion assays, transfected 293T cells were recovered 6 h posttransfection and cocultured with TZM-bl cells. Luciferase activities were measured 30 h posttransfection using a luciferase assay. For the infection of cell-cell fusion assays, 293T cells were transfected with pcDNA-1E-RE using Ag ELISA kit (Zeptometrix, Buffalo, NY, USA) according to the manufacturer’s instructions. Cell-to-cell infection assays were performed as previously described (6) using Jurkat cells as the targets. Briefly, 293T cells were cotransfected with pUCHR-intGLUT1, pHp-dl-N/A, pcMV-rev, pAdvantage, and pcDNA-1E-RE in the presence of pcDNA-GT1-FLAG, pcDNA-GT3-FLAG, or empty vector. Transfected cells were recovered 6 h posttransfection and cocultured with Jurkat cells. The luciferase activities were measured after 48 h of coculture.

Flow cytometry. The 293T cells were transfected with pcDNA-GT1-FLAG, pcDNA-1E-RE, pNL43ΔBglII, pcCMV-rev, and pAdvantage as described above. The cells were recovered 24 h posttransfection using cell dissociation solution (Sigma) and stained with anti-gp46 rat monoclonal antibody (MAB) LAT-27 (28), followed by staining with an allophycocyanin (APC)-conjugated anti-rat IgG (BioLegend, San Diego, CA, USA). The cells were fixed with fluorescein isothiocyanate (FITC)-conjugated anti-GLUT1 (R&D Systems, Minneapolis, MN, USA), fixed with 4% paraformaldehyde for 15 min, and analyzed using a

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http://jvi.asm.org/Downloaded from
FACS Calibur fluorescent-activated cell sorter (BD Biosciences, San Jose, CA, USA).

**Laser scanning confocal microscopy.** The HeLa cells were plated on poly-l-lysine-coated eight-well glass slides (Matsunami Glass, Osaka, Japan), transfected with pEGFP-GLUT1 and pcDNA-1-E-RRE using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions, and cultured for 24 h. The cells were fixed with 4% paraformaldehyde for 15 min. The fixed cells were permeabilized with 0.2% Triton X-100 and stained with the anti-gp46 rat MAb LAT-27 (28), stained with anti-FLAG conjugated with Cy3 (Jackson ImmunoResearch), and analyzed using an LSM-700 Zen confocal laser scanning microscope (Carl Zeiss, Gottingen, Germany) with a 60× objective lens. The images were processed using the LSM imaging browser (Carl Zeiss).

**Immunoprecipitation and Western blotting.** The 293T cells in six-well plates were transfected with 3.5 μg of pNL43ABglIII, 1.5 μg of pcDNA-1-E-RRE, 0.5 μg of pCMV-rev, and 0.5 μg of pAdvantage (Promega) using Lipofectamine 2000 (Invitrogen) and then cultured for 24 h at 37°C. The cells were then solubilized using 1% Brij O10 (Sigma-Aldrich) lysis buffer (1% Brij O10, 20 mM Tris-Cl, pH 8.0, 150 mM NaCl) with a protease inhibitor cocktail (Nakalai Tesque, Kyoto, Japan). For the preparation of VLPs, culture supernatants from transfected 293T cells were harvested after 24 h of culture and filtered through 0.45-μm pore-size filters. The filtered supernatants then were centrifuged at 10,000 × g for 1 h at 4°C. The pellets were resuspended in 2× loading buffer. The cell lysates or VLPs then were separated by SDS-PAGE and blotted onto PVDF (polyvinylidenefluoride) Immobilon-P Membrane, Billerica, MA, USA) membranes. The membranes were treated with antibody against gp46 (Wako, Osaka, Japan) and anti-FLAG mouse MAb (Wako). Thesignals were detected using horseradish peroxidase (HRP)-conjugated anti-mouse IgG. Thesignals were detected using horseradish peroxidase (HRP)-conjugated anti-mouse IgG. 

