Comparison of cDNAs encoding the gibbon ape leukaemia virus receptor from susceptible and non-susceptible murine cells

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The gibbon ape leukaemia virus (GaLV) family of type C retroviruses consists of five closely related viral isolates, GaLV SF, GaLV SEATO, GaLV Br, GaLV H and simian sarcoma-associated virus. The cDNA encoding the human receptor for GaLV SEATO had previously been isolated. We now demonstrate that all of the above GaLVs can use the human form of the GaLV receptor to infect cells. All murine cells analysed to date have been found to be resistant to infection by GaLVs owing to the absence of a functional GaLV receptor. We have now identified a murine cell line which is unique in its susceptibility to GaLV infection. This cell line was established from a Japanese feral mouse, Mus molossinus. We cloned and sequenced the cDNA for the receptor expressed in these cells and compared it to the cDNA for the GaLV receptor expressed in resistant murine cells such as NIH 3T3 (derived from M. m. musculus) and MDTF (derived from M. dunni tail fibroblasts). The crucial region for GaLV infection (the fourth extracellular domain) from the functional M. m. molossinus GaLV receptor is quite divergent from the same region of the M. m. musculus and M. dunni proteins, but similar to that of the functional human GaLV receptor. These results confirm the importance of the amino acids of this region in GaLV receptor function.

Introduction

The members of the gibbon ape leukaemia virus (GaLV) family of primate type C retroviruses have been grouped together on the basis of nucleic acid hybridization studies and immunological cross-reactivity (Benveniste & Todaro, 1973; Rangan, 1974; Thiel et al., 1978). Members of the GaLV family have been isolated from primates in a variety of different disease states. The first isolate of GaLV, known as GaLV SF, was obtained from a gibbon ape with lymphocytic leukaemia in a colony in San Francisco (Kawakami et al., 1972; Snyder et al., 1973). To date, other members include a virus isolated from a gibbon in the SEATO Laboratory gibbon ape colony in Thailand where five spontaneous cases of granulocytic leukaemia had been reported (DePaoli et al., 1973; Kawakami & Buckley, 1974). The GaLV SEATO virus is the only GaLV that has been demonstrated to be a causative agent of leukaemia in gibbons. Kawakami and co-workers were able to induce chronic granulocytic leukaemia in 8 to 9 month old gibbon apes after the injection of concentrated GaLV SEATO virus (Kawakami & Holmberg, 1980). A third GaLV was obtained from a gibbon ape with B cell leukaemia housed in a colony on Hall's Island off the coast of Bermuda and is designated GaLV H (Gallo et al., 1978; Reitz et al., 1979). Brain tissue from three tumour-free gibbon apes was the source of the GaLV Br isolates. Interestingly, two of these gibbon apes had been injected with brain extracts from kuru patients (Todaro et al., 1975). The last member of the GaLV family, simian sarcoma-associated virus (SSAV) was isolated from a woolly monkey with multiple fibrosarcomas; this woolly monkey had been housed with a gibbon ape (Theilen et al., 1971). Although clearly related to each other on structural and genetic bases, it has not been determined whether all GaLVs use the same receptor to infect cells. Furthermore, the xenotropic murine leukaemia virus (X-MuLV) isolated from the Asian feral mouse Mus caroli interferes with superinfection by GaLV SEATO and SSV in mink cells (Lieber et al., 1975). This finding suggests that some X-MuLVs and GaLVs may use a common receptor. X-MuLVs constitute a class of MuLVs in which not all members have the same host range and interference properties, indicating that specific X-MuLVs may use different receptors to infect cells.

The human receptor for GaLV SEATO has recently been identified to be a protein with multiple membrane-spanning domains (O'Hara et al., 1990). Murine NIH
3T3 cells, like other murine cell lines such as SC-1 and MDTF, are resistant to GaLV infection (Egillis et al., 1993). NIH 3T3 cells express a non-functional form of the GaLV receptor (Johann et al., 1992). Transfer of human DNA encoding the GaLV receptor into NIH 3T3 fibroblasts renders them sensitive to infection by GaLV SEATO (O’Hara et al., 1990). Mutagenesis studies of the human and murine forms of the GaLV receptor have allowed identification of a region in the fourth extracellular domain which is critical for susceptibility to the virus (Johann et al., 1993; Tailor et al., 1993).

