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A human amphotropic retrovirus receptor is a second member of the gibbon ape leukemia virus receptor family

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ABSTRACT **Retrovirus infection is initiated by binding of the viral envelope glycoprotein to a cell-surface receptor. The envelope proteins of type C retroviruses of mammals demonstrate similarities in structural organization and protein sequence. These similarities suggest the possibility that retroviruses from different interference groups might use related proteins as receptors, despite the absence of any relationship between retrovirus receptors isolated to date. To investigate this possibility, we have identified a human cDNA clone encoding a protein closely related to the receptor for gibbon ape leukemia virus and have found that it functions as the receptor for the amphotropic group of murine retroviruses. Expression of this protein (GLVR-2) is likely to be a requirement for infection of human cells by amphotropic retroviral vectors for purposes of gene therapy.**

Type C retroviruses found in vertebrates have similar morphology, genomic organization, and also replication pathways. Despite their similarities, independent isolates differ strikingly in their ability to infect potential hosts. This property is a consequence of variation in the viral envelope glycoproteins that results in binding to different receptor proteins. Type C retroviruses have been classified on the basis of their envelope–receptor interactions by superinfection interference assays (1). By using these assays, five distinct groups of murine retroviruses (murine leukemia viruses; MuLVs) and eight distinct groups of retroviruses that can infect human cells in culture have been identified (2, 3). In addition, the existence of distinct receptors for each murine interference group has been supported by chromosome-mapping studies with somatic cell hybrids (4, 5). Indeed, the gene encoding the receptor for ecotropic MuLV is syntenic with *Rec-1*, the genetic locus on mouse chromosome 5 identified by testing mouse–Chinese hamster hybrid cell lines for infection (6).

Murine type C retroviruses have been engineered to serve as vectors that can introduce specified genes into susceptible target cells. These vectors are packaged in virions containing the amphotropic MuLV (A-MuLV) envelope to create viruses that can infect human cells and are, therefore, suitable for gene therapy in patients (7, 8). Previous reports have noted that the envelope of A-MuLV is closely related to the envelope of xenotropic and polytropic MuLVs but defines a distinct interference group (9, 10). In addition, studies of somatic cell hybrids have demonstrated that susceptibility to A-MuLV infection maps to mouse chromosome 8 and can be segregated from susceptibility to infection by the related viruses, which require mouse chromosome 1 (5, 11). Recently, similar studies using human–hamster hybrids identified a locus with the same properties on human chromosome 8 (12). Therefore, the existence of a distinct gene encoding the amphotropic receptor in both mice and humans has been suspected.

Five receptors for retroviruses have been cloned. The gibbon ape leukemia virus (GALV) receptor 1 (GLVR-1) has 10 presumed transmembrane domains (13). Murine cationic amino acid transporter 1 is the receptor for ecotropic MuLV and also contains multiple (14) putative transmembrane domains (14). However, in contrast to these proteins, the subgroup A Rous sarcoma virus receptor has only a single transmembrane domain (15); this receptor is more similar to the cloned receptors for the lentiviruses, which also have a single transmembrane domain. These receptors are CD4, the human immunodeficiency virus receptor (16, 17), and the receptor for bovine leukemia virus (18). Apart from being integral membrane proteins, until now no further similarities have been found among retrovirus receptors. In this study we report the identification of a cDNA from human cells that encodes a protein related to GLVR-1 that maps to human chromosome 8 and confers susceptibility to infection by A-MuLV.¶

MATERIALS AND METHODS

Cells and Retroviral Vectors. NIH 3T3, human 293, and CHO-K1 cells were cultured in Dulbecco's modified Eagle medium/10% calf serum. CHO-K1 cells transfected with expression plasmids for GLVR-2 (CHO-K1-G7) or for the ecotropic MuLV receptor, murine cationic amino acid transporter MCAT (CHO-K1-MCAT1), were made as described (19) and were cultured as CHO-K1 cells. The amphotropic retroviral vectors PAP3 and PA317/LN, which encode β-galactosidase and G418 resistance, respectively, were described elsewhere (7, 21), as was the ecotropic retroviral vector ecoBAG encoding β-galactosidase (20).

Virus Infection. To test the susceptibility of cells for infection by retroviral vectors, 3×10^5 cells per dish were plated in 6-cm dishes 1 day before exposure to virus for 4 hr. Thirty-six hours later, cells exposed to PAP3 or ecoBAG virus were examined for acquired β-galactosidase activity (21). The cells exposed to PA317/LN were refed with medium/G418 for 14 days, at which time G418-resistant colonies were stained with 1% crystal violet and counted.