**Western blotting (Fig. 1B),** increased the surface expression level of GLUT1 (Fig. 1A). GLUT1 also was preferentially incorporated into VLPs during the overexpression of GLUT1 compared with the level during overexpression of GLUT3, irrespective of the expression of HTLV-1 Env (Fig. 1B). When GLUT1 and HTLV-1 Env were coexpressed in VLP-producing 293T cells, the surface expression level of gp46 did not appear to be affected by overexpression of GLUT1 compared with the expression level in mock-transfected 293T cells (Fig. 1B).

We also noticed that mature gp46 was not incorporated into VLPs produced from 293T cells expressing large amounts of GLUT1. The larger molecular size recognized by anti-gp46 in VLPs (~53 kDa), which corresponds to the size of an underglycosylated and uncleaved form of Env, most likely was due to the contamination of intracellular materials of the cells. In contrast, mature gp46 was incorporated into the VLPs produced from mock- or GLUT3-transfected 293T cells (Fig. 1B). The cell-cell fusion activity, cell-to-cell infectivity, and cell-free virus infectivity also were profoundly reduced by the overexpression of GLUT1 but not by the overexpression of GLUT3 in VLP-producing 293T cells (Fig. 1C). In contrast, fusion activity and infectivity mediated by HIV-1 Env, including cell-cell fusion, cell-to-cell infection, and cell-free infection, were not inhibited by overexpression of GLUT1 or GLUT3 (data not shown). To exclude the effect of VLPs produced by HIV-1 vectors, we also checked the effect of VLPs produced by HTLV-1 vectors. We found that overexpression of GLUT1 in HTLV-1-VLP-producing cells also specifically and profoundly inhibited HTLV-1 Env-mediated cell-to-cell infection (Fig. 1D). These results indicate that GLUT1 specifically inhibits HTLV-1 Env-mediated cell fusion and infectivity, probably owing to the loss of mature gp46 incorporation into VLPs.

**Inverse correlation between the expression level of GLUT1 and the fusion activity of HTLV-1 Env in VLP-producing 293T cells.** We further checked whether the expression level of GLUT1 is correlated with the inhibition of HTLV-1 Env-mediated fusion. When 293T cells were transfected with increasing amounts of the plasmid encoding FLAG-tagged GLUT1, GLUT1 was increasingly incorporated into VLPs (Fig. 2A), while HTLV-1 Env-mediated fusion activity and infectivity were inversely inhibited (Fig. 2B) in a dose-dependent manner.

**RESULTS**

**Inhibition of HTLV-1 Env-mediated cell fusion and infection by overexpression of GLUT1 in VLP-producing 293T cells.** It has been shown that the expression of GLUT1 in target cells is essential for entry for HTLV-1 (3). However, it is unknown whether the expression of GLUT1 in virus-producing cells affects the efficiency of virus entry, because GLUT1 is physically associated with HTLV-1 Env (3). To address this issue, we selected 293T cells as VLP-producing cells, because 293T cells typically express low levels of GLUT1 on their cell surfaces (Fig. 1A). For the efficient production of VLPs, HIV-1 vectors pseudotyped with HTLV-1 Env also were used in this study. The overexpression of FLAG-tagged GLUT1 in VLP-producing cells, which was confirmed by Western blotting (Fig. 1B), increased the surface expression level of GLUT1 (Fig. 1A). GLUT1 also was preferentially incorporated into VLPs during the overexpression of GLUT1 compared with...
each chimera expressed in VLP-producing 293T cells was evaluated by a cell-cell fusion assay. We first confirmed that all chimeras were expressed at similar levels in 293T cells (Fig. 3B). The inhibition of the cell-cell fusion at levels similar to those induced by GLUT1 was observed in chimeras harboring extracellular loop 6 (ECL6) from GLUT1, though the GLUT1 (3-ECL6) chimera, which harbors GLUT1 with ECL6 from GLUT3, also had inhibitory activity against the cell-cell fusion to some extent. These results indicate that GLUT1 ECL6 is sufficient for the inhibition of cell fusion activity mediated by HTLV-1 Env.

