Cell, Vol. 115, 449–459, November 14, 2003, Copyright ©2003 by Cell Press

The Ubiquitous Glucose Transporter GLUT-1 Is a Receptor for HTLV

Nicolas Manel, Felix J. Kim,[1] Sandrina Kinet,[1] Naomi Taylor, Marc Sitbon,* and Jean-Luc Battini*
Institut de Génétique Moléculaire de Montpellier
CNRS UMR 5535/IFR 122
F-34293 Montpellier Cedex 5
France

Summary

The human T cell leukemia virus (HTLV) is associated with leukemia and neurological syndromes. The physiopathological effects of HTLV envelopes are unclear and the identity of the receptor, present on all vertebrate cell lines, has been elusive. We show that the receptor binding domains of both HTLV-1 and -2 envelope glycoproteins inhibit glucose transport by interacting with GLUT-1, the ubiquitous vertebrate glucose transporter. Receptor binding and HTLV envelope-driven infection are selectively inhibited when glucose transport or GLUT-1 expression are blocked by cytochalasin B or siRNAs, respectively. Furthermore, ectopic expression of GLUT-1, but not the related transporter GLUT-3, restores HTLV infection abrogated by either GLUT-1 siRNAs or interfering HTLV envelope glycoproteins. Therefore, GLUT-1 is a receptor for HTLV. Perturbations in glucose metabolism resulting from interactions of HTLV envelope glycoproteins with GLUT-1 are likely to contribute to HTLV-associated disorders.

Introduction

The human T cell leukemia virus (HTLV) type 1 and 2 are present in all areas of the world as endemic or sporadic infectious agents (Slattery et al., 1999). The etiological role of HTLV-1 in adult T cell leukemia (ATL) and tropical spastic paraparesis/HTLV-associated myelopathy (TSP/HAM) has been well established (Gessain et al., 1985; Osame et al., 1986; Poiesz et al., 1980; Yoshida et al., 1982). The apparently restricted tropism of HTLV to T lymphocytes in infected patients (Cavrois et al., 1996; Hanon et al., 2000) contrasts with the ability of the viral-encoded envelope glycoprotein (Env) to bind to and direct entry into all vertebrate cell types tested in vitro (Kim et al., 2003b; Sutton and Littman, 1996; Trejo and Ratner, 2000). Retroviral infections depend on early interactions between Env and cellular receptors. Identification of cellular receptors and coreceptors for other retroviral envelopes have helped to elucidate certain aspects of retrovirus physiopathology as well as their transmission and spreading within organisms and populations (Overbaugh et al., 2001). However, no clear association between HTLV Env and HTLV-associated diseases has been established and the identity of the receptor(s) for HTLV-1 and HTLV-2 Env has remained elusive.

Numerous cell surface components have been shown to play a role in HTLV Env-mediated syncytia formation (Daenke et al., 1999; Hildreth et al., 1997) and in enhancing virus adsorption and infection (Pinon et al., 2003). Nevertheless, HTLV Env-dependent cell membrane fusion and syncytia formation appear to be distinct from receptor binding per se (Daenke and Booth, 2000; Denesvre et al., 1996; Kim et al., 2000, 2003b). The search for the HTLV Env receptor has been hindered in part by its ubiquitous presence (Jassal et al., 2001; Kim et al., 2003b; Sutton and Littman, 1996). Additionally, the induction of rampant syncytium formation in cell culture upon expression of HTLV Env has prevented its efficient and persistent expression. Based on our observation that the HTLV Env amino-terminal domain shares striking structural and functional homology with that of murine leukemia viruses (MLV) (Battini et al., 1995; Kim et al., 2000, 2003a), we defined the HTLV Env receptor binding domain (RBD) and derived HTLV Env-based tools that overcome the problem of syncytia formation (Kim et al., 2000, 2003b). We were thus able to follow specific interactions between the Env RBD and the HTLV receptor. We found that the HTLV receptor is not expressed on the surface of quiescent T lymphocytes, the major HTLV reservoir in vivo, but is induced following T cell activation (Manel et al., 2003).

Here, we describe striking metabolic alterations in cells expressing HTLV envelopes as well as truncated HTLV receptor binding domains. These alterations are independent of syncytia formation and are characterized by a defect in the acidification of the cell culture medium associated with decreased extracellular lactate production and diminished glucose uptake and consumption. These observations, as well as the knowledge that Env receptors for the related MLV and most gammaretroviruses belong to the family of multimembrane-spanning transporters (Overbaugh et al., 2001), prompted us to test ubiquitous lactate and glucose transport-associated molecules that are upregulated on activated T cells, as receptors for HTLV Env. We show that the ubiquitous glucose transporter GLUT-1 is a specific component of the HTLV receptor and plays an essential role in HTLV-envelope mediated infection. Our observation that interaction of GLUT-1 with the entire HTLV-1 and HTLV-2 envelopes as well as truncated HTLV-1 and HTLV-2 RBD alters glucose metabolism provides new leads regarding the mechanisms implicated in HTLV-associated physiopathological disorders.

Results

HTLV Envelopes and Lactate Metabolism

Cell proliferation in standard culture media is accompanied by acidification of the milieu that translates into a color change from red to yellow tones in the presence of the phenol-red pH indicator. Upon transfection of

*Correspondence: sitbon@igm.cnrs-mop.fr (M.S.), battini@igm.cnrs-mop.fr (J.-L.B.)

[1]These authors contributed equally to this work.

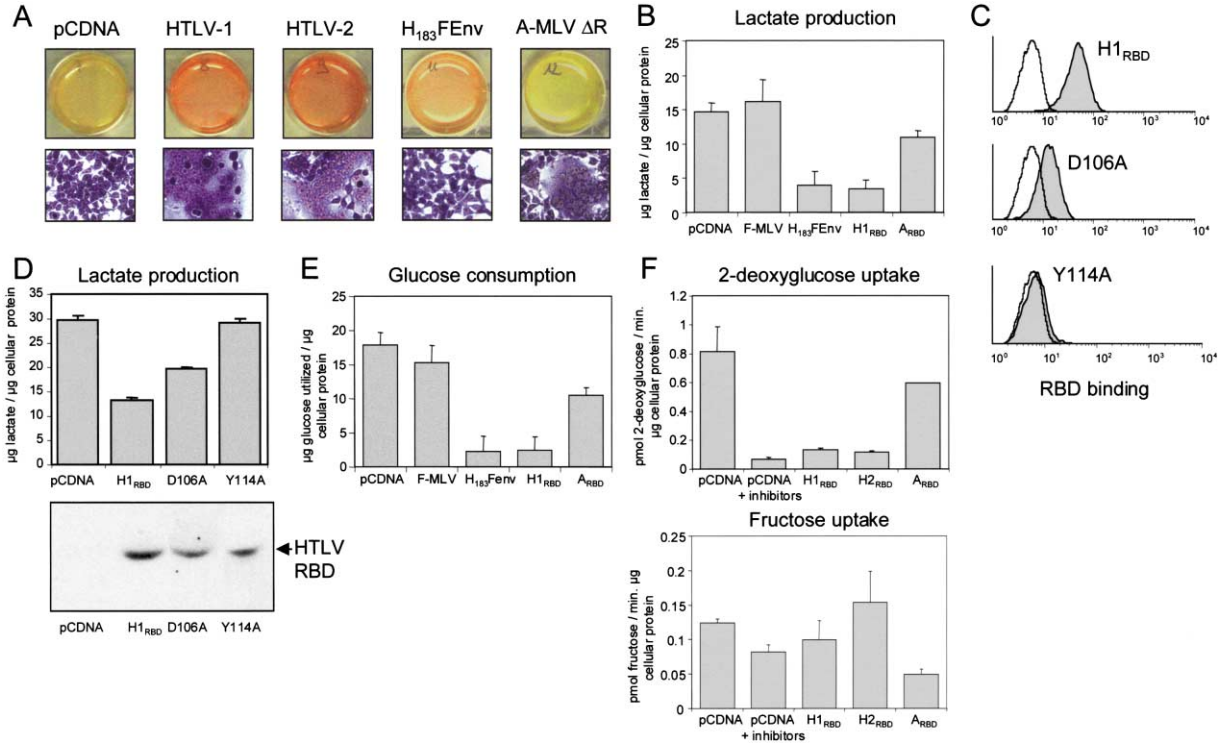

Figure 1. HTLV Envelope-Receptor Binding Alters Extracellular Lactate Production and Glucose Transport

(A) Medium acidification and syncytia formation (Giemsa staining) in 293T cells one day post-transfection with control DNA (pCDNA) or Env expression vectors, including syncytial wild-type HTLV-1 Env and HTLV-2 Env, a non-syncytial chimeric HTLV/MLV Env harboring the HTLV receptor binding domain (H_{183}FEnv), and a syncytial amphotropic-MLV Env (A-MLV ΔR). Yellow medium indicates normal acidification during cell culture.

