The Cellular Receptor for Gibbon Ape Leukemia Virus Is a Novel High Affinity Sodium-dependent Phosphate Transporter

Zoltan Olah*, Csaba Lehel*, Wayne B. Anderson†, Maribeth V. Eiden*, and Carolyn A. Wilson‡

From the §Laboratory of Cellular Oncology, NCI, National Institutes of Health, Bethesda, Maryland 20892 and the \Laboratory of Cell Biology, National Institute of Mental Health, Bethesda, Maryland 20892

The primate type C retrovirus gibbon ape leukemia virus (GaLV) has been shown to use a widely expressed, multiple membrane-spanning protein of unknown function as its cell surface receptor on human cells (GLVR1) (Johann, S. V., Gibbons, J. J., and O’Hara, B. (1992) J. Virol. 66, 1635–1640; O’Hara, B., Johann, S. V., Klinger, H. P., Blair, D. G., Rubinson, H., Dunn, K. J., Sass, P., Vitek, S. M., and Robins, T. (1990) Cell Growth Diff. 1, 119–127). Here we present evidence that the receptor for GaLV (GLVR1) functions as a sodium-dependent transporter of inorganic phosphate. GLVR1 is shown to have approximately 3–4-fold higher affinity for phosphate than other mammalian phosphate transporters described to date. Productive infection of GLVR1-expressing cells by GaLV, but not other retroviruses, results in the complete blockade of GLVR1-specific uptake of inorganic phosphate. Since productive infection of cells with GaLV is generally not cytotoxic, it is likely that more than one phosphate transporter exists on the cell surface. Our data suggest that GLVR1 represents a sodium-dependent phosphate transporter that differs from other mammalian phosphate transporters in structure, affinity for phosphate, and function.

Virus receptors play a critical role in viral infection. At present, the cellular functions for only two retroviral receptors have been identified. The receptor for the lentivirus, human immunodeficiency virus (HIV), has been identified as the CD4 molecule (1–3), and the receptor for the type C ecotropic murine leukemia virus (E-MuLV) has been demonstrated to function as a sodium-independent cell surface transporter of basic amino acids (4–6). The discovery that the CD4 molecule serves as the receptor for HIV has increased the understanding of the role of CD4 in viral-mediated pathology and enabled the development of CD4-based antiviral strategies (7, 8).

As a transporter of basic amino acids, the E-MuLV receptor serves a critical cell function. Paradoxically, E-MuLV infection is not cytotoxic to most cell types, even though productive infection of cells by E-MuLV renders the receptor inaccessible to incoming virus (9). The puzzle of how cells maintain productive infection with E-MuLV in the absence of a cytotoxic effect was solved when it was shown that mink cells that express the E-MuLV receptor have an incomplete blockade of E-MuLV receptor-specific arginine transport after E-MuLV infection. This suggests that the E-MuLV receptor can still transport arginine even though the receptor is not accessible for virus entry (10).

A receptor for a second type C retrovirus, gibbon ape leukemia virus (GaLV), has been cloned and sequenced (11). This receptor (designated GLVR1) is ubiquitously expressed in a wide variety of species. Functional GaLV receptors are present on cells derived from cat, dog, cow, some birds, bat, mink, rabbit, monkey, primate, and most rodents with the notable exception of mice (13). The murine GaLV receptor homologue shares 50% amino acid identity with the functional human form of the receptor (12). It has been shown previously that substitution of as few as 2 amino acids in the murine form of GLVR1 allows for GaLV receptor function (14). These subtle differences in the primary structure of the form of the protein expressed on murine cells account for the resistance of murine cells to GaLV infection.

The cellular function of this widespread receptor has not been determined. It has been reported that GLVR1 shares 31% amino acid identity with a phosphate permease from Neurospora crassa, Pho-4° (12, 15). Furthermore, we noted that GLVR1 also shares 17% amino acid identity with the sodium-dependent inorganic phosphate transporter identified from human kidney cortex (16). Based on these observations, we sought to determine whether GLVR1 may function as a transporter of inorganic phosphate and how cells are able to maintain productive infection with GaLV in the absence of cytotoxic effect. We report here that GLVR1 is a ubiquitously expressed, high affinity phosphate transporter, which presumably functions in maintaining cellular phosphate levels.

MATERIALS AND METHODS

Cells, Retroviral Vector, and Wild Type Virus Infections—NIH 3T3 murine fibroblasts (ATCC CRL 1658) and MDTF (Mus dunni tail fibroblasts) were maintained in Dulbecco’s modified Eagle’s medium supplemented with 5% (v/v) fetal bovine serum.