Bafilomycin A1 inhibits HTLV-1 Env-mediated fusion by increasing the expression level of GLUT1 in the plasma membrane. It has been reported that an endosomal acidification inhibitor, bafilomycin A1 (BFLA1), which blocks vacuolar proton pump activity mediated by V-ATPase, induces the translocation of GLUT1 from intracellular storage sites to the plasma membrane in adipocytes (30). Flow-cytometric analyses revealed that the surface expression level of GLUT1 was increased by BFLA1 not only in GLUT1-transfected cells but also in untransfected 293T cells (Fig. 4A), while the total levels of cellular GLUT1 were unchanged (Fig. 4B). These results indicate that BFLA1 induces the translocation of endogenous GLUT1 from intracellular storage sites to the plasma membrane in 293T cells. However, the surface expression level of gp46 was not affected by the treatment with BFLA1 (Fig. 4A).

The incorporation of GLUT1 in VLPs also was not affected by BFLA1 treatment (Fig. 4B). However, mature gp46 was not incorporated into VLPs in the presence of BFLA1, irrespective of the overexpression of GLUT1 or GLUT3 (Fig. 4B).
BFLA1 inhibits HTLV-1 Env function by inducing the accumulation of GLUT1 in the plasma membrane, producing results similar to those observed during the overexpression of GLUT1.

**Association of GLUT1 and gp46 in intracellular compartments by bafilomycin A1.** Inhibition of HTLV-1 Env function by the translocation of GLUT1 to the plasma membrane suggests that under normal conditions, GLUT1 and HTLV-1 Env reside in separate intracellular compartments. To test this hypothesis, we coexpressed FLAG-tagged GLUT1 and HTLV-1 Env in 293T cells and checked whether anti-FLAG antibody immunoprecipitates gp46 of HTLV-1 Env. We found that FLAG-tagged GLUT1 was able to coimmunoprecipitate gp46 in untreated cells, and that BFLA1 treatment largely enhanced this association of GLUT1 with gp46 (Fig. 5A). We next sought to check whether GLUT1 is colocalized with gp46 in intracellular compartments using laser scanning confocal microscopy. When GFP-tagged GLUT1 and HTLV-1 Env were coexpressed in 293T cells, both were partly colocalized, while treatment with BFLA1 substantially induced colocalization of both in intracellular compartments (Fig. 5B). These results indicate that most of the GLUT1 and HTLV-1 Env are localized in separate intracellular compartments, resulting in marginal association between them under normal conditions.

**Overexpression of GLUT1 and bafilomycin A1 treatment both inhibit HTLV-1 Env processing.** As shown in Fig. 4B, when the cells overexpressed GLUT1 or were treated with BFLA1, mature gp46 was not incorporated into VLPs, suggesting that these conditions cause an impairment in the processing of Env. To access the processing of Env, we constructed an expression vector for HTLV-1 Env with a FLAG tag in the C-terminal end (Fig. 6A). Successful processing of HTLV-1 Env by a cellular enzyme(s) should result in a cleaved TM region of 21 kDa, while unsuccessful processing should result in an uncleaved size of ~53 kDa. We found that overexpression of GLUT1, but not overexpression of GLUT3, in 293T cells inhibited the processing of Env (Fig. 6B). We further observed that treatment of 293T cells with BFLA1 also inhibited the processing of Env irrespective of the overexpression of GLUT1 or GLUT3 (Fig. 6B). These results imply that the cleavage of Env precursor is inhibited by the association of Env with GLUT1 in intracellular compartments.