Cloning and Sequencing. An HL-60 cDNA library (Stratagene HL1020B) in λgt11 was screened with a mixture of two *Eco*RI fragments containing bases 1–2659 of the human GLVR-1 cDNA-containing clone pHGR6-1 (22). Filters were hybridized at 30°C in 50% (vol/vol) formamide/10× Den-

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Abbreviations: GALV, gibbon ape leukemia virus; GLVR, GALV receptor; MuLV, murine leukemia virus; A-MuLV, amphotropic MuLV.

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¶The sequence reported in this paper has been deposited in the GenBank data base (accession no. L20852).

hardt's solution/5× standard saline/citrate (SSC)/0.1% SDS/ autoclaved salmon sperm DNA at 100 μg/ml. The filters were washed at 45°C in 0.2× SSC/0.2% SDS for 20 min. Exposure to film showed ≈3 plaques per 40,000 screened. Washing the filters in the same solution at 65°C resulted in loss of 50% of the plaques. Several plaques evident only after the low-stringency wash were plaque-purified. The inserts from two of these phage (pOJ40A and -B) were subcloned by using *Eco*RI. Partial sequencing of these clones revealed that they represented the same locus and were highly homologous to GLVR-1 nt 1890–2250 (22). However, both clones represented partial cDNAs. To obtain a full-sized clone, a human placental cDNA library (Stratagene 936203) in Lambda ZAP II was screened by using phage pOJ40B. The screening was done at 42°C in the hybridization solution above, and the filters were washed at 65°C in 0.2× SSC/0.2% SDS. Ten hybridizing phage were plaque-purified, and the inserts were excised in pBluescript (SK)$^-$ using helper phage per the manufacturer's instructions. Sequencing was performed by using single-stranded DNA templates and synthetic oligonucleotide primers (23, 24). The clones isolated from the second round of screening represented the same locus as pOJ40B, as determined by sequencing. One clone, pGLVR2-1, contained an entire open reading frame homologous to GLVR-1. An expression plasmid for GLVR-2 was made as follows. pcDNA1-tkpA, constructed by Tom Jones, Lederle Laboratories, was derived from pcDNA1 (Invitrogen). For convenience in manipulation, the ampicillin-resistance gene was first cloned into pcDNA1 by cloning in a blunt 1.1-kb fragment from pBR322 encoding ampicillin resistance into the *Nru* I site of pcDNA1 between *supF* and the cytomegalovirus immediate early promoter. The 1.23-kb *Xba* I–*Acc* I fragment [containing the splice, poly(A) signal, and SP6 promoter] was removed, the vector was filled in with Klenow fragment, and a 180-bp *Bam*HI–*Hae* III fragment (filled in with Klenow fragment) containing the Herpes simplex virus thymidine kinase poly(A) signal (25) was inserted. pcDNA1-tkpA therefore contains the cytomegalovirus promoter, a multiple cloning site, and a poly(A) signal, with no splice signals. To clone GLVR-2 into this plasmid, the *Hin*dIII–*Sac* I fragment of pGLVR2-1 (nt 184–2745, containing the complete open reading frame with 59 untranslated nucleotides upstream of the open reading frame and 543 untranslated nucleotides downstream of the open reading frame) was cloned between the *Hin*dIII and *Eco*RV sites of pcDNA1-tkpA. The clone was designated pOJ74.

Chromosomal Localization. Human–mouse hybrid cell lines were made and were characterized by karyotypic analysis and by mapped enzyme markers (26–28). Southern analysis was done as described (22); hybridization was done at 40°C in 50% formamide/5× Denhardt's solution/5× SSC/ 0.2% SDS/salmon sperm DNA at 100 μg/ml. The probe used was a 1-kb *Bam*HI fragment from a partial GLVR-2 cDNA clone, pOJ40A, containing 3′ translated and untranslated sequence, labeled with the random primer method. The blots were washed twice in 0.2% SSC/0.2% SDS for 15 min at 55°C followed by 10 min at 60°C.

RESULTS

Cloning and Characterization of GLVR-2. In an attempt to identify additional members of a virus-receptor family, we have cloned a cDNA encoding GLVR-2, a protein closely related to GLVR-1. Low-stringency hybridization with a cDNA encoding GLVR-1 (22) was used to isolate related phage clones from human HL-60 and placental cDNA libraries. One clone, pGLVR2-1, contained an open reading frame specifying a 652-amino acid protein that is closely related to

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GLVR-1  MATLITSTTAATAASGPLVDYLWMLILGFIIAFVLAFSVGANDVANSFGTAVGSGVVTLK  60
GLVR-2                MAMDEYLWMVILGFIIAFILAFSVGANDVANSFGTAVGSGVVTLR  45