MDTF cells expressing the human cDNA for the GaLV receptor were infected with wild type GaLV, strain SEATO, as described previously (17). Infection was monitored by measuring the reverse transcriptase activity from the cell media of the infected cells (18). By 2–3 weeks post-infection, the reverse transcriptase activity had reached a peak level, at which time productive GaLV infection was assessed by a superinfection interference assay. GLVR1-expressing MDTF cells were also infected with two different molecular clones of ecotropic murine leukemia virus (E-MuLV), Friend murine leukemia virus (57A) (19), or Moloney murine leukemia virus (MLV-K) (20). Productive infection by E-MuLV was assessed by reverse transcriptase assays, followed by superinfection interference assays.

Superinfection interference assays were performed as described previously (17). Briefly, uninfected or productively infected cells were exposed to retroviral vectors bearing either GaLV envelope glycoproteins (PG13/G1B/S/N) (21, 22) or ecotropic murine leukemia virus envelope.
were carried out for a 2-min time period on control and GLVR1-expressing NIH 3T3 and MDTF cells, as described under "Material and Methods." Values are expressed as the mean ± S.E. of triplicate determinations.

Functional Properties of the GLVR1 Protein—As described above, murine cells are resistant to GaLV infection because the murine form of the GLVR1 protein does not function as a GaLV receptor (12, 14). This property of murine cells provides a selective advantage to leukemia virus-infected cells over uninfected cells. To determine whether GLVR1 can function as a phosphate transporter, two murine fibroblast cell lines, NIH 3T3 and MDTF (M. dunni tail fibroblasts), were infected by a retroviral vector containing the GLVR1 cDNA (17). Expression of the human GLVR1 cDNA in MDTF cells correlated with their acquired susceptibility to retroviruses bearing GaLV envelopes. Both NIH 3T3/ GLVR1 and MDTF/GLVR1 cells were efficiently infected by the GaLV-enveloped retroviral vector, PG13/G1BgsN. A 2-fold increase in phosphate uptake was evident in both the NIH 3T3/GLVR1 and MDTF/GLVR1 cells, as compared to control NIH 3T3 and MDTF cells expressing exclusively murine GLVR1 (Fig. 1). A similar increase in anion transport was not observed when sulfate was substituted for phosphate under identical experimental conditions, suggesting that transport was selective for P, anions.

Several functional parameters of GLVR1-specific P, transport were assessed and compared to the previously reported parameters established for the endogenous P, transport function in NIH 3T3 cells (26) and for the Na/P, transporter in human kidney cortex (16). The effects of different monovalent cations on GLVR1-dependent phosphate uptake were compared by substituting potassium, lithium, NH₄, or choline chloride for sodium chloride in the uptake medium of MDTF and MDTF/GLVR1 cells. As shown in Fig. 2, replacement of sodium chloride with each of these cations reduced phosphate transport dramatically. These results indicate that both the human GLVR1 transporter and the endogenous murine phosphate transporter(s) are sodium-dependent.

The phosphate transporter identified in human kidney cortex has a pH optimum of 8.0 for phosphate uptake (16). Phosphate uptake was measured in uptake media with pH values ranging from 5.5 to 8.5. The endogenous murine transporter and the human GLVR1 transporter appear to exhibit two peaks of phosphate uptake: one occurring at pH 6.5 and another at pH 7.5 (Fig. 3).

The kinetic parameters (Vₘₐₓ and Kₐ) of phosphate uptake were determined for the endogenous transporter(s) expressed in MDTF cells and for the human form of the GLVR1 transporter overexpressed in MDTF cells (Fig. 4). The Vₘₐₓ determined for both the endogenous MDTF transporter and the GLVR1 transporter (2.3 and 1.9 nmol of P/min/mg of protein, respectively) is approximately 10 times higher than that reported previously for phosphate uptake in NIH 3T3 fibroblasts (Vₘₐₓ = 0.25 nmol of P/min/mg of protein) (26). The affinity for P, of the sodium-dependent transporter present in MDTF cells (Kₐ = 133 μM) is in the same range as that reported for the human kidney cortex Na/P, transporter (Kₐ = 170 μM) (16), but higher than that observed with NIH 3T3 cells (Kₐ = 220 μM).
Methods.