(B) Extracellular lactate production in the culture medium of 293T cells was measured two days following transfection with control DNA (pCDNA), with expression vectors containing Friend-MLV (F-MLV) Env, chimeric H_{183}FEnv, HTLV-1 ($H1_{RBD}$), or amphotropic MLV RBD (A_{RBD}). Mean extracellular lactate production normalized to cellular protein content of triplicate samples is shown.

(C) Binding of rFc-tagged $H1_{RBD}$, D106A, and Y114A $H1_{RBD}$ mutants on 293T cells was monitored by FACS analysis following incubation with a FITC-conjugated anti-rabbit IgG antibody.

(D) Extracellular lactate production in the medium of 293T cells one day post-transfection with an irrelevant DNA (pCDNA), $H1_{RBD}$, or the D106A and Y114A $H1_{RBD}$ mutants. Data are representative of three independent experiments. The secretion or presence of HTLV Env mutants in the 293T-transfected culture medium was monitored by immunoblotting with a peroxidase-conjugated anti-rabbit IgG antibody.

(E) Extracellular glucose in the culture medium of 293T cells was measured two days following transfection with pCDNA, F-MLV Env, H_{183}FEnv, $H1_{RBD}$, or A_{RBD}. This measure was used to calculate mean glucose consumption after normalization to cellular protein content.

(F) 2-deoxy[1-^{3}H]glucose and [U-^{14}C]fructose uptake following either transfection of 293T cells with control DNA (pCDNA), $H1_{RBD}$, $H2_{RBD}$, or A_{RBD} expression vectors, or treatment with the cytochalasin B and phloretin glucose transporter inhibitors (pCDNA+inhibitors). Data are the means of triplicate measures and are representative of two to three independent experiments.

293T cells with either highly syncytial HTLV-1 and HTLV-2 envelopes, or a non-syncytial chimeric envelope that harbors the HTLV-1 RBD in an MLV Env backbone (H_{183}FEnv), culture medium did not readily become acidic, and harbored red tones for several days post-transfection (Figure 1A). Moreover, expression of truncated soluble HTLV RBD proteins fused with either EGFP, an influenza hemagglutinin peptide tag (HA), or a rabbit immunoglobulin fragment (rFc) also inhibited medium acidification (not shown). In contrast, envelope constructs that lacked HTLV RBD did not have this effect, including different MLV group envelopes, feline, porcine, lentiviral, and Jaagsiekte retroviral Env, as well as VSVG and Ebola glycoproteins (not shown). Syncytium formation was not in itself responsible for this effect; medium acidification was observed in cells expressing a syncytial amphotropic-MLV (A-MLV ΔR) Env (Figure 1A), and was blocked when HTLV Env was expressed in cells that are resistant to HTLV-Env mediated syncytia formation (NIH3T3 TK^{-} cells) (Kim et al., 2003b) (not shown).

The acidification of cell culture media is associated with the extracellular accumulation of lactate (Warburg, 1956). Lactate is the major byproduct of anaerobic glycolysis in vitro and its excretion is mediated by an H^{+}/lactate symporter (Halestrap and Price, 1999). We monitored lactate content in culture supernatants following transfection of various retroviral envelopes and RBD. Extracellular lactate production was consistently 3-fold lower in H_{183}FEnv- and HTLV RBD-transfected cells than in control- or MLV Env-transfected cells (Figure 1B). This difference was detected as early as 4 hr after the addition of fresh media (not shown). Moreover, this decrease in extracellular lactate production after HTLV RBD transfection was DNA dose dependent (not shown).

To examine whether there is a direct relationship be-

tween binding of the HTLV envelope receptor and the subsequent diminution in extracellular lactate production and acidification, we used HTLV RBD mutants with impaired receptor binding capacities. For this purpose, mutations resulting in single alanine substitutions were introduced in $H1_{RBD}$, at either the D106 or Y114 positions, both of which are highly conserved among primate T-lymphotropic viruses (F.J.K., unpublished observations). D106A and Y114A RBD mutants exhibited significantly reduced (D106A) or non-detectable (Y114A) binding to the HTLV receptor as monitored by FACS analysis (Figure 1C) and did not lead to detectable infection (not shown). Moreover, extracellular lactate accumulation was not reduced in cells expressing the non binding Y114A RBD mutant and was only partially reduced in cells harboring the D106A RBD, although both D106A and Y114A RBD mutants were expressed and secreted as efficiently as the wild-type $H1_{RBD}$ (Figure 1D). Similar results were obtained with $H2_{RBD}$ harboring the same allelic mutations (not shown), following transfection of these mutants in HeLa cells. Therefore, perturbations in lactate metabolism directly correlated with binding of HTLV RBD to the HTLV receptor.

Extracellular lactate accumulates in cell cultures following its transport across cellular membranes by the multimembrane-spanning MCT1 monocarboxylate transporter (Garcia et al., 1994). Because HTLV and MLV envelopes share a common modular organization (Kim et al., 2000, 2003a) and the receptors for MLV Env are multimembrane-spanning metabolite transporters (Overbaugh et al., 2001), we assessed whether the HTLV RBD bound to MCT1. Moreover, expression of the MCT1 chaperone CD147 (Kirk et al., 2000) increases during T cell activation (Kasinrerk et al., 1992), a characteristic of the HTLV receptor (Manel et al., 2003; Nath et al., 2003). Nevertheless, separate and combined overexpression of MCT1 and CD147 did not result in increased $H1_{RBD}$ binding (not shown), arguing against a role for these molecules as receptors for HTLV Env.

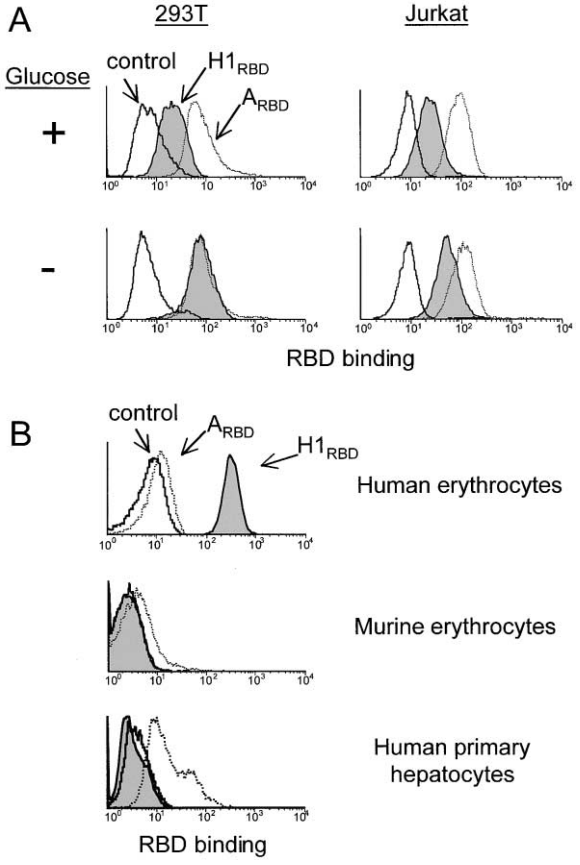

Figure 2. Expression of the HTLV Receptor on Glucose-Starved Cell Lines and Primary Cells

(A) Expression of the HTLV and amphotropic-MLV receptors on 293T and Jurkat T cells cultured overnight in the presence (1 g/l) or absence of glucose was monitored by binding of $H1_{RBD}$ and A_{RBD}, respectively.

(B) Expression of the HTLV and amphotropic-MLV receptors on the surface of freshly isolated human and murine erythrocytes, and primary human hepatocytes was monitored by binding of $H1_{RBD}$ and A_{RBD}, respectively. Binding of the HTLV envelope RBD is shown in filled histograms, A_{RBD} binding is shown in dotted histograms, and control immunofluorescence is shown in solid line histograms.

HTLV Envelopes Alter Glucose Metabolism

In addition to a decrease in extracellular lactate accumulation, expression of the HTLV RBD also led to decreased intracellular lactate content (not shown), indicative of metabolic alterations upstream of lactate transport. In cell cultures, lactate production results from the degradation of glucose during anaerobic glycolysis. Therefore, we assessed whether the decreased accumulation of lactate observed upon expression of HTLV RBD was linked to glucose metabolism. We measured glucose consumption as an indicator of the rate of glycolysis. Glucose consumption of cells expressing an HTLV RBD within the context of the H_{183}FEnv entire envelope or the tagged $H1_{RBD}$ was significantly decreased as compared to that of control cells (Figure 1E) and this defect was detected as early as 8 hr post-transfection (not shown). Notably, the transfection efficiencies in the experiments ranged from 80%–95% (not shown).