In this study we used NIH 3T3 cells containing human DNA encoding the GaLV SEATO receptor to determine whether all members of the GaLV family of viruses can use this receptor to infect cells. In addition, we identified a murine cell line which differs from other murine cells in its susceptibility to GaLV SEATO infection. We have cloned and sequenced the GaLV receptor cDNA from this GaLV-susceptible murine cell line and compared its sequence to other murine-derived cell cDNAs encoding non-functional GaLV receptors.

**Methods**

**Cell lines and viruses.** The following cell lines were used in this study: Rat-2 embryo fibroblasts (ATCC CRL 1764); Tb/Lu, bat lung fibroblasts (ATCC CCL 68); HOS, human osteosarcoma cells (ATCC CRL 1543); Mv-1-Lu, mink lung fibroblasts (ATCC CCL 64); NIH 3T3 murine fibroblasts (ATCC CRL 1658); NIH 3T3/GRT-5; NIH 3T3 cells expressing the human GaLV receptor (O’Hara et al., 1990); MDTF, M. dunnii tail fibroblasts (provided by Dr Janet Hartley, National Institute of Allergy and Infectious Diseases, Bethesda, Md., U.S.A.); MMK, M. musculus molossinus kidney cells (ATCC CRL 6439); PA317 (ATCC CRL 9078); CRIP/BAG and CRE/BAG [provided by Dr C. Cepko, Harvard Medical School, Boston, Mass., U.S.A. (Danos & Mulligan, 1988; Price et al., 1987)]; and PG13/BAG (ATCC CRL 10683; Miller et al., 1991). All cell lines were maintained in Dulbecco’s modified Eagle’s medium (DMEM, Whittaker Bio-products) supplemented with 5% fetal bovine serum (FBS), 100 μg/ml of penicillin, 100 μg/ml of streptomycin and 40 mM-glutamine.

GaLV wild-type viruses were obtained from the following productively infected cell lines: SEATO-88, GaLV SEATO-infected bat lung fibroblasts; GaLV-4-88, GaLV-Br-infected bat lung fibroblasts; 71-AP-1, SAV-infected marmoset fibroblasts; UCD-144, GaLV SF-infected primate T cells; 6G1-PB, GaLV-H-infected lymphocytes. Xenotropic MuLV Cas No. 1 (Cloyd et al., 1979, 1985), X-MuLV(C), viral supernatant and BALB/c-Ju #1 xenotropic MuLV (Cloyd et al., 1979), X-MuLV(B)-infected mink lung fibroblasts were obtained from Dr Christine Kozak (National Institute of Allergy and Infectious Diseases, Bethesda, Md., U.S.A.).

**Virus pseudotypes and GaLV vector infections.** PA317 cells producing MoMuLV-based packageable genomes [either the pLNSX genome (Miller & Rosman, 1989) or the BAG genome (Price et al., 1987)] were used to infect either GaLV or X-MuLV producer cells to generate GaLV and X-MuLV pseudovirions, respectively. GaLV retroviral vectors containing the GaLV SEATO envelope, Moloney (Mo) MuLV core components and MoMuLV-based genomes were produced from either PG13 cells containing the BAG genome (Wilson & Eiden, 1991) or from ES/10-GAS cells (Wilson, 1989) expressing the F-EVX genome (Wilson et al., 1989).

Viral titres were determined as previously described (Wilson & Eiden, 1991). Briefly, 3 × 10⁷ cells were exposed to filtered cell supernatant containing viral pseudotypes and adjusted to 3 to 6 μg/ml of polybrene (Abbott Laboratories). Titres of GaLV-resistant colonies were determined by reseeding the cells 24 h after exposure to virus at various densities in 10 cm dishes. The next day the medium was changed to G418-containing DMEM in the following concentrations: 300 μg/ml G418 (active component) for HOS cells, 400 μg/ml G418 for bat lung fibroblasts, 450 μg/ml G418 for Rat-2 fibroblasts, and 800 μg/ml G418 for mink lung fibroblasts. After 10 to 14 days in selective medium, G418-resistant colonies were fixed, stained and counted. Titres were calculated to represent the number of cells/ml of cell supernatant. Assays for cells expressing β-galactosidase were performed 48 to 72 h after exposure to viral pseudotypes as described (Sanes et al., 1986). Foci of blue-staining cells were visualized with a microscope and counted. The number of blue foci per ml of cell supernatant was calculated.