**DISCUSSION**

Because GLUT1 has been shown to directly associate with gp46 of HTLV-1 Env as the receptor for HTLV-1 (32), we expected that a large amount of GLUT1 in virus-producing cells with HTLV-1
Env would inhibit fusion activity and nascent progeny virus infectivity. Indeed, the overexpression of GLUT1 in VLP-producing cells impaired the HTLV-1 Env-mediated virus fusion and infection in a dose-dependent manner. Furthermore, a reduction of GLUT1 in virus-producing cells by an siRNA knockdown of endogenous GLUT1 enhanced the HTLV-1 Env-mediated infection. These results indicate that HTLV-1 Env-mediated fusion activity and infectivity are inversely correlated with the expression level of GLUT1 in cells productively infected with virus. Chimeric studies with GLUT3, which had no inhibitory activity against cell fusion and infection mediated by HTLV-1 Env, revealed that the ECL6 domain was crucial for the inhibitory activity of GLUT1. This domain previously has been shown to be sufficient for binding gp46, while other domains, such as ECL1 and ECL5, have some role(s) in the infection process (32). Taken together, GLUT1 in virus-infected cells should be regulated to low levels to avoid the interaction of HTLV-1 Env with GLUT1.

It has been shown that some of the enveloped viruses regulate their receptor(s) in virus-infected cells through their gene products. For example, to release the virus from the infected cells, influenza virus neuraminidase (NA) enzymatically cleaves sialic acids from sialic acid-containing receptors that are bound to the hemagglutinin (HA) (33–35). In the case of retroviruses, the downregulation of their receptors is believed to be necessary for preventing the infected cells from causing superinfection, which is known as superinfection interference (reviewed in reference 36). However, downregulation of the viral receptor also has been shown to maintain viral infectivity. Indeed, overexpression of CD4, which is the receptor for another human retrovirus, HIV-1, has been shown to impair the infectivity of HIV-1 (20, 37–39). To reduce the amount of CD4 in the infected cells, however, the HIV-1 accessory gene products, Nef and Vpu, induce downregulation and degradation, respectively, of the principal receptor CD4. Although HTLV-1 is a complex retrovirus that, like HIV-1, has several accessory genes, no HTLV-1 gene product has been shown to regulate its receptor molecules. In the present study, the overexpression of GLUT1 completely inhibited the HTLV-1 Env-mediated fusion and infection in VLPs made with HTLV-1-packing plasmids, suggesting that HTLV-1 gene products do not involve the regulation of GLUT1, although this has not yet been fully confirmed.

It also has been reported that HIV-1 Nef downregulates its coreceptor, CCR5, in HIV-1-infected cells, although the effect was not as striking as the ability of Nef to downregulate CD4 (40, 41). We previously showed that the incorporation of larger amounts of HIV-1 coreceptor CCR5 into virions impaired the infectivity of HIV-1 when a CCR5-high CD4+ T-cell line was infected with a distinct HIV-1 molecular clone, while a CCR5-low CD4+ T-cell line was able to support virus infectivity (42). Because primary CD4+ T cells express a low level of CCR5 in general (43, 44), the incorporation of CCR5 into virions and the interaction of CCR5 with HIV-1 gp120 are expected to be limited. Similarly, the incorporation of GLUT1 into VLPs was dependent on the expression level of GLUT1 in VLP-producing cells in the present study. Thus, it is likely that cells expressing a low level of GLUT1 should be selected as the target cells for HTLV-1 to produce nascent progeny virus.

It has been shown that CD4+ and CD8+ T cells, B cells, macrophages, myeloid cells, and fibroblasts have been infected with HTLV-1 (13, 45–48), which is not surprising, because GLUT1 is

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**FIG 3** Determination of the region of GLUT1 responsible for the inhibitory activity against HTLV-1 Env-mediated fusion. (A) Schematic of chimeras between GLUT1 and GLUT3. (B) VLP-producing 293T cells were cotransfected with HTLV-1 Env and chimeras between GLUT1 and GLUT3. Cell lysates were analyzed by Western blotting using anti-FLAG MAb. CypA levels were used as the internal control. (C) Cell-cell fusion activity for each of the chimeras is expressed as the percentage of the level for empty vector.
ubiquitously expressed in these cells. It has been reported that CD8+ T cells express much more GLUT1 than CD4+ T cells (49, 50). Nonetheless, HTLV-1 is found primarily in CD4+ T cells in infected individuals (15). The results from the present study suggest it is possible that CD8+ T cells produce fusion-incompetent virus because of their higher expression of GLUT1. To efficiently use the small amount of GLUT1 in CD4+ T cells for HTLV-1 entry, however, HTLV-1 may need to infect the cells via cell-to-cell contact.