We found that this decrease in glucose consumption correlated with a decrease in glucose transport across the cellular membrane. Transfection of either HTLV-1 or HTLV-2 RBD resulted in an approximately 4-fold decrease in 2-deoxyglucose uptake (Figure 1F), comparable to the decrease observed when glucose uptake was inhibited by cytochalasin B and phloretin. The envelope-mediated inhibition was specific to HTLV, since the A-MLV RBD (A_{RBD}) control envelope had only a minor effect on glucose uptake. Moreover, the inhibitory effect of HTLV envelopes was specific to glucose uptake, since 3-O-methylglucose transport was similarly inhibited (not shown) whereas fructose uptake was not altered by the presence of either HTLV-1 or HTLV-2 RBD (Figure 1F). The decrease in glucose transport was likely due to the steady state expression of the HTLV RBD following transfection, as the level of the secreted RBD was insufficient to modulate glucose metabolism.

We next evaluated the effect of glucose deprivation on the availability of the HTLV receptor in both adherent 293T cells and suspension Jurkat T cells. After overnight culture of cells in the absence of glucose, binding of $H1_{RBD}$ was surprisingly increased by 2-fold in both cell types (Figure 2A). This effect of glucose deprivation was

specific to HTLV as A_{RBD} binding was only marginally altered under the same conditions (Figure 2A). This phenomenon is reminiscent of a general metabolite transport feedback loop, whereby transporter availability at the cell surface increases upon substrate starvation.

GLUT-1 Expression Parallels HTLV RBD Binding

Inhibition of glucose consumption via binding of the HTLV RBD to a glucose transporter is compatible with the metabolic effects described above. Upon evaluation of the different glucose transporter candidates, GLUT-1 was the only one to present all the known properties of the HTLV receptor. Indeed, GLUT-1 expression is increased upon glucose deprivation and is involved in glucose transport in all vertebrates (Mueckler et al., 1985). Furthermore, GLUT-1 is not expressed on quiescent primary T cells and its expression is induced upon T cell activation (Chakrabarti et al., 1994; Rathmell et al., 2000) with kinetics that are strikingly similar to what we and others have reported for the HTLV receptor (Manel et al., 2003; Nath et al., 2003). Since human, but not murine, erythrocytes have been described to exhibit the highest concentration of GLUT-1 (Mantych et al., 1991; Mueckler, 1994), we compared HTLV receptor availability on freshly isolated human and murine red blood cells. Binding of $H1_{RBD}$ to human erythrocytes was remarkably efficient, reaching levels higher than those observed on any other tested cell type (Figure 2B and not shown). In marked contrast, no significant $H1_{RBD}$ binding was detected on murine erythrocytes although comparably low levels of A_{RBD} binding were observed on murine and human erythrocytes. Similarly, primary human hepatocytes which do not express GLUT-1 (Mueckler, 1994) did not bind $H1_{RBD}$, while A_{RBD} binding on these cells was readily detected (Figure 2B). Thus, the expression of GLUT-1 parallels that of the HTLV receptor.

HTLV Envelopes Bind GLUT-1

In order to directly test the ability of HTLV envelopes to bind GLUT-1, we derived a tagged GLUT-1 expression vector and overexpressed this protein in 293T cells. Binding of both $H1_{RBD}$ and $H2_{RBD}$ were dramatically increased upon GLUT-1 overexpression (Figure 3A). This interaction required a functional HTLV RBD as binding of the D102A HTLV-2 mutant did not increase following overexpression of GLUT-1 (Figure 3A). Moreover, this interaction was specific to GLUT-1, since $H1_{RBD}$ and $H2_{RBD}$ binding remained at background levels upon overexpression of the A-MLV envelope receptor, the inorganic phosphate transporter PiT-2 (Miller et al., 1994; van Zeijl et al., 1994). However, as expected, binding of A_{RBD} was specifically increased upon transfection of PiT-2, but remained unchanged after GLUT-1 overexpression (Figure 3A).

The specificity of the interaction between GLUT-1 and HTLV RBD was further confirmed by experiments using GLUT-3, the closest isoform to GLUT-1 whose glucose transport kinetics are similar to that of GLUT-1 (Asano et al., 1992; Joost and Thorens, 2001). GLUT-3 is expressed at similar levels as GLUT-1 (See Supplemental Data available at http://www.cell.com/cgi/content/full/115/4/449/DC1), and like GLUT-1, alleviates the block in glucose metabolism induced by the HTLV RBD (see below). However, unlike GLUT-1, overexpression of GLUT-3 did not increase $H1_{RBD}$ and $H2_{RBD}$ binding (Figure 3A). These data demonstrate that increased HTLV RBD binding is specific to GLUT-1 expression and not an indirect consequence of increased glucose uptake. To confirm that increased $H1_{RBD}$ binding was directly due to those cells that overexpressed GLUT-1, we derived DsRed2 fluorochrome-tagged GLUT-1 and GLUT-3 expression vectors to unequivocally identify GLUT-overexpressing cells. In this context, we found that those cells overexpressing DsRed2-tagged GLUT-1 displayed significantly increased $H1_{RBD}$ binding, as demonstrated by FACS analysis (Figure 3B). $H1_{RBD}$ binding was not altered in cells overexpressing the tagged-GLUT-3.

A direct interaction between GLUT-1 and HTLV envelopes was demonstrated by coimmunoprecipitation of $H1_{RBD}$ and GLUT-1. As shown in Figure 4, GLUT-1 was detected upon immunoprecipitation of $H1_{RBD}$-rFc with anti-rabbit-IgG-coated beads. The coimmunoprecipitation of GLUT-1 with the $H1_{RBD}$ Y114A receptor binding defective mutant was dramatically diminished. No coimmunoprecipitation was observed in the absence of HTLV RBD.

GLUT-1 Mediates HTLV Infection

As demonstrated above, HTLV envelopes physically associate with GLUT-1 and this interaction modulates glucose metabolism. To determine whether this interaction is essential for infection, it would have been opportune to use GLUT-1-deficient cell lines. However, since there are no GLUT-1 null cell lines and since quiescent primary cells lacking GLUT-1 are not amenable to HTLV infection assays, we developed three different models of HTLV envelope-driven infection in which either receptor availability or GLUT-1 expression were limiting.

In the first model, we monitored HTLV envelope-driven infection in the presence of cytochalasin B which inhibits glucose transport (Kasahara and Hinkle, 1977) and which we found to inhibit HTLV RBD binding. Indeed, treatment of Jurkat T cells with cytochalasin B dramatically inhibited binding of $H1_{RBD}$, whereas binding of A_{RBD} remained unchanged (Figure 5A). To determine whether this effect was due to the role of cytochalasin B as a ligand for GLUT-1 or, alternatively, was indirectly due to its indirect effects on microfilaments, cells were treated with the related molecule cytochalasin D. This latter molecule, which is not a ligand of GLUT-1, led to a low-level, nonspecific decrease in both HTLV and A_{RBD} binding (see Supplemental Data at above URL). Thus, these data suggest that the cytochalasin B-mediated inhibition of HTLV RBD binding is largely due to its interaction with GLUT-1 and not to an impact on actin microfilaments.

To determine whether the cytochalasin B-mediated decrease in receptor availability would translate into an effect on HTLV envelope-driven infection, control and cytochalasin B-treated cells were infected with virions harboring either the HTLV or A-MLV envelopes (Figure 5B). A 75% inhibition of HTLV envelope-driven infection was observed after cytochalasin B treatment. In contrast, A-MLV envelope-driven infection was not reduced, but was in fact increased.