**Cloning and sequencing of GaLV receptor cDNA homologues.** A PCR-based strategy, similar to that previously described, was used to clone regions of the cDNA for the GaLV receptor homologue (Eiden et al., 1993). Polyadenylated RNA was isolated from either MMK or MDTF cells (Okayama et al., 1987). Random hexanucleotides were used to prime cDNA synthesis from mRNA using avian myeloblastosis virus reverse transcriptase (Promega). Using synthetic oligonucleotide primers corresponding to the nucleotide sequence of the human GaLV receptor (O’Hara et al., 1990) regions of the receptor cDNA were selectively amplified from MMK cells by PCR. Four overlapping segments were generated: the 5’ segment was with primers GR1 (nt 370 to 396) and GR5 (nt 1091 to 1071), the second fragment with primers GR10 (nt 1008 to 1041) and GR11 (nt 1720 to 1691), the third with GR13 (nt 1538 to 1570) and hGR4A (nt 2383 to 2336) and the 3’ fragment with primers GR3 (nt 1734 to 1745) and GR14 (nt 2414 to 2386). One region of the MDTF GaLV receptor cDNA was amplified by using synthetic oligonucleotide primers based on the M. m. musculus nucleotide sequence: muGR15 (nt 1698 to 1732) and muGR4 (nt 2478 to 2453). The PCR-amplified products were ligated into the TA cloning vector (Invitrogen) and sequenced by direct dideoxynucleotide sequencing (Sanger et al., 1977; Mierendorf & Pfeffer, 1987). Several PCR clones were sequenced to ensure that changes were authentic and not PCR-induced. Comparison and alignment of the deduced amino acid sequences of different GaLV receptor cDNAs was performed using Geneworks software (Intelligenetics) which scores mismatches and gaps rather than gaps as distances.
Table 1. Receptor interference patterns of SSAV and four GaLV isolates

<table>
<thead>
<tr>
<th>Pseudotype of superinfecting virus*</th>
<th>Ratio of virus titre on infected/uninfected cell types†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mink/4070A</td>
</tr>
<tr>
<td>A-MuLV</td>
<td>0.004</td>
</tr>
<tr>
<td>X-MuLV(B)</td>
<td>ND</td>
</tr>
<tr>
<td>X-MuLV(C)</td>
<td>2.3</td>
</tr>
<tr>
<td>GaLV SEATO</td>
<td>2.5</td>
</tr>
<tr>
<td>GaLV SF</td>
<td>ND</td>
</tr>
<tr>
<td>GaLV Br</td>
<td>ND</td>
</tr>
<tr>
<td>GaLV H</td>
<td>ND</td>
</tr>
<tr>
<td>SSAV</td>
<td>2.5</td>
</tr>
</tbody>
</table>

* Viral pseudotypes were generated and titrated as described. All of the pseudotyped viruses contain a MoMLV-based recombinant genome.
† Values represent ratios of the pseudovirion titre on infected cells to the pseudovirion titre on uninfected cells. Values shown in bold indicate cross-interference.
ND, Not determined.

Table 2. Susceptibility of NIH 3T3/GRT-5 cells to infection by members of GaLV family

<table>
<thead>
<tr>
<th>Virus pseudotype</th>
<th>Rat-2</th>
<th>NIH 3T3</th>
<th>NIH 3T3/GRT-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>MoMLV</td>
<td>++++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>GaLV SEATO</td>
<td>++++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>GaLV SF</td>
<td>++++</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>GaLV Br</td>
<td>++++</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>GaLV H</td>
<td>++++</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>SSAV</td>
<td>++++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>X-MuLV(B)</td>
<td>++++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>X-MuLV(C)</td>
<td>++++</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

* Titres were determined by counting the number of blue foci 48 to 72 h after exposing 3 x 10⁴ target cells to 1.0 ml of filtered infected cell supernatant such that +++, +++, +++, +++, 10 to 10² blue foci/ml; +++, 1 to 10² blue foci/ml; +, 1 to 10 blue foci/ml; –, < 1 blue focus/ml.