        QACILASIFETVGSVLLGAKVSETIRKGLIDVEMYNSTQGLLMAGSVSAMFGSAVWQLVA  120
        QACILASIFETTGSVLLGAKVGETIRKGIIDVNLYNETVETLMAGEVSAMVGSAVWQLIA  105

        SFLKLPISGTHCIVGATIGFSLVAKGQEGVKWSELIKIVMSWFVSPLLSGIMSGILFFLV  180
        SFLRLPISGTHCIVGSTIGFSLVAIGTKGVQWMELVKIVASWFISPLLSGFMSGLLFVLI  165

        RAFILHKADPVPNGLRALPVFYACTVGINLFSIMYTGAPLLGFDKLPLWGTILISVGCAV  240
        RIFILKKEDPVPNGLRALPVFYAATIAINVFSIMYTGAPVLGLV-LPMWAIALISFGVAL  224

        FCALIVWFFVCPRMKRKIEREIKCSPSESPLMEKKNSLKEDHEETKLSVGDIENKHPVSE  300
        LFAFFVWLFVCPWMRRKITGKLQ---KEGALSRVSDESLSKVQEAESPV--FKELPGAKA  279

        VGPATVPLQAVVEERTVSFKLGDLEEAPERERLPSVDLKEETSIDSTVNGAVQLPNGNLV  360
        NDDSTIPLTGAAGE-TLGTSEGTSAGSHPRAAYGRA-L---SMTHGSVKSPI--SNGTFG  332

        QFSQAVSNQINSSGHSQYHTVHKDSGLYKELLHKLHLAKVGDCM--GDSGDKPLRRNNSY  418
        -FDGHTRSD----GHV-YHTVHKDSGLYKDLLHKIHIDRGPEEKPAQESNYRLLRRNNSY  386

        TSYTMAICGMPLD-SFRAKEGEQKGEEMEKLTWPNADSKKRIRMDSYTSYCNAV--SDLH  475
        TCYTAAICGLPVHATFRAADSSAPEDSEKLVGDTVSYSKKRLRYDSYSSYCNAVAEAEIE  446

        S-ASEIDMSVKAAMGLGDRKGSNGSLEEWYDQDKPEVSLLFQFLQILTACFGSFAHGGND  534
        AEEGGVEMKLASELADPDQPREDPAEEEKEEKDAPEVHLLFHFLQVLTACFGSFAHGGND  506

        VSNAIGPLVALYLVYDTGDVSSKVATPIWLLLYGGVGICVGLWVWGRRVIQTMGKDLTPI  594
        VSNAIGPLVALWLIYKQGGVTQEAATPVWLLFYGGVGICTGLWVWGRRVIQTMGKDLTPI  566

        TPSSGFSIELASALTVVIASNIGLPISTTHCKVGSVVSVGWLRSKKAVDWRLFRNIFMAW  654
        TPSSGFTIELASAFTVVIASNIGLPVSTTHCKVGSVVAVGWIRSRKAVDWRLFRNIFVAW  626

        FVTVPISGVISAAIMAIFRYVILRM                                     679
        FVTVPVAGLFSAAVMALLMYGILPYV                                    652
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FIG. 1. Comparison of deduced protein sequences of GLVR-1 and GLVR-2. Identical residues are indicated in boldface type. Gaps are indicated by dashes. Proposed transmembrane domains (identified as in ref. 13) are underlined. Numbers of residues at line ends are indicated at right.

Table 1. Segregation of GLVR-2 with human chromosomes in mouse–human hybrids

Chromosome	Concordant hybrids, no.	Discordant hybrids, no.	Discordance, %
1	27	11	34
2	17	19	53
3	21	14	40
4	19	16	46
5	20	16	44
6	20	16	44
7	20	14	41
8	36	0	0
9	18	14	44
10	23	13	36
11	12	19	61
12	25	11	31
13	21	15	42
14	20	15	43
15	24	11	31
16	24	12	33
17	19	14	42
18	21	15	42
19	19	17	47
20	19	17	47
21	16	20	56
22	18	17	49
X	13	18	58

GLVR-1. A comparison of the amino acid sequences of GLVR-1 and GLVR-2 is shown in Fig. 1. GLVR-2 is 62% identical overall to GLVR-1 and shows a similar distribution of hydrophobic and hydrophilic regions. Like GLVR-1, these regions are ordered as an overall hydrophobic region containing multiple potential transmembrane domains [residues 1–235; 71% identity with GLVR-1-(1–251)], a hydrophilic region [residues 236–482; 34% identity with GLVR-1-(252–511)], and a second hydrophobic region again with multiple potential transmembrane domains [residues 483–652; 81% identity with GLVR-1-(512–679)]. GLVR-2 contains a repeated sequence between residues 1–165 and 483–652 that is also present in GLVR-1 (22). Nine of the 10 hydrophobic domains of GLVR-1 predicted to cross the cell membrane (22) are conserved in GLVR-2, but one domain (GLVR-2 residues 483–504) contains several amino acid residues with charged side chains that are not found in the corresponding region of GLVR-1 (residues 512–532).