In the second model, HTLV receptor availability was

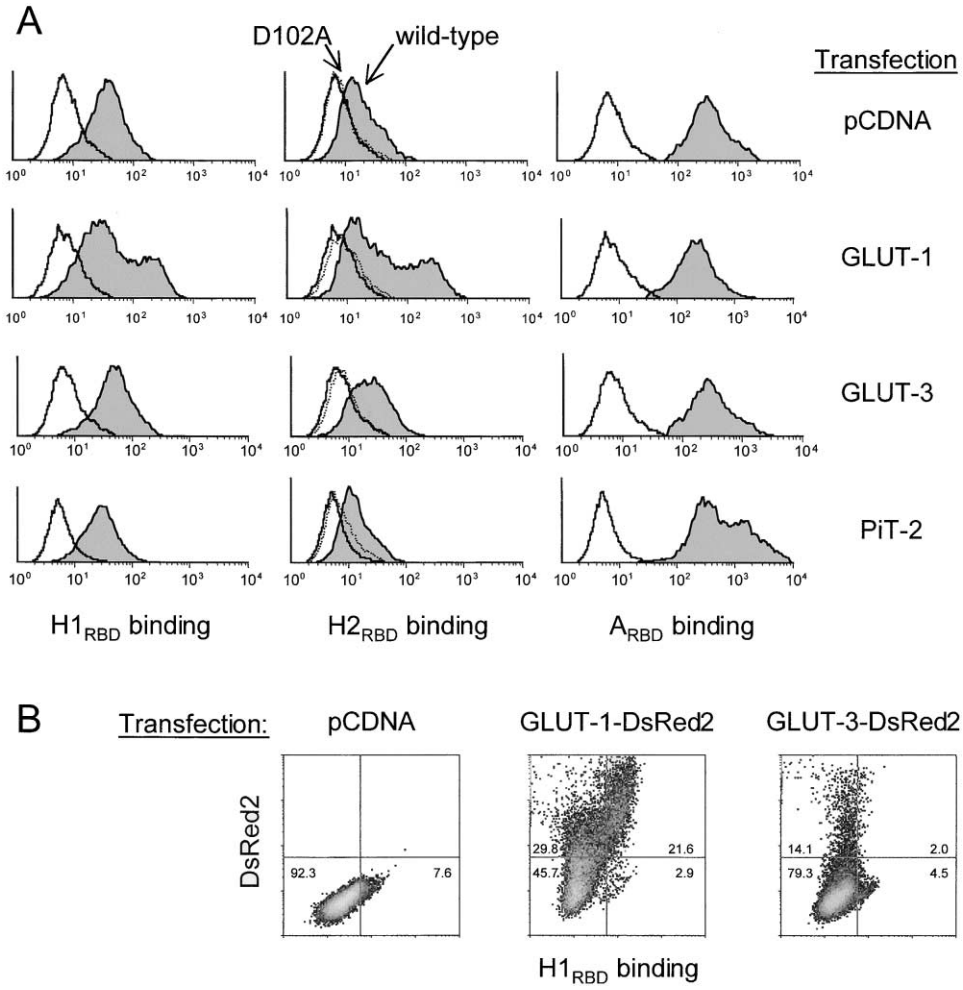

Figure 3. HTLV Envelope-Receptor Binding Increases in GLUT-1 Overexpressing Cells

(A) Binding of $H1_{RBD}$, $H2_{RBD}$, $H2_{RBD}$ D102A mutant, and A_{RBD} to control 293T cells or 293T cells overexpressing either GLUT-1, GLUT-3, or PiT-2. Intrinsic fluorescence (solid line histograms), wild-type RBD binding (filled histograms), and mutant D102A binding (dotted lines histograms) are shown.

(B) Correlation between GLUT-1 expression and HTLV RBD binding in individual cells. FACS analyses of $H2_{RBD}$-EGFP binding to 293T cells transfected with control DNA (pCDNA), GLUT-1-DsRed2, or GLUT-3-DsRed2 expression vectors are shown. Quadrants were drawn to separate cells with basal HTLV RBD binding from cells with high binding. The percentage of cells in each quadrant is indicated.

specifically limited by the intracellular expression of an interfering HTLV envelope RBD ($H2_{RBD}$). In these conditions, both extracellular binding of HTLV envelope (Figure 6A) and infection by HTLV envelope-harboring virions (Figure 6B) were dramatically reduced. Overexpression of GLUT-1 in these cells rescued HTLV envelope-mediated binding and infection. Although overexpression of GLUT-3 in these cells reversed defects in lactate and glucose metabolism (Figure 6C and not shown), HTLV envelope binding and viral titers remained significantly reduced. Expression of the interfering $H2_{RBD}$ was equivalent in all conditions of glucose transporter overexpression (Figure 6D). These results confirmed the specific relationship between HTLV receptor availability and GLUT-1 expression.

In the third model, we used siRNAs directed against the GLUT-1 3′UTR to specifically down-modulate endogenous GLUT-1 expression. Transfection of those GLUT-1 siRNAs led to a concomitant decrease in HTLV RBD binding (Figure 7A) and a 75% inhibition of HTLV envelope-mediated infection (Figure 7B). As observed in the receptor interference experiments, overexpression of GLUT-1, but not GLUT-3, restored HTLV RBD binding and HTLV envelope-mediated infection (Figures 7A and 7B). The effect of the GLUT-1 siRNAs on HTLV envelope-driven infection was not due to metabolic perturbations per se as both GLUT-1 and GLUT-3 overexpression reestablished glucose transport and lactate production (Figure 7C and data not shown). Furthermore, GLUT-1 siRNAs did not inhibit A-MLV envelope-mediated binding and infection. Down-modulation of GLUT-1 by siRNAs, and overexpression of ectopic GLUT-1 following transfection were directly verified in these different cell culture conditions by immunoblotting with an anti-GLUT-1 polyclonal antibody (Figure 7D). Altogether, these data demonstrate that HTLV envelope-mediated viral entry occurs through a direct interaction with GLUT-1.

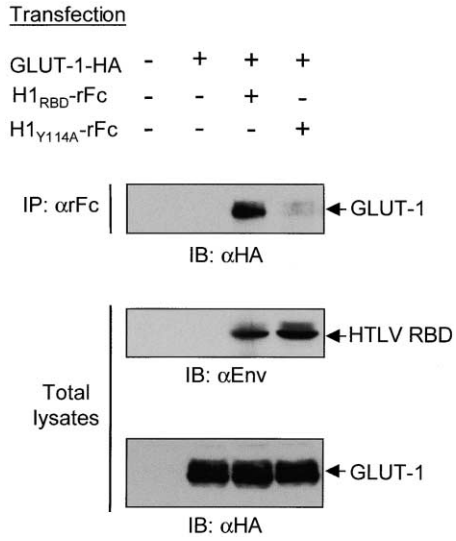

Figure 4. GLUT-1 Physically Associates with the HTLV Envelope

Immunoprecipitation of HA-tagged GLUT-1 from 293T cells transfected with expression vectors carrying control DNA, GLUT-1 alone, or a combination of GLUT-1 with either wild-type $H1_{RBD}$ or the Y114A $H1_{RBD}$ mutant. Immunoprecipitations were performed using anti-rabbit-IgG beads and blots were probed with an anti-HA antibody. Total cell extracts were blotted using a monoclonal anti-HTLV envelope antibody (1C11) and an anti-HA antibody to monitor HTLV RBD and GLUT-1 levels, respectively.

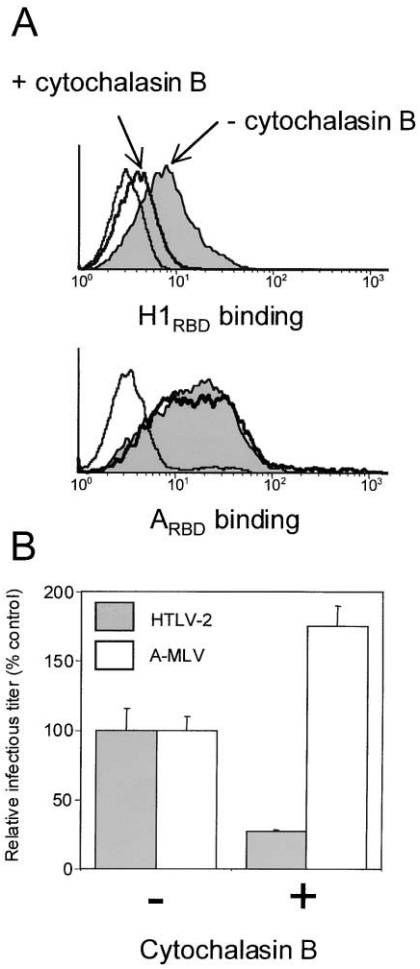

Figure 5. Cytochalasin B Inhibits HTLV Envelope Binding and Infection

(A) $H1_{RBD}$ and A_{RBD} binding were monitored on Jurkat cells pretreated with or without 20 μM of the GLUT-1 inhibitor cytochalasin B.

(B) Relative infectious titers of virions pseudotyped with either HTLV-2 (shaded) or A-MLV (open) envelopes were assessed in control and cytochalasin B-treated HeLa cells. Relative infectious titers were calculated from input virions ranging from 10 to 80 PFU per well for both HTLV-2 and A-MLV.

Discussion

Here we show that the HTLV-1 and -2 receptor binding domains physically associate with the glucose transporter GLUT-1. This interaction strongly inhibited glucose uptake and consumption, leading to decreased lactate production and a drop in the acidification of the extracellular milieu. This effect was specific as HTLV-1 and 2 envelopes with mutations that interfered with receptor binding did not block glucose consumption (not shown). There was a rapid increase in HTLV envelope binding upon glucose starvation, highlighting a nutrient-sensing negative feedback loop between glucose availability and cell surface HTLV receptor expression. In this context, we explored whether GLUT-1, the ubiquitous glucose transporter, was a receptor for HTLV Env-mediated viral entry. Because antibodies that reliably interact with all the glycosylated forms of GLUT-1 have been raised against internal determinants, they could not be used to inhibit HTLV Env-mediated viral entry (M. Mueckler, personal communication). Nevertheless, extensive evidence converged to identify GLUT-1 as the receptor for HTLV envelope-mediated binding and infection: HTLV envelopes bind all vertebrate cell lines but not insect cells and GLUT-1 orthologs are conserved among vertebrates but are highly divergent between vertebrates and insects (Escher and Rasmuson-Lestander, 1999); highest binding of HTLV RBD is observed on human erythrocytes, where GLUT-1 is the major glucose transporter isoform (Concha et al., 1997); HTLV RBD binding is not detected on human primary hepatocytes and murine erythrocytes where GLUT-1 is known to be only minimally expressed (Mantych et al., 1991; Mueckler, 1994); HTLV RBD binding is increased upon overexpression of GLUT-1 but not GLUT-3; and GLUT-1 coimmunoprecipitates with $H1_{RBD}$. Moreover, as we previously demonstrated for the HTLV receptor (Manel et al., 2003), GLUT-1 is not expressed on resting T lymphocytes whereas the GLUT 2-4 isoforms are present (Chakrabarti et al., 1994; Korgun et al., 2002), and GLUT-1 is induced upon immunological (Frauwirth et al., 2002; Yu et al., 2003) or pharmacological (Chakrabarti et al., 1994) activation. Finally, GLUT-1 overexpression specifically restores HTLV envelope-mediated infection after receptor blocking with either an interfering HTLV envelope or GLUT-1 siRNAs. GLUT-1 is thus a new member of the multimembrane spanning metabolite transporter family known to serve as receptors for retroviral envelopes. Interestingly, until now, all envelopes that recognize such receptors have been shown to be encoded by retroviruses that have a "simple" genetic organization,