Table 3. Susceptibility of cell lines from two feral Asian mice to GALV infection

<table>
<thead>
<tr>
<th>Virus pseudotype</th>
<th>Rat-2</th>
<th>NIH 3T3</th>
<th>MDTF</th>
<th>MMK</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-MuLV</td>
<td>1.0</td>
<td>1.0</td>
<td>1.18</td>
<td>0.97</td>
</tr>
<tr>
<td>GaLV SEATO</td>
<td>1.0</td>
<td>0</td>
<td>0.12</td>
<td>0.16</td>
</tr>
<tr>
<td>X-MuLV(B)</td>
<td>1.0</td>
<td>0</td>
<td>0.34</td>
<td>0.04</td>
</tr>
<tr>
<td>X-MuLV(C)</td>
<td>1.0</td>
<td>0</td>
<td>0.34</td>
<td>0.04</td>
</tr>
</tbody>
</table>

* Cells exposed to virus pseudotypes were assessed for viral infection by histochemical staining for β-galactosidase activity. Titres were determined by counting the number of blue-staining plaques and then normalized to the titres observed on Rat-2 fibroblasts exposed to the same viral pseudotype.

Fig. 1. Viral interference properties on M. m. molossinus kidney (MMK) cells. (a) MMK cells exposed to GaLV SEATO/BAG pseudotypes. (b) MMK cells productively infected with GaLV SEATO wild-type virus and then exposed to GaLV SEATO/BAG pseudotypes. (c) MMK cells exposed to X-MuLV(C)/BAG pseudotypes. (d) MMK cells productively infected with GaLV SEATO wild-type virus and then exposed to X-MuLV(C)/BAG pseudotypes. Forty-eight to 72 h post-exposure to pseudotype virus, all cells were fixed and histochemically stained for the presence of β-galactosidase. Bar marker in (d) represents 250 μm.

Lake Casitas feral mouse in California. The Lake Casitas mice have been shown to be closely related to certain strains of Asian mice (Inaguma et al., 1991). Mink cells productively infected with A-MuLV were resistant to superinfection by A-MuLV pseudotypes, but not to either of the X-MuLVs or any of the GaLV pseudotypes (Table 1). Mink cells infected with either of the X-MuLVs were resistant to infection by both of the X-
MuLVs but A-MuLV and all of the GaLV pseudotypes were able to infect these cells. GaLV SEATO-infected mink, human, bat or rat cells were resistant to super-infection by all of the GaLV pseudotypes but were susceptible to infection by either A-MuLV or the X-MuLVs (Table 1). These results demonstrate that all of the GaLVs belong to the same interference group and that this group is separate from that including A-MuLV or any of the X-MuLVs tested in this assay.

Classification of viruses into the same interference group suggests that these viruses use a common receptor in a particular cell. To demonstrate that all members of the GaLV family of viruses could use the human GaLV SEATO receptor to infect cells, we infected NIH 3T3/GRT-5 cells with the GaLV pseudovirions. NIH 3T3/GRT-5 cells are NIH 3T3 cells which express the human form of the receptor. As shown in Table 2, we exposed Rat-2 fibroblasts, NIH 3T3 cells and NIH 3T3/GRT-5 cells to either MoMuLV, GaLV, or X-MuLV pseudovirions. As expected, MoMuLV pseudovirions, derived from an ecotropic murine leukaemia virus, were able to infect all three cell types. X-MuLV pseudovirions were unable to infect either NIH 3T3 or NIH 3T3/GRT-5 cells. None of the GaLV pseudovirions were able to infect NIH 3T3 cells, whereas all of the GaLVs infected NIH 3T3/GRT-5 cells and control Rat-2 fibroblasts. Therefore, all of the GaLV isolates were able to use the human receptor for cell entry.
Analysis of the susceptibility of feral murine cell lines to GaLV infection

We next examined murine cell lines derived from two Asian feral mice for their susceptibility to infection by GaLV SEATO, A-MuLV or X-MuLV pseudovirions. As shown in Table 3, MDTF cells, a tail fibroblast cell line derived from an Indian feral mouse, M. dunni, like NIH 3T3 cells, are resistant to infection by GaLV SEATO but are susceptible to infection by X-MuLV(B) and X-MuLV(C) as well as A-MuLV pseudovirions. Thus MDTF cells serve as a host cell for A-MuLV and X-MuLV but not GaLV infection. Further, we have previously shown that the block to GaLV infection in MDTF cells is receptor-mediated (Eglitis et al., 1993).