Chromosomal Localization. To determine which virus GLVR-2 could be a receptor for, we determined its chromosomal location and compared this location with the known chromosomal locations of other retrovirus receptors. The chromosomal locations of five retrovirus receptor genes have been determined in the human genome. Two of these, which have been cloned, are CD4 on chromosome 12 (29, 30) and GLVR-1 on chromosome 2 (31). Three others, which have not been cloned, are the human T-cell leukemia virus types I and II (HTLV-I and -II) receptor on chromosome 17 (32), the RD114 receptor on chromosome 19 (33), and the A-MuLV receptor (*Ram-1*) on chromosome 8 (12). We screened DNA of 36 mouse–human somatic cell hybrids by Southern analysis for the presence of GLVR-2 (26–28). The GLVR-2 probe hybridized to an 8.6-kb *Eco*RI fragment (data not shown). The blots were scored for the presence or absence of this band, the results of which are shown in Table 1. These results show unambiguously that GLVR-2 is located on chromosome 8.

Amphotropic Receptor Function. To test the hypothesis that GLVR-2 can act as a receptor for A-MuLV, the cDNA encoding GLVR-2 was introduced into the nonpermissive Chinese hamster fibroblast cell line CHO-K1 (which lacks a functional receptor for A-MuLV; 34), under transcriptional control of a cytomegalovirus promoter. Three independent clones of CHO-K1 cells that express GLVR-2 acquired susceptibility to infection by PAP3 and PA317/LN, two amphotropic retroviral vectors encoding β-galactosidase and G418 resistance, respectively (7, 21). The results for one of these clones, CHO-K1-G7, are shown in Fig. 2 and Table 2. The levels of infection are comparable to, if not better than, those for the human kidney cell line 293 and to murine NIH 3T3 fibroblasts. The GLVR-2-expressing clones were not infected by the vector bearing the ecotropic retroviral envelope (Table 2). There was no detectable infection of mock-transfected CHO-K1 cells by either virus. The susceptibility to infection by PAP3 was 19.4 × 10^3 β-galactosidase-expressing cells per 10^5 cells and to PA317/LN was 35.0 ×

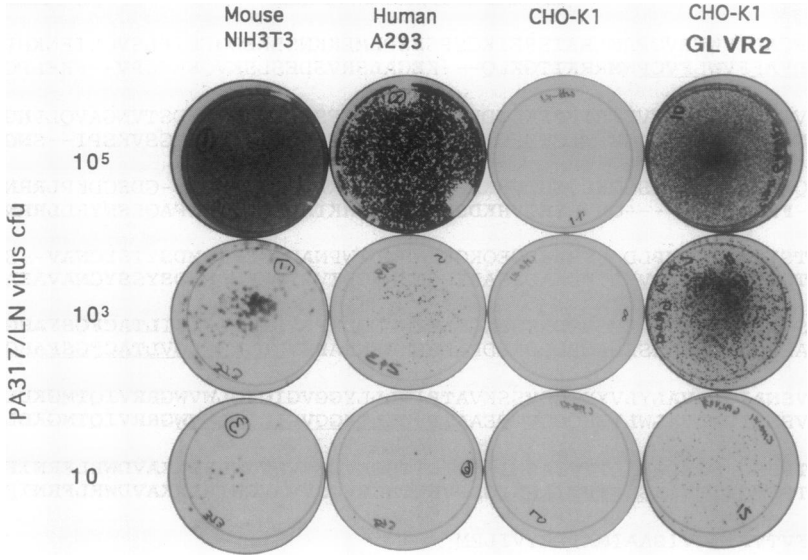

FIG. 2. Infection of amphotropic virus. Cell lines were exposed to serial 100-fold dilutions of PA317/LN virus (7 × 10^5 colony-forming units per ml on mouse NIH 3T3 cells) in medium containing Polybrene (8 μg/ml) for 4 hr. Two days later, cells were refed in medium containing G418 (1 mg/ml), and selection medium was replaced every 3 days. After 14 days surviving cells were stained with 1% crystal violet. CHO-K1 GLVR-2 refers to the CHO-K1-G7 cell line transfected with the expression plasmid encoding GLVR-2.