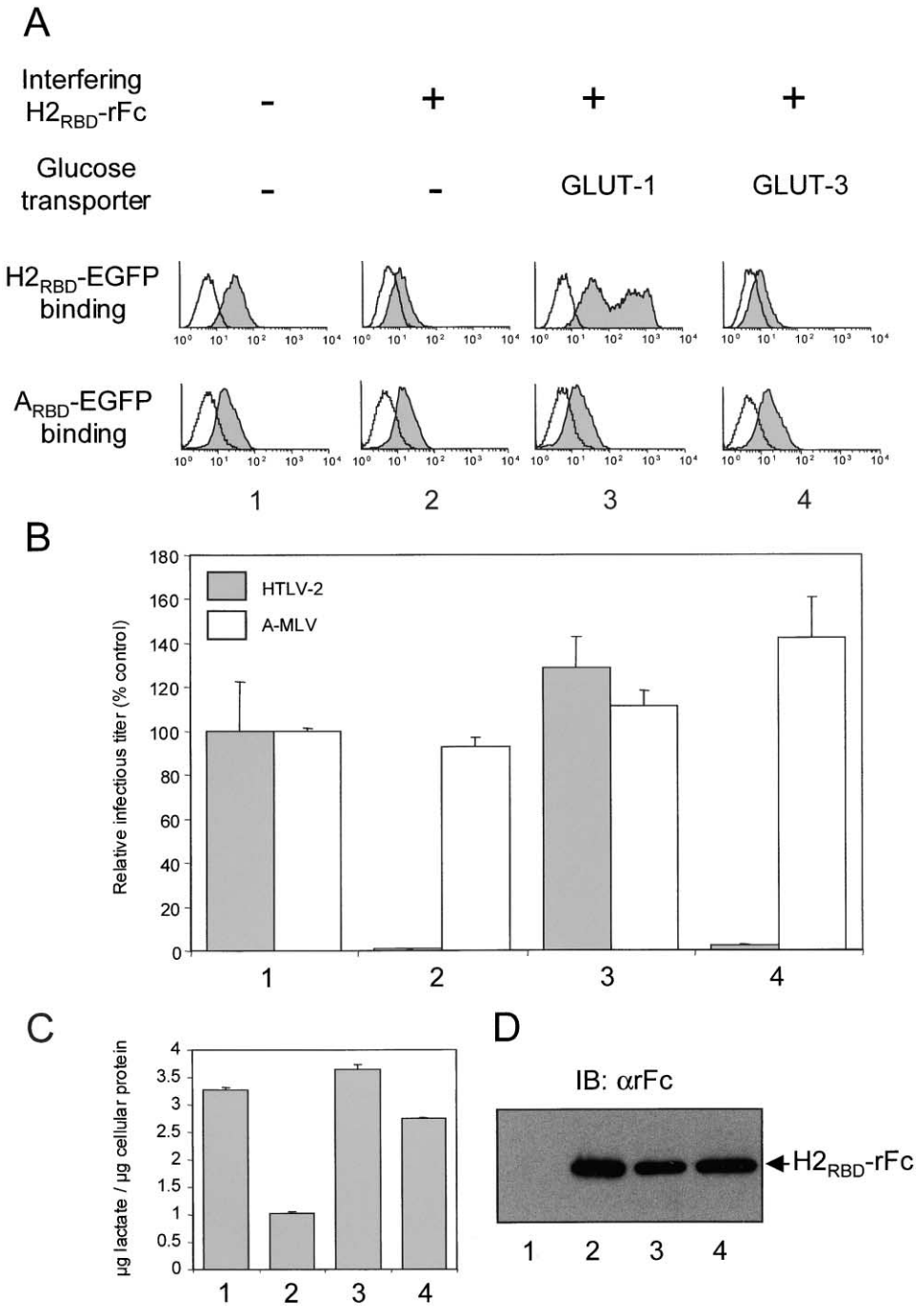

Figure 6. RBD Interference to HTLV Envelope Binding and Infection Is Alleviated by GLUT-1

293T cells were transfected with an irrelevant or interfering HTLV RBD expression vector, alone or together with GLUT-1 or GLUT-3 expression vectors. Transfected cells were assayed for (A) $H1_{RBD}$ and A_{RBD} binding. Specific binding and intrinsic fluorescence are indicated by filled and solid histograms, respectively. (B) Relative infection titers of virions pseudotyped with either HTLV-2 (shaded) or A-MLV (open) envelopes. Input virions ranged from 50 to 300 PFU per well. (C) Extracellular lactate accumulation in 293T cell culture medium one day after transfection. (D) HTLV RBD ($H2_{RBD}$) expression was monitored by immunoblotting of total protein extracts with an anti-rabbit-IgG antibody.

such as MLV, feline leukemia viruses, porcine endogenous retrovirus, and the gibbon ape leukemia virus (Overbaugh et al., 2001; Tailor et al., 2003), whereas HTLV is a "complex" retrovirus that codes for several additional regulatory proteins. However, we have shown that in contrast to the wide phylogenetic divergence of their genomes, the HTLV and MLV envelopes share a similar modular organization with highly conserved amino acid motifs in their respective receptor binding domains (Kim et al., 2000, 2003a).

Cell-to-cell contact and oriented cytoskeleton movements appear to be required for HTLV transmission (Iga-

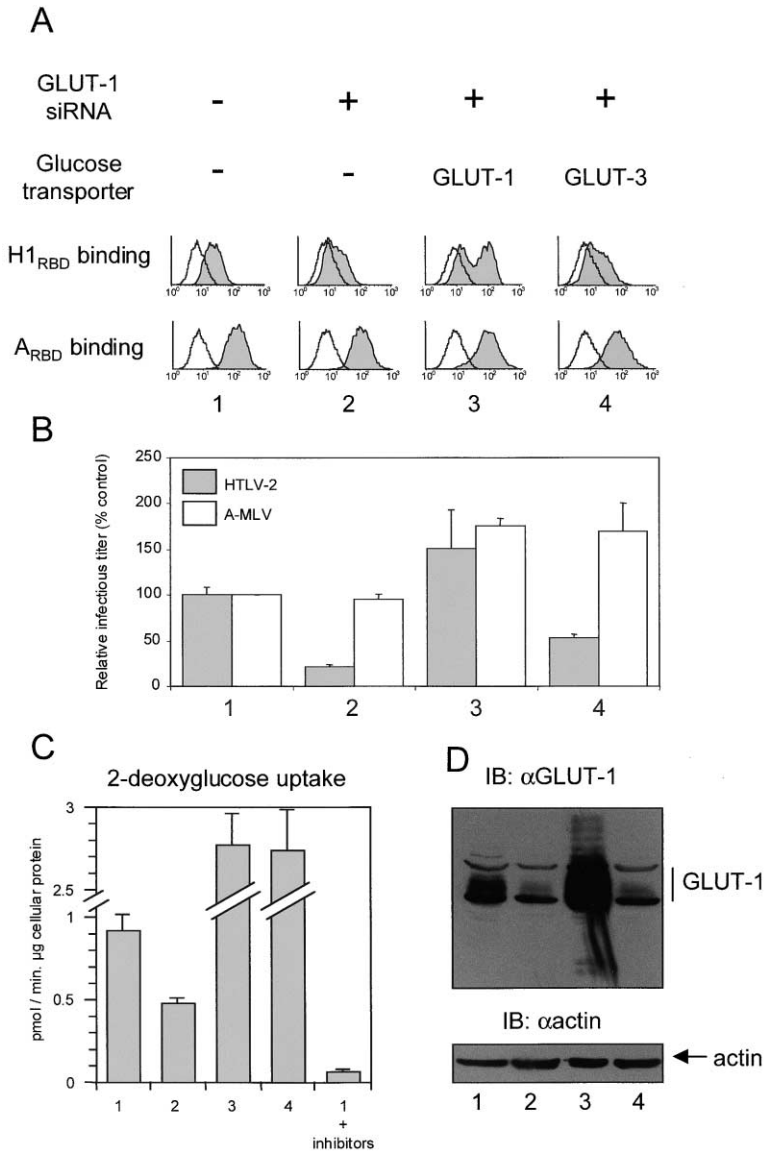

Figure 7. GLUT-1 Is Required for HTLV Envelope Binding and Infection

293T cells were transfected with a mixture of three GLUT-1 specific siRNAs or irrelevant siRNAs in combination with either an empty, a GLUT-1, or a GLUT-3 expression vector. Transfected cells were assayed for (A) $H1_{RBD}$ and A_{RBD} binding. Specific binding and intrinsic fluorescence are indicated by filled and solid-line histograms, respectively. (B) Relative infection titers of virions pseudotyped with either HTLV-2 (shaded) or A-MLV (open) envelopes. Input virions ranged from 50 to 400 PFU per well. (C) 2-deoxy[1-^{3}H]glucose uptake. Uptake was measured in cells transfected with the GLUT-1 siRNAs and GLUT-1 and GLUT-3 expression vectors. Cells incubated with cytochalasin B and phloretin inhibitors were used as controls for glucose transport inhibition. (D) Endogenous and ectopic GLUT-1 expression; expression was monitored by immunoblotting total protein extracts with an anti-GLUT-1 antibody. Equivalent protein loading was confirmed by immunoblotting with an anti-actin antibody.