MMK cells, a kidney cell line derived from a Japanese feral mouse, M. m. molossinus, are susceptible to infection by GaLV SEATO as well as X-MuLV(B), X-MuLV(C) and A-MuLV pseudovirions (Table 3). Efficient infection by a GaLV makes MMK cells unusual, since other murine cells are resistant to GaLV infection. We hypothesized that a novel form of the GaLV receptor may be used in MMK cells by both GaLV and X-MuLVs. To test this hypothesis, we exposed MMK cells productively infected with GaLV SEATO to either GaLV SEATO or X-MuLV(C) pseudovirions (Fig. 1). The ratio of the GaLV SEATO pseudovirion titre on GaLV-infected cells to the titre on uninfected MMK cells was 0.262, demonstrating that infection by GaLV but not X-MuLV(C) pseudovirions was inhibited in GaLV SEATO-infected MMK cells. Therefore, these viruses use distinct receptors to enter MMK cells.

Cloning of the cDNAs for the GaLV receptor from MMK cells and MDTF cells; comparison with M. m. musculus-derived GaLV receptor homologue

A PCR-based strategy was used to amplify, clone and sequence the entire cDNA for the GaLV receptor from MMK cells. The deduced amino acid sequence of the MMK GaLV receptor was compared to that of the GaLV receptor homologue previously isolated from the thymus of M. m. musculus (Johann et al., 1992) (Fig. 2). Cells derived from M. m. musculus are resistant to GaLV infection, hence this murine receptor homologue is non-functional. The MMK GaLV receptor shares an overall 88% amino acid identity with the M. m. musculus-derived GaLV receptor. Differences in amino acid residues are found throughout the protein, however several regions are quite divergent between these two receptors. Most of the differences are found in the putative cytoplasmic domains; the N and C termini of the protein contain several amino acid substitutions, the second cytoplasmic loop contains seven amino acid substitutions out of the 23 amino acids that make this loop and several clusters of amino acid substitutions are found in the third cytoplasmic loop. In this region, the MMK GaLV receptor shares only 22% amino acid identity with the M. m. musculus GaLV receptor.

Comparison of the putative extracellular domains of these two receptors reveals a region encompassing amino acid residues 553 to 562 (based on the M. m. musculus numbering) in which the M. m. musculus receptor has eight amino acid residues out of 11 which differ from the M. m. molossinus GaLV receptor sequence. This region of the human GaLV receptor has been demonstrated to be critical for GaLV infection (Johann et al., 1993; Tailor et al., 1993). In an effort to define the residues in this region that may be implicated in the functional differences between these two receptors, we isolated and sequenced this portion of the GaLV receptor cDNA from a second feral mouse cell line, MDTF, which unlike MMK cells is resistant to GaLV infection. Comparison of this region of the MDTF receptor homologue to the MMK receptor reveals several amino acid residues which are conserved: Asp-553, Ser-555, Lys-557 and Ala-559 (Fig. 3). Conservation of these residues between the MMK receptor which functions as a GaLV receptor and the MDTF receptor which does not serve as a GaLV receptor would suggest that these residues do not contribute directly to the function of this protein as a GaLV receptor.

Discussion

We have demonstrated that five different isolates of GaLV (GaLV SEATO, GaLV SF, GaLV H, GaLV Br and SAV) can use the same receptor to infect cells. Superinfection interference assays demonstrated that in human, mink, bat and rat cells, all members of the GaLV family cross-interfere. Furthermore, all five GaLVs could...
The N-terminal 88 to 200 amino acids of various MuLV infect NIH 3T3 cells expressing the human form of the GaLV receptor. Takeuchi et al. have demonstrated that feline leukaemia virus subgroup B (FeLV-B) can also use this receptor for entry into cells (Takeuchi et al., 1992). The N-terminal 88 to 200 amino acids of various MuLV surface (SU) envelope proteins have been shown to affect host range and receptor interference properties (Battini et al., 1992; Morgan et al., 1993; Ott & Rein, 1992). Similarly, the N-terminal 87 to 92 amino acids of the FeLV-A and -C SU proteins were shown to influence host range (Brojatsch et al., 1992). Comparison of the amino acid sequences of the SU envelope glycoproteins from the various GaLV isolates and FeLV-B will reveal regions of conserved sequences which might be important in receptor binding.