GaLV infection blocks GLVR1-specific Pi transport. When cells expressing a functional GaLV receptor are infected with GaLV, their viral receptors are no longer accessible (27). To determine what effect GaLV infection and the concomitant loss of functional GaLV receptors might have on GLVR1-specific Pi transport, MDTF/GLVR1 cells were exposed to GaLV strain SEATO. At 2–3 weeks post-exposure, viral infection assays were performed (as described under “Materials and Methods”) to insure virus had spread throughout the culture. The absence of functional human GLVR1 encoded viral receptors on the surface of infected MDTF/GLVR1 cells was demonstrated by the more than 100-fold diminution in viral-induced foci observed when infected MDTF/GLVR1 cells were exposed to homologous challenge virus (e.g. E-MuLV or GaLV) (data not shown). Infection of MDTF/GLVR1 cells with GaLV resulted in a reduction of Pi uptake to the level of the control MDTF cells (1240 and 1212.5 pmol of Pi/min/mg of protein, respectively), compared to a nearly 2-fold increase observed in uninfected MDTF/GLVR1 cells (2129 pmol of Pi/min/mg of protein) (Fig. 5). As a control for the possibility that productive retroviral infection contributes to a reduction of Pi uptake by a nonspecific mechanism, we productively infected MDTF/GLVR1 cells with retroviruses that use a receptor other than GLVR1 to infect cells. MDTF/GLVR1 cells were infected with two different strains of E-MuLV, Friend murine leukemia virus, and Moloney murine leukemia virus as described under “Materials and Methods.” E-MuLVs have been shown to use a cell surface transporter of cationic amino acids as a viral receptor (5,6) and therefore provide a useful control for the general effects of retroviral infection on Pi transport. In contrast to the results obtained following GaLV infection, infection of MDTF/GLVR1 cells with either Friend or Moloney murine leukemia virus did not result in a marked reduction in the level of Pi uptake relative to that of uninfected MDTF/GLVR1 cells (Fig. 5). This result shows that transport of phosphate via the GLVR1 receptor is completely and specifically blocked in GaLV-infected cells. The observation that cells productively infected by GaLV do not demonstrate any cytopathic effect, despite the fact that phosphate uptake is presumably critical to the viability of the cells, argues for the presence of more than one phosphate transporter in these cell types.

**DISCUSSION**

The receptor for the gibbon ape leukemia virus (GaLV) is an integral membrane protein found on most types of cells. We have now determined that the GaLV receptor functions as a high affinity sodium-dependent phosphate transporter. Murine cells express a form of the GLVR1 protein that, in contrast to the human GLVR1 protein, does not function as a GaLV receptor (12,14). Expression of the human form of this
receptor (GLVR1) in murine cells results in a marked increase in the level of sodium-dependent Pi transport in these cells. This increase correlates with the presence of functional GaLV receptors. The increase in Pi uptake mediated by the human form of GLVR1 is blocked when this form of the receptor is rendered nonfunctional as a consequence of GaLV infection. Infection with either Moloney or Friend murine leukemia virus, retroviruses that do not utilize either human GLYR1 or the murine GLVR1 as viral receptors, does not result in the loss of either GLVR1 function or the concomitant increase in Pi uptake. Therefore, the increase in Pi transport observed in MDTF/GLVR1 requires the presence of functional GaLV receptors.

GLVR1 has several structural features that are found in other transporters/phosphate permeases. A hydropathy plot of the deduced amino acid sequence of GLVR1 indicates 10 membrane-spanning α-helical segments (11). According to determinations of the mean hydrophobicity and hydrophobic moments of each of the membrane-spanning domains, four of the segments (segments 1, 6, 9, and 10) are highly hydrophobic, while six of the segments (2–5, 7, and 8) are more amphipathic in nature (Table I). Since each of these α-helical structures can take on a cylinder-like shape, a model was prepared to indicate the location of the hydrophilic and hydrophobic amino acid residues that make up each of the α-helical segments. As depicted in Fig. 6, the amino acid residues within the six amphipathic transmembrane segments (2–5, 7, and 8) are arrayed such that the hydrophilic residues would be on one side of the cylinder and the hydrophobic residues would be on the other. In this model, with the amphipathic transmembrane segments oriented so that their hydrophobic sides face toward the hydrophobic lipid environment of the membrane, the hydrophilic sides of these six transmembrane segments form a channel or pore to facilitate phosphate uptake. A similar pentagonal pore structure has been hypothesized for members of the glucose transporter family (29).

**Fig. 5. Effect of virus infection on GLVR1-specific phosphate uptake.** Phosphate uptake was measured during a 2-min uptake incubation at 37°C in MDTF cells (MDTF), and GLVR1-expressing MDTF cells (MDTF/GLVR1) (control uninfected cells or cells infected with GaLV (+GaLV), Friend murine leukemia virus (+FrMuLV), or Moloney murine leukemia virus (+MoMuLV)). The values represent the mean ± standard error of triplicate determinations.