kura et al., 2003). A role for envelope and receptor interactions in these processes has been suggested (Derse and Heidecker, 2003). In agreement with these observations, we found that the HTLV receptor, despite pancellular expression, is specifically concentrated to mobile membrane regions and cell-to-cell contact areas (Supplemental Data). This suggests that the HTLV envelope receptor associates with the cytoskeleton. Indeed, a cytoplasmic binding partner of GLUT-1, GLUT1CBP, which encodes a PDZ domain, has been reported to link GLUT-1 to the cytoskeleton (Bunn et al., 1999). It will therefore be of interest to evaluate the respective impact of the HTLV envelope on membrane organization, its cytoskeleton-associated cellular partners, such as GLUT-1 and GLUT1CBP, and other known or yet to be identified interacting cell surface components.

Because expression of the HTLV receptor is induced upon glucose starvation, transmission of HTLV may be more efficient among cells that are locally starved of glucose and express high levels of GLUT-1, such as immature thymocytes (Feuer et al., 1996; Yu et al., 2003). The ability of circulating erythrocytes to dock HTLV, as shown here, might provide a means of distributing HTLV to such tissues, provided that they are not readily eliminated by the reticuloendothelial system. Elucidation of GLUT-1 cell surface recycling upon HTLV envelope binding to different cell types, including murine and human erythrocytes, may offer further clues to the in vivo distribution of HTLV during infection.

The identification of GLUT-1 as a receptor for HTLV envelopes provides insight into the paradox between the ubiquitous in vitro expression of the receptor on cell lines and the apparent restriction of HTLV tropism to T lymphocytes in vivo. Early after infection, rapid and dramatic metabolic alterations associated with decreased glucose consumption are likely to occur upon expression of the HTLV envelope. It is tempting to propose that during in vivo infection, HTLV initially spreads with a large tropism, but that the vast majority of cells that are dependent on GLUT-1 activity and concomi-

tantly express the HTLV envelope are rapidly eliminated. In contrast, resting T lymphocytes that have a low metabolic rate and as such are much less dependent on glucose uptake, are more likely to tolerate this effect and survive infection. Furthermore, local imbalances in glucose uptake, following HTLV infection may lead to specific physiological alterations (Akaoka et al., 2001). In this regard, the potential relationship between HTLV-associated neuropathologies and the specific dependence of neurons on GLUT-1 mediated glucose consumption (De Vivo et al., 1991; Siegel et al., 1998) becomes particularly relevant.

Experimental Procedures

Cell Culture and Virus Preparations

Human 293T and HeLa cells were grown in DMEM with high glucose (4.5 g/l) and Jurkat T cells were grown in RPMI with 10% fetal bovine serum (FBS) at 37°C. Freshly isolated hepatocytes were obtained following surgery in accordance with the Hospital Ethics Board as published (Pichard et al., 1990). For glucose starvation experiments, cells were grown in either glucose-free DMEM (Life Technologies) or glucose-free RPMI (Dutscher) with 10% dialyzed FBS (Life Technologies) and glucose (1 g/l) was supplemented when indicated. Replication-defective LacZ retroviral vector was pseudotyped with either HTLV or A-MLV envelopes as previously described (Kim et al., 2003b).

Expression Vectors and siRNAs

Full-length envelope expression vectors have been described elsewhere (Denesvre et al., 1995 1996; Rosenberg et al., 1998). The chimeric HTLV/MLV Env, H_{183}FEnv, was obtained by replacing the first 269 amino acid residues of the F-MLV Env with the 183 amino-terminal residues of the HTLV-1 Env. $H1_{RBD}$, $H2_{RBD}$, and A_{RBD} comprise the first 215 aa, 178 aa, and 397 aa of the HTLV-1, HTLV-2, and A-MLV SU, respectively, fused to either a carboxy-terminal rabbit immunoglobulin Fc (rFc) tag or to the enhanced green fluorescence protein (EGFP) coding sequence. Construction of the chimeric envelope as well as wild-type and mutant truncated envelope expression vectors will be detailed elsewhere. Human *Glut-1* and *Glut*-3 cDNA were amplified by PCR from the pLib HeLa cDNA library (Clontech) from the first AUG to the last codon before the termination codon, and individually inserted into pCHIX, a modified version of the pCSI vector (Battini et al., 1999) that includes a C-terminal factor Xa cleavage site, and the hemagglutinin (HA) and histidine tags. *Glut-1* and *Glut*-3 cDNA were also inserted into a modified pCSI vector that includes a DsRed2 C-terminal tag. Similarly, human CD147 was amplified from 293T total RNA and inserted into pCHIX. The rat MCT1 expression vector was a gift from J. Mercier and G. Py and the VSVG-tagged human PiT-2 expression vector was a gift from J.-M. Heard (Rodrigues and Heard, 1999). The sequences of the three synthetic annealed siRNAs complementary to the GLUT-1 3′UTR, and which are not present in the GLUT-1 expression vector, are UGAUGUCCAGAAGAAUAUUTT, AAUAUUCUUCUGGACAUCATT, and UAUUAAAUACAGACACUAATT (Eurogentec, plus strand indicated).

Envelope Expression and Metabolic Measurements

293T cells were transfected with the envelope expression vectors using the calcium phosphate method. After an overnight transfection, cells were washed in phosphate-buffered saline (PBS) and fresh medium was added. Media were harvested, filtered through a 0.45 μm pore-size filter, and lactate and glucose were measured with enzymatic diagnostic kits (Sigma). Values were normalized to cellular protein content using the Bradford assay (Sigma) after solubilization of cells in lysis buffer (Kim et al., 2000) before clarification by centrifugation.

Hexose Uptake Assays

The hexose uptake assay was adapted from Harrison et al. (1990) using 2-deoxy-D[1-^{3}H]glucose, 3-O-[^{14}C]methyl-D-glucose and D[U-^{14}C]fructose (Amersham). After transfection of Env expression vectors, approximately 2.5×10^5 293T cells per well were seeded in 24-well plates. The next day, cells were washed in PBS, incubated in serum-free DMEM, washed in serum/glucose-free DMEM, and incubated for 20 min in 500 μl serum/glucose-free DMEM in the presence or absence of inhibitors (20 μM cytochalasin B and 300 μM phloretin; Sigma). Uptake was initiated by adding labeled hexoses to a final concentration of 0.1 mM (2 μCi/ml for 2-deoxy-D[1-^{3}H]glucose and 0.2 μCi/ml for D[U-^{14}C]fructose and 3-O-[^{14}C]methyl-D-glucose) and cells were incubated for an additional five minutes. Cells were then resuspended in 500 μl cold serum/glucose-free DMEM, washed in serum/glucose-free DMEM, and solubilized in 400 μl of 0.1% SDS. Three μl were used for Bradford normalization, and ^{3}H or ^{14}C incorporation in the remainder of the samples was counted.

Immunoblots

Culture media (10 μl) from 293T cells transfected with HTLV RBD, or lysates from cells overexpressing GLUT-1 or GLUT-3, alone or in combination with HTLV-2 RBD, were electrophoresed in SDS–12.5% acrylamide gels, transferred and probed with either a peroxidase-conjugated anti-rabbit immunoglobulin antiserum or mouse monoclonal antibodies directed against HTLV Env (1C11), HA tag (12CA5), or actin (C4, ICN), or a rabbit polyclonal serum raised against a GLUT-1 carboxy terminal synthetic peptide (generous gift form M. Mueckler), followed by the corresponding peroxidase-conjugated anti-immunoglobulin antiserum. Proteins were visualized using the ECLplus kit (Amersham).