Some xenotropic MuLVs from Asian feral mice have been reported to interfere with GaLV superinfection and/or to be antigenically related to GaLV or SSAV (Lieber et al., 1975; Benveniste & Todaro, 1973). We examined two xenotropic MuLVs, one derived from an inbred mouse [X-MuLV(B)] and one derived from a feral mouse in California [X-MuLV(C)] for receptor interference with GaLV infection. X-MuLV(C) was derived from mice which have been shown to be genetically similar to M. m. molossinus (Gardner et al., 1991; Inaguma et al., 1991). Neither of these xenotropic MuLVs appear to use the GaLV receptor to infect human, bat, rat, mink or M. m. molossinus cells. The murine homologue of the GaLV receptor maps to mouse chromosome 2 (Adamson et al., 1991) whereas susceptibility to X-MuLV infection maps to chromosome 1 of wild mice (Kozak, 1985), suggesting that these viruses use distinct receptors. However, the GaLV-related Asian xenotropic MuLVs isolated from M. cervicolor and M. caroli may use a different receptor than the X-MuLVs tested here. An ecotropic murine leukaemia virus isolated from wild mice (Kozak, 1985), suggesting that these viruses use distinct receptors. However, the GaLV-related Asian xenotropic MuLVs isolated from M. cervicolor and M. caroli may use a different receptor than the X-MuLVs tested here. An ecotropic murine leukaemia virus isolated from M. cervicolor, which does not seem to be GaLV-related on the basis of serological cross-reactivity and nucleic acid hybridization, has been shown to use a receptor which maps to mouse chromosome 2 (named the Rec-2 receptor) (Rapp & Marshall, 1980) and may in fact be the GaLV receptor homologue.

X-MuLVs and GaLVs have for the most part an identical host range. Most murine cells are resistant to infection by both types of viruses whereas a broad variety of mammalian cells are infected by these viruses, notably human, bovine, buffalo and certain hamster cells (Cloyd et al., 1985; Weiss & Long, 1977; Teich et al., 1975; C. A. Wilson, unpublished data). The tail fibroblast cell line derived from the Indian feral mouse M. dunni provides an example of a cell susceptible to X-MuLV infection but resistant to GaLV infection. The MMK cell line is the only murine cell known to be susceptible to efficient infection by both GaLV and X-MuLV. M. m. molossinus is thought to have arisen from a cross between Northern Chinese M. m. musculus and the Southeastern Asian mouse, M. m. castaneus (Inaguma et al., 1991; Yonekawa et al., 1986). M. m. molossinus have been shown by analysis of Fv-4 loci to be closely related to the California wild mice from Lake Casitas and genetically distinct from other feral mouse populations which are found in Europe (M. m. musculus) and America (M. m. domesticus) (Gardner et al., 1991).

The amino acid residues which compose the putative fourth extracellular loop have been shown to be critical for GaLV, SSAV and FeLV-B infection (Johann et al., 1993; Tailor et al., 1993). Analysis of mutagenesis of this domain had suggested certain requirements for infectivity. For example, Tailor and co-workers demonstrated that the human receptor functions as a GaLV receptor with either glutamic acid or aspartic acid in amino acid position 550. Substitution of a lysine at amino acid residue 550 produced a receptor incapable of mediating GaLV infection (Tailor et al., 1993). These results might suggest that a negatively charged residue is required in this position. However, the M. m. molossinus receptor has a neutral amino acid, an isoleucine, in this position. Substitution of the amino acid residues found in this domain of the GaLV receptor from M. dunni for the corresponding amino acid residues of the human GaLV receptor results in a loss of GaLV receptor function (Johann et al., 1993), suggesting that the fourth extracellular domain of the M. dunni receptor contributes to loss of GaLV receptor function in the MDTF form.

We sequenced the cDNA derived from RNA encoding the GaLV receptor isolated from the murine cell line MMK (from the Asian feral mouse M. m. molossinus) which is susceptible to infection by GaLV. Comparison of the putative fourth extracellular domain of this MMK-derived murine receptor cDNA to that of the functional human GaLV receptor showed a striking degree of conservation of this domain. Comparison of this MMK GaLV receptor cDNA with other homologous cDNAs isolated from GaLV-resistant murine cells (such as MDTF and NIH 3T3) has shown that this region is quite divergent among the functional and non-functional forms of the murine GaLV receptors. However, the fourth extracellular region of two non-functional murine GaLV receptors (those of M. dunni and M. m. musculus) are highly related to each other. Other amino acid residues which differ between functional and non-functional forms of the murine GaLV receptors are found in transmembrane and cytoplasmic regions. These regions may mediate the post-binding steps of GaLV infection (i.e. internalization).

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References


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