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Although our 293T cells endogenously express GLUT1 at low
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levels on their cell surfaces, similar to CD4\(^+\) T cells, BFLA1 treatment enhanced the surface expression of endogenous GLUT1, while the total GLUT1 level was not affected (30). This result indicated that endogenous GLUT1 is localized mainly in intracellular compartments in 293T cells, although its exact location is unknown. In addition, BFLA1 treatment induced the enhanced binding of gp46 with GLUT1 and the colocalization of both molecules in the same intracellular compartments. Therefore, it is assumed that the overexpression of GLUT1 or translocation of endogenous GLUT1 into the plasma membrane induces the association of gp46 with GLUT1, thereby inhibiting the cell fusion and infection mediated by HTLV-1 Env.

When VLPs were produced in 293T cells expressed with larger amounts of GLUT1 or treated with BFLA1, mature gp46 was not incorporated into VLPs. FLAG-tagged HTLV-1 Env in VLP-producing cells confirmed that the precursor Env was not efficiently cleaved in GLUT1-overexpressed or BFLA1-treated 293T cells, probably owing to the direct association of Env with GLUT1. Previous studies have shown that the cleavage of retroviral Env is essential for the surface expression of Env, incorporation of Env into virosomes, and fusion activity of Env (51, 52). The lack of precursor Env cleavage of HTLV-1 Env by several experimental conditions, such as the treatment of cells with various inhibitors, also has been shown to reduce the surface expression of Env, leading to the loss of fusion activity (53). In our case, we showed that GLUT1-associated HTLV-1 Env was translocated to the cell surface, but mature gp46 was not incorporated into VLPs from GLUT1-overexpressed or BFLA1-treated 293T cells. These results indicate that trafficking of HTLV-1 Env occurs in spite of its association with GLUT1, but in cells with overexpressed GLUT1 or cells treated with BFLA-1 the conformational maturation of Env is impaired.

It should be noted that colocalization of GLUT1 with gp46 was partly observed in untreated 293T cells, while BFLA1 treatment enhanced the colocalization of both molecules not only in the plasma membrane but also in the cytoplasm. These results indicate that GLUT1 localizes in different intracellular compartments from gp46 under normal conditions. Because the processing of Env was inhibited by the association of Env with GLUT1, this association likely occurs in specific intracellular compartments, presumably in the endoplasmic reticulum or Golgi apparatus, though the exact location is not known. Although GLUT1 is thought to be an unregulated transporter responsible for the basal uptake of glucose in general, recycling between intracellular storage sites and the cell surface has been reported not only in adipocytes following BFLA1 treatment (30) but also in T cells following CD28 stimulation (54, 55). However, regulatory T (Treg) cells, which are CD4\(^+\) and thought to be the principal target of HTLV-1 (56–58), do not express large amounts of GLUT1 upon stimulation (59–61). Thus, GLUT1 likely is regulated in HTLV-1-infected cells, thereby supporting HTLV-1 virus infectivity.

In conclusion, our present study provides new insight into how HTLV-1 regulates its receptor molecule(s) in virus-infected cells. Regulation of the receptor molecules is achieved not only through the viral gene products but also by the spatial regulation of the receptor molecules in virus-infected cells. However, our findings should be confirmed using natural target cells for HTLV-1 infection, such as CD4\(^+\) T cells. Additionally, it remains to be determined how other receptor molecules are regulated during productive infection in HTLV-1-infected cells, such as DSPG and NRP-1. Further studies are necessary to understand the underlying molecular mechanism(s) in the regulation of receptors for HTLV-1 in infected cells.

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