Coimmunoprecipitation Assay

293T cells were transfected with the various envelope expression vectors, alone or in combination with the GLUT-1 expression vector. Twenty-four hours following transfection, cells were lysed in Brij buffer (1% Brij97, 150 mM NaCl, 20 mM Tris [pH 7.5], and 5 mM EDTA); 10% of the lysate was used for immunoblots of total protein extracts, while 90% of the lysate was immunoprecipitated with agarose beads coupled to goat anti-rabbit IgG immunoglobulins (Sigma) for 4 hr at 4°C. Beads were washed four times with Brij buffer, denatured in loading buffer, and processed for immunobloting.

Binding Assays

Binding assays were carried out as previously described (Manel et al., 2003). Briefly, 5×10^5 cells were washed with PBA (PBS containing 1% bovine serum albumin [BSA] and 0.1% sodium azide), incubated with 300 μl of control, H_{RBD}, or A_{RBD} supernatants for 30 min at 37°C, washed, and labeled for 30 min on ice with an FITC-conjugated sheep anti-rFc antibody (1:500 dilution; Sigma). Cells were immediately analyzed on a FACSCalibur (Becton Dickinson). When indicated, cytochalasin B (20 μM; Sigma) was added to cells for 1 hr prior to binding analyses.

Infections

10^5 293T target cells were treated as follows prior to infection: (1) incubation for 30 min with 20 μM cytochalasin B, (2) transfection with either the interfering $H2_{RBD}$ or pCDNA in the presence or absence of GLUT-1 or GLUT-3 expression vectors, or (3) transfection with 20 pmol of an equimolar mixture of three siRNAs complementary to the endogenous GLUT-1 3′UTR in the presence or absence of GLUT-1 or GLUT-3 cDNA expression vectors. Infections were carried out with serial dilutions of LacZ retroviral vectors pseudotyped with either HTLV-2 or A-MLV envelopes. The following day, fresh medium supplemented with fructose (5 g/l) was added. Twenty-four hours later, LacZ expression was monitored by X-Gal staining.

Acknowledgments

We are grateful to R.K. Naviaux for key input on lactate metabolism; P. Sonigo and J. Leibowitch for stimulating discussions on cell metabolism; C. Denesvre for constant interest in this work; P. Travo for expert assistance in imaging; O. Adjali, I. Du Chêné, G. Mandon, C. Mongellaz, L. Swainson, C. Valle, and S. Wieckowski for their help with some of the experiments; M. Mueckler for his expert advice on GLUT-1 biochemistry and for generously providing us with an anti-GLUT-1 antibody; J. Mercier, G. Py, J.M. Heard, M.-C. Dokhelar,

and R. Hipskind for kindly sharing plasmid DNA and antibodies; M. Daujat and P. Maurel for primary hepatocytes; P. Jeanteur and J.M. Blanchard for continuous support; and all members of our laboratories. N.M. was successively funded by the Ecole Normale Supérieure de Lyon and the Ministère de la Recherche (AC); F.J.K. was funded by an award from the Philippe Foundation and successive fellowships from the Association pour la Recherche sur le Cancer (ARC) and the Fondation de France; and S.K. was funded by the European Community and the CNRS. N.T., M.S.and J.-L.B. are supported by INSERM. This work was supported by grants from ARC, AFM, ANRS (M.S. and N.T.), the March of Dimes (N.T.), and Fondation de France (M.S.).

Received: June 6, 2003
Revised: October 14, 2003
Accepted: October 21, 2003
Published: November 13, 2003

References

Akaoka, H., Szymocha, R., Beurton-Marduel, P., Bernard, A., Belin, M.F., and Giraudon, P. (2001). Functional changes in astrocytes by human T-lymphotropic virus type-1 T- lymphocytes. Virus Res. *78*, 57–66.

Asano, T., Katagiri, H., Takata, K., Tsukuda, K., Lin, J.L., Ishihara, H., Inukai, K., Hirano, H., Yazaki, Y., and Oka, Y. (1992). Characterization of GLUT3 protein expressed in Chinese hamster ovary cells. Biochem. J. *288*, 189–193.

Battini, J.L., Danos, O., and Heard, J.M. (1995). Receptor-binding domain of murine leukemia virus envelope glycoproteins. J. Virol. *69*, 713–719.

Battini, J.L., Rasko, J.E., and Miller, A.D. (1999). A human cell-surface receptor for xenotropic and polytropic murine leukemia viruses: possible role in G protein-coupled signal transduction. Proc. Natl. Acad. Sci. USA *96*, 1385–1390.

Bunn, R.C., Jensen, M.A., and Reed, B.C. (1999). Protein interactions with the glucose transporter binding protein GLUT1CBP that provide a link between GLUT1 and the cytoskeleton. Mol. Biol. Cell *10*, 819–832.

Cavrois, M., Gessain, A., Wain-Hobson, S., and Wattel, E. (1996). Proliferation of HTLV-1 infected circulating cells in vivo in all asymptomatic carriers and patients with TSP/HAM. Oncogene *12*, 2419–2423.

Chakrabarti, R., Jung, C.Y., Lee, T.P., Liu, H., and Mookerjee, B.K. (1994). Changes in glucose transport and transporter isoforms during the activation of human peripheral blood lymphocytes by phytohemagglutinin. J. Immunol. *152*, 2660–2668.

Concha, I.I., Velasquez, F.V., Martinez, J.M., Angulo, C., Droppelmann, A., Reyes, A.M., Slebe, J.C., Vera, J.C., and Golde, D.W. (1997). Human erythrocytes express GLUT5 and transport fructose. Blood *89*, 4190–4195.

Daenke, S., and Booth, S. (2000). HTLV-1-induced cell fusion is limited at two distinct steps in the fusion pathway after receptor binding. J. Cell Sci. *113*, 37–44.

Daenke, S., McCracken, S.A., and Booth, S. (1999). Human T-cell leukaemia/lymphoma virus type 1 syncytium formation is regulated in a cell-specific manner by ICAM-1, ICAM-3 and VCAM-1 and can be inhibited by antibodies to integrin beta2 or beta7. J. Gen. Virol. *80*, 1429–1436.

De Vivo, D.C., Trifiletti, R.R., Jacobson, R.I., Ronen, G.M., Behmand, R.A., and Harik, S.I. (1991). Defective glucose transport across the blood-brain barrier as a cause of persistent hypoglycorrhachia, seizures, and developmental delay. N. Engl. J. Med. *325*, 703–709.

Denesvre, C., Sonigo, P., Corbin, A., Ellerbrok, H., and Sitbon, M. (1995). Influence of transmembrane domains on the fusogenic abilities of human and murine leukemia retrovirus envelopes. J. Virol. *69*, 4149–4157.

Denesvre, C., Carrington, C., Corbin, A., Takeuchi, Y., Cosset, F.L., Schulz, T., Sitbon, M., and Sonigo, P. (1996). TM domain swapping of murine leukemia virus and human T-cell leukemia virus envelopes confers different infectious abilities despite similar incorporation into virions. J. Virol. *70*, 4380–4386.

Derse, D., and Heidecker, G. (2003). Virology. Forced entry–or does HTLV-I have the key? Science *299*, 1670–1671.

Escher, S.A., and Rasmuson-Lestander, A. (1999). The Drosophila glucose transporter gene: cDNA sequence, phylogenetic comparisons, analysis of functional sites and secondary structures. Hereditas *130*, 95–103.

Feuer, G., Fraser, J.K., Zack, J.A., Lee, F., Feuer, R., and Chen, I.S. (1996). Human T-cell leukemia virus infection of human hematopoietic progenitor cells: maintenance of virus infection during differentiation in vitro and in vivo. J. Virol. *70*, 4038–4044.

Frauwirth, K.A., Riley, J.L., Harris, M.H., Parry, R.V., Rathmell, J.C., Plas, D.R., Elstrom, R.L., June, C.H., and Thompson, C.B. (2002). The CD28 signaling pathway regulates glucose metabolism. Immunity *16*, 769–777.

Garcia, C.K., Goldstein, J.L., Pathak, R.K., Anderson, R.G., and Brown, M.S. (1994). Molecular characterization of a membrane transporter for lactate, pyruvate, and other monocarboxylates: implications for the Cori cycle. Cell *76*, 865–873.

Gessain, A., Barin, F., Vernant, J.C., Gout, O., Maurs, L., Calender, A., and de The, G. (1985). Antibodies to human T-lymphotropic virus type-I in patients with tropical spastic paraparesis. Lancet *2*, 407–410.

Halestrap, A.P., and Price, N.T. (1999). The proton-linked monocarboxylate transporter (MCT) family: structure, function and regulation. Biochem. J. *343*, 281–299.

Hanon, E., Stinchcombe, J.C., Saito, M., Asquith, B.E., Taylor, G.P., Tanaka, Y., Weber, J.N., Griffiths, G.M., and Bangham, C.R. (2000). Fratricide among CD8(+) T lymphocytes naturally infected with human T cell lymphotropic virus type I. Immunity *13*, 657–664.