**TABLE I**

Calculations of mean hydrophobicity and hydrophobic moments of the transmembrane domains of GLVR1

| Transmembrane domain | Amino acid sequence | Mean hydrophobicity | Hydrophobic moments |
|----------------------|---------------------|---------------------|---------------------|
| 1                    | Leu22–Val69         | 2.72                | 0.26                |
| 2                    | Ala62–Ala87         | 1.61                | 0.14                |
| 3                    | Met105–Leu113       | 1.04                | 1.11                |
| 4                    | Met115–Leu167       | 1.45                | 1.21                |
| 5                    | Val207–Thr215       | 1.43                | 0.76                |
| 6                    | Ile232–Phe303       | 2.70                | 0.33                |
| 7                    | Ser312–Ala330       | 1.52                | 1.24                |
| 8                    | Ala335–Leu376       | 1.71                | 0.78                |
| 9                    | Ser362–Asn368       | 1.22                | 0.33                |
| 10                   | Met369–Met408       | 1.61                | 0.61                |

Another feature shared by GLVR1 and other Na+-dependent transporters is the presence of a proposed Na+ binding domain identified by Deguchi et al. (30). This sequence, Gly-X-X-X-Leu-X-X-Gly-Arg, is present in the kidney-specific Na+/phosphate transporters, the rabbit and human Na+/glucose transporters in the intestine, and the Na+/glutamate and the Na+/proline transporters of Escherichia coli (31). We have identified a sequence spanning amino acid residues 571–581 that conforms to these consensus sequence requirements. Two possibilities that might account for the ability of GaLV to block GLVR1-mediated Pi transport are as follows: 1) GaLV binding could directly block the ability of Pi to interact with GLVR1 due to the physical proximity of the Pi and GaLV binding sites; or 2) GaLV binding could inhibit the ability of Na+ to interact with GLVR1. Since residues 550–558 in the fourth extracellular do-
main have been shown to be critical for GaLV infection (14, 32), the proximity of the putative Na' binding site and the virus binding site may account for the reduction in GLVR1-specific sodium-dependent phosphate transport that accompanies GaLV infection.

Although GLVR1 shares a high degree of homology and a similar topology with Pho-4', the phosphate permease of N. crassa, it is structurally and functionally distinct and exhibits a different distribution pattern than the NaP-1, -2, and -3 kidney-specific phosphate transporters. The NaP-1, -2, and -3 transporters are tissue-restricted in their expression (16, 28), whereas GLVR1 is expressed in all tissues examined (12).

Functional parameters also distinguish GLVR1-specific P; transport from P; transport by the human kidney NaP-3 transporter. First, NaP-3 transport responds dramatically to increasing pH, doubling the level of P; transport as the pH is changed from 7.0 to 8.0. This phenomenon is thought to be due to allosteric regulation by protons of the NaP; cotransport system (31). Both the endogenous Na/P; transporter in MDTF cells and the GLVR1-specific P; transporter exhibit peaks of maximal phosphate uptake at pH 6.5 and 7.5, with a dramatic decrease in P; uptake at pH 8.0 and above. This suggests a different effect of pH on the regulation of GLVR1-specific P; transport compared to the NaP-3 transporter. Second, the affinity for phosphate of the GLVR1 sodium-dependent phosphate transporter is approximately 3–4-fold higher than that of other mammalian phosphate transporters (16, 28), suggesting that the human form of GLVR1 transporter is a high affinity transporter for phosphate. Finally, unlike the kidney transporters, GLVR1 is highly evolutionary conserved and is expressed in a wide variety of tissues and cell lines (11, 12).

The process by which mammalian retroviruses choose their receptors is an opportunistic one. Since productive infection by retroviruses results in loss of available receptor for infection, there are essentially three types of cell surface molecules that are candidates as retroviral receptors, namely (a) molecules whose functional or substrate binding site is different from that of the retrovirus, so that cellular function is not abrogated by infection (e.g. the receptor for ecotropic murine leukemia viruses (10); (b) molecules whose function is a luxury one, so that loss of cell function does not result in cell death and loss of viral host function (for example, CD4, the receptor for HIV), and (c) molecules whose function in the cell is redundant, so that loss of cellular function is not synonymous with cell death. The GaLV receptor appears to be the first example of the third type; our data suggest that cells susceptible to GaLV contain at least one phosphate transporter in addition to the GaLV receptor so that infection with GaLV does not inhibit all phosphate transport. Finally, given the role of inorganic phosphate as an important regulator of cellular metabolism, it will be of interest to better understand how this novel ubiquitous transporter functions in maintaining cellular phosphate homeostasis.

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