Table 2. Susceptibility of cell lines to infection by amphotropic and ecotropic retroviral vectors

	β-Galactosidase activity*			
	Amphotropic virus		Ecotropic virus	
Cell line	PAP3	PA317/LN	ecoBAG	None
NIH 3T3	10.5	25.3	11.2	0
CHO-K1-G7	19.4	35.0	0	0
CHO-K1-MCAT1	0	0	2.7	0
CHO-K1	0	0	0	0
Human 293	0.9	3.1	0	0

*Results represent numbers of cells with acquired β-galactosidase activity (from amphotropic PAP3 or ecotropic ecoBAG) or colonies resistant to G418 (from amphotropic PA317/LN) × 10^3 per 10^5 cells exposed to virus.

10^3 neomycin-resistant colonies per 10^5 cells (Table 2). These findings demonstrate that expression of GLVR-2 is sufficient to confer susceptibility to infection by A-MuLV. GLVR-2 does not confer susceptibility to infection by GALV (data not shown).

DISCUSSION

We show here that expression of GLVR-2 in CHO-K1 cells renders these cells susceptible for infection by the amphotropic retrovirus. Previously, we demonstrated that the murine cationic amino acid transporter is a receptor for ecotropic retrovirus in that it binds the envelope surface protein (gp70) of these viruses (35) and is sufficient to confer susceptibility to infection by these viruses when expressed in CHO-K1 cells. The PAP3 and PA317/LN vectors used in this study are derived from ecotropic proviruses that have acquired amphotropic host range after substitution of their envelope glycoprotein gene with the A-MuLV 4070 glycoprotein gene. Therefore, infection of CHO-K1-G7 by PAP3 and PA317/LN, but not by the ecotropic vector ecoBAG, must result from an interaction between the amphotropic envelope protein and GLVR-2. These findings indicate that GLVR-2 allows infection by providing a binding site for the amphotropic envelope surface protein. The fact that GLVR-1 acts as a receptor for viruses in the GALV interference group by relieving a block at the receptor level (36) lends further support to the premise that GLVR-2 is a receptor for A-MuLV.

It was previously demonstrated that human–Chinese hamster hybrid cells carrying human chromosome 8 were susceptible to infection by A-MuLV and that human chromosome 8 therefore carries the A-MuLV receptor locus *Ram-1* (12). We show here that GLVR-2 localizes to chromosome 8 and is, therefore, likely to identify the human analogue of *Ram-1*. The rat amphotropic receptor was recently identified by expression cloning and has an overall identity to the human amphotropic receptor of 92% (39).

As mentioned, GLVR-1 and GLVR-2 are highly homologous in the C-terminal hydrophobic region. However, within this region, a cluster of differences exists at GLVR-1 residues 550–558 and GLVR-2 residues 522–530 (Fig. 1). We have recently found that GLVR-1-(550–558) plays a critical role in infection by GALV and that the corresponding residues in the mouse Glvr-1 homologue are responsible for the resistance of mouse cells to infection by GALV (37). The finding that GLVR-1 and -2 differ substantially in this same region leads to the supposition that GLVR-2-(522–530) will have a similar critical role in infection. Given the overall similarity of the proteins, it may be that simple exchange of these regions between GLVR-1 and -2 would convert a GALV receptor to an amphotropic receptor and vice versa.

Different retroviruses using the same or related proteins as receptors might be expected to show homology in their envelope glycoproteins as a result of similar needs in receptor recognition. Such homology might be evident not only in the relatively conserved regions of the glycoproteins but also in the first variable region, which at least in mammalian C-type viruses is thought to be the main determinant of host range (38). GALV and feline leukemia virus B use GLVR-1 as a receptor (36) but show little homology in their envelope glycoproteins. However, feline leukemia virus B and amphotropic virus, which use GLVR-1 and -2 as receptors, respectively, are very similar, particularly in the length and sequence of their variable regions (38). Thus, at least in one instance, there is homology between envelope glycoproteins of viruses using related receptors. These data, which represent an example of separate retroviruses using related proteins as receptors, raise the possibility that a similar situation might exist for other retroviruses. The noteworthy similarities between feline leukemia virus B, amphotropic, mink cell focus-forming (MCF), and xenotropic viruses (38) suggests that MCF and xenotropic viruses use GLVR-like proteins as receptors.

Human gene therapy, to date, has relied almost exclusively on amphotropic retroviral vectors. One limitation to their use has been difficulty in infecting some potential target cells, such as self-renewing hematopoietic progenitor cells. The identification of GLVR-2 should permit examination of the role of receptor expression in determining cell-type specificity of amphotropic viruses.

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