Harrison, S.A., Buxton, J.M., Helgerson, A.L., MacDonald, R.G., Chlapowski, F.J., Carruthers, A., and Czech, M.P. (1990). Insulin action on activity and cell surface disposition of human HepG2 glucose transporters expressed in Chinese hamster ovary cells. J. Biol. Chem. *265*, 5793–5801.

Hildreth, J.E., Subramanium, A., and Hampton, R.A. (1997). Human T-cell lymphotropic virus type 1 (HTLV-1)-induced syncytium formation mediated by vascular cell adhesion molecule-1: evidence for involvement of cell adhesion molecules in HTLV-1 biology. J. Virol. *71*, 1173–1180.

Igakura, T., Stinchcombe, J.C., Goon, P.K., Taylor, G.P., Weber, J.N., Griffiths, G.M., Tanaka, Y., Osame, M., and Bangham, C.R. (2003). Spread of HTLV-I between lymphocytes by virus-induced polarization of the cytoskeleton. Science *299*, 1713–1716.

Jassal, S.R., Pohler, R.G., and Brighty, D.W. (2001). Human T-cell leukemia virus type 1 receptor expression among syncytium- resistant cell lines revealed by a novel surface glycoprotein- immunoadhesin. J. Virol. *75*, 8317–8328.

Joost, H.G., and Thorens, B. (2001). The extended GLUT-family of sugar/polyol transport facilitators: nomenclature, sequence characteristics, and potential function of its novel members (review). Mol. Membr. Biol. *18*, 247–256.

Kasahara, M., and Hinkle, P.C. (1977). Reconstitution and purification of the D-glucose transporter from human erythrocytes. J. Biol. Chem. *252*, 7384–7390.

Kasinrerk, W., Fiebiger, E., Stefanova, I., Baumruker, T., Knapp, W., and Stockinger, H. (1992). Human leukocyte activation antigen M6, a member of the Ig superfamily, is the species homologue of rat OX-47, mouse basigin, and chicken HT7 molecule. J. Immunol. *149*, 847–854.

Kim, F.J., Seiliez, I., Denesvre, C., Lavillette, D., Cosset, F.L., and Sitbon, M. (2000). Definition of an amino-terminal domain of the human T-cell leukemia virus type 1 envelope surface unit that extends the fusogenic range of an ecotropic murine leukemia virus. J. Biol. Chem. *275*, 23417–23420.

Kim, F.J., Manel, N., Battini, J.-L., and Sitbon, M. (2003a). Emergence of vertebrate retroviruses and envelope capture. Virology, in press.

Kim, F.J., Manel, N., Boublik, Y., Battini, J.L., and Sitbon, M. (2003b).

Human T-cell leukemia virus type 1 envelope-mediated syncytium formation can be activated in resistant Mammalian cell lines by a carboxy-terminal truncation of the envelope cytoplasmic domain. J. Virol. *77*, 963–969.

Kirk, P., Wilson, M.C., Heddle, C., Brown, M.H., Barclay, A.N., and Halestrap, A.P. (2000). CD147 is tightly associated with lactate transporters MCT1 and MCT4 and facilitates their cell surface expression. EMBO J. *19*, 3896–3904.

Korgun, E.T., Demir, R., Sedlmayr, P., Desoye, G., Arikan, G.M., Puerstner, P., Haeusler, M., Dohr, G., Skofitsch, G., and Hahn, T. (2002). Sustained hypoglycemia affects glucose transporter expression of human blood leukocytes. Blood Cells Mol. Dis. *28*, 152–159.

Manel, N., Kinet, S., Battini, J.L., Kim, F.J., Taylor, N., and Sitbon, M. (2003). The HTLV receptor is an early T-cell activation marker whose expression requires de novo protein synthesis. Blood *101*, 1913–1918.

Mantych, G., Devaskar, U., deMello, D., and Devaskar, S. (1991). GLUT 1-glucose transporter protein in adult and fetal mouse lung. Biochem. Biophys. Res. Commun. *180*, 367–373.

Miller, D.G., Edwards, R.H., and Miller, A.D. (1994). Cloning of the cellular receptor for amphotropic murine retroviruses reveals homology to that for gibbon ape leukemia virus. Proc. Natl. Acad. Sci. USA *91*, 78–82.

Mueckler, M. (1994). Facilitative glucose transporters. Eur. J. Biochem. *219*, 713–725.

Mueckler, M., Caruso, C., Baldwin, S.A., Panico, M., Blench, I., Morris, H.R., Allard, W.J., Lienhard, G.E., and Lodish, H.F. (1985). Sequence and structure of a human glucose transporter. Science *229*, 941–945.

Nath, M.D., Ruscetti, F.W., Petrow-Sadowski, C., and Jones, K.S. (2003). Regulation of the cell-surface expression of an HTLV-I binding protein in human T cells during immune activation. Blood *101*, 3085–3092.

Osame, M., Usuku, K., Izumo, S., Ijichi, N., Amitani, H., Igata, A., Matsumoto, M., and Tara, M. (1986). HTLV-I associated myelopathy, a new clinical entity. Lancet *1*, 1031–1032.

Overbaugh, J., Miller, A.D., and Eiden, M.V. (2001). Receptors and entry cofactors for retroviruses include single and multiple transmembrane-spanning proteins as well as newly described glycophosphatidylinositol-anchored and secreted proteins. Microbiol. Mol. Biol. Rev. *65*, 371–389.

Pichard, L., Fabre, I., Fabre, G., Domergue, J., Saint Aubert, B., Mourad, G., and Maurel, P. (1990). Cyclosporin A drug interactions. Screening for inducers and inhibitors of cytochrome P-450 (cyclosporin A oxidase) in primary cultures of human hepatocytes and in liver microsomes. Drug Metab. Dispos. *18*, 595–606.

Pinon, J.D., Klasse, P.J., Jassal, S.R., Welson, S., Weber, J., Brighty, D.W., and Sattentau, Q.J. (2003). Human T-cell leukemia virus type 1 envelope glycoprotein gp46 interacts with cell surface heparan sulfate proteoglycans. J. Virol. *77*, 9922–9930.

Poiesz, B.J., Ruscetti, F.W., Gazdar, A.F., Bunn, P.A., Minna, J.D., and Gallo, R.C. (1980). Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. Proc. Natl. Acad. Sci. USA *77*, 7415–7419.

Rathmell, J.C., Vander Heiden, M.G., Harris, M.H., Frauwirth, K.A., and Thompson, C.B. (2000). In the absence of extrinsic signals, nutrient utilization by lymphocytes is insufficient to maintain either cell size or viability. Mol. Cell *6*, 683–692.

Rodrigues, P., and Heard, J.M. (1999). Modulation of phosphate uptake and amphotropic murine leukemia virus entry by posttranslational modifications of PIT-2. J. Virol. *73*, 3789–3799.

Rosenberg, A.R., Delamarre, L., Preira, A., and Dokhelar, M.C. (1998). Analysis of functional conservation in the surface and transmembrane glycoprotein subunits of human T-cell leukemia virus type 1 (HTLV-1) and HTLV-2. J. Virol. *72*, 7609–7614.

Siegel, G.J., Agranoff, B.W., Wayne Albers, W., Fisher, S.K., and Uhler, M.D. (1998). Circulation and Energy Metabolism of the Brain. Basic Neurochemistry, Chapters 5–31.

Slattery, J.P., Franchini, G., and Gessain, A. (1999). Genomic evolution, patterns of global dissemination, and interspecies transmission of human and simian T-cell leukemia/lymphotropic viruses. Genome Res. *9*, 525–540.

Sutton, R.E., and Littman, D.R. (1996). Broad host range of human T-cell leukemia virus type 1 demonstrated with an improved pseudotyping system. J. Virol. *70*, 7322–7326.

Tailor, C.S., Lavillette, D., Marin, M., and Kabat, D. (2003). Cell surface receptors for gammaretroviruses. Curr. Top. Microbiol. Immunol. *281*, 29–106.

Trejo, S.R., and Ratner, L. (2000). The HTLV receptor is a widely expressed protein. Virology *268*, 41–48.

van Zeijl, M., Johann, S.V., Closs, E., Cunningham, J., Eddy, R., Shows, T.B., and O'Hara, B. (1994). A human amphotropic retrovirus receptor is a second member of the gibbon ape leukemia virus receptor family. Proc. Natl. Acad. Sci. USA *91*, 1168–1172.

Warburg, O. (1956). On the origin of cancer cells. Science *123*, 309–314.

Yoshida, M., Miyoshi, I., and Hinuma, Y. (1982). Isolation and characterization of retrovirus from cell lines of human adult T-cell leukemia and its implication in the disease. Proc. Natl. Acad. Sci. USA *79*, 2031–2035.

Yu, Q., Erman, B., Bhandoola, A., Sharrow, S.O., and Singer, A. (2003). In vitro evidence that cytokine receptor signals are required for differentiation of double positive thymocytes into functionally mature CD8(+) T cells. J. Exp. Med. *197*, 475–487.