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Chimeras of Receptors for Gibbon Ape Leukemia Virus/Feline Leukemia Virus B and Amphotropic Murine Leukemia Virus Reveal Different Modes of Receptor Recognition by Retrovirus

LENE PEDERSEN,[1] STEPHEN V. JOHANN,[1] MARJA VAN ZEIJL,[1] FINN SKOU PEDERSEN,[2] AND BRYAN O'HARA[1]*

Lederle Laboratories, American Cyanamid Company, Pearl River, New York,[1] and Department of Molecular Biology, University of Aarhus, Aarhus, Denmark[2]

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***Glvr1* encodes the human receptor for gibbon ape leukemia virus (GALV) and feline leukemia virus subgroup B (FeLV-B), while the related gene *Glvr2* encodes the human receptor for amphotropic murine leukemia viruses (A-MLVs). The two proteins are 62% identical in their amino acid sequences and are predicted to have 10 transmembrane domains and five extracellular loops. A stretch of nine amino acids (region A) in the predicted fourth extracellular loop was previously shown to be critical for the function of Glvr1 as receptor for GALV and FeLV-B. Glvr1 and -2 show clusters of amino acid differences in several of their predicted extracellular loops, with the highest degree of divergence in region A. Chimeras were made between the two genes to further investigate the role of Glvr1 region A in defining receptor specificity for GALV and FeLV-B and to map which regions of Glvr2 control receptor specificity for A-MLVs. Region A from Glvr1 was sufficient to confer receptor specificity for GALV upon Glvr2, with the same chimera failing to act as a receptor for FeLV-B. However, introduction of additional N- or C-terminal Glvr1-encoding sequences in addition to Glvr1 region A-encoding sequences resulted in functional FeLV-B receptors. Therefore, FeLV-B is dependent on Glvr1 sequences outside region A for infectivity. The receptor specificity of Glvr2 for A-MLV could not be mapped to a single critical region; rather, N-terminal as well as C-terminal Glvr2-encoding sequences could confer specificity for A-MLV infection upon Glvr1. Surprisingly, though GALV/FeLV-B and A-MLV belong to different interference groups, some chimeras functioned as receptors for all three viruses.**

Retroviral infection of a cell is dependent on the presence of a specific receptor on the cell surface. The human gene *Glvr1* encodes the receptor for gibbon ape leukemia virus (GALV), simian sarcoma-associated virus, and feline leukemia virus subgroup B (FeLV-B) (12, 16). The related human gene *Glvr2* (18) and the rat homolog of *Glvr2*, *Ram-1* (11), encode the receptor for amphotropic murine leukemia virus (A-MLV), which belongs to an interference group distinct from that to which GALV and FeLV-B belong (14). Recently, Kavanaugh et al. showed that both Glvr1 and Glvr2 are sodium/phosphate symporters (6). A hydropathy plot of Glvr1 predicts 10 transmembrane domains and five extracellular loops (Fig. 1) (4). Glvr1 and -2 show 62% amino acid identity, with the highest degree of divergence clustered in the predicted intra- and extracellular loops, and are therefore presumed to have very similar topologies (Fig. 1) (18). It must be anticipated that some of the differences between the two proteins define the specificity of the receptors for viruses from the GALV group or for A-MLV.

Sequence comparison between Glvr1 and homologous Glvr1 proteins from noninfectible species has provided insight into which regions of the protein are critical for its function as a receptor (5, 15, 19). For example, the mouse homolog of Glvr1 shows 90% identity to human Glvr1 at the amino acid level (65 amino acid differences) (4); however, cells expressing the mouse homolog are not infectible with GALV or FeLV-B (5, 15). In previous work involving hybrids between Glvr1 and the mouse homolog, it was shown that a cluster of only 7 of the 65 amino acid differences between the two proteins were critical in defining receptor function (5, 15). These seven amino acids are confined to a stretch of nine amino acids, region A (Glvr1 position 550 through 558), in the predicted fourth extracellular loop (Fig. 1) (5). However, the highly homologous human and murine proteins show only two additional amino acid differences in the remaining predicted extracellular loops (4). It is therefore possible that other sequences which are also critical for receptor function, but which could not be identified by using the hybrids between Glvr1 and the mouse homolog because of their presence in both proteins, exist. Glvr1 and -2 show 21% amino acid divergence in their predicted extracellular loops (18); however, as mentioned, they are predicted to have very similar topological maps. Hybrids between Glvr1 and -2 therefore offer the possibility of studying a large number of alterations with minimal danger of disturbing the overall topology of the receptors. We have therefore made hybrids between Glvr1 and -2, in order to extend our analysis of what is important for the receptor function of Glvr1 for GALV and FeLV-B and in order to analyze what is important for the function of Glvr2 as a receptor for A-MLV. Our main focus was on the role of region A in the fourth extracellular loop, because this region is critical for the function of Glvr1 as a receptor for GALV and FeLV-B and because it is highly divergent between Glvr1 and -2.

When region A of Glvr1 was introduced into Glvr2, the chimera acted as a receptor for GALV but not for FeLV-B.

* Corresponding author. Mailing address: Lederle Laboratories, American Cyanamid Company, MRD 205/209, 401 N. Middletown Rd., Pearl River, NY 10965. Phone: (914) 732-4576. Fax: (914) 732-2480.

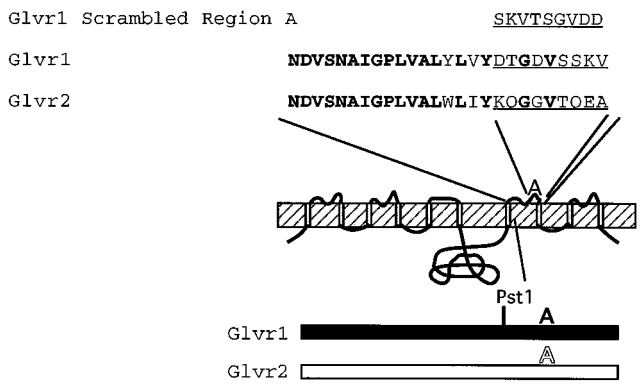


FIG. 1. Topological map of Glvr1, Glvr2, and chimeras. Speculative topological map of Glvr1, Glvr2, and chimeras. The proteins are predicted to encode 10 transmembrane domains and five extracellular loops; a large hydrophilic domain is predicted to be intracellular. All but the third extracellular domain harbor short stretches of highly hydrophobic sequences which are shown as dipping in and out of the membrane. Region A is a stretch of nine amino acids in the predicted fourth extracellular domain (Glvr1 position 550 through 558; Glvr2 position 522 through 530). The entire sequences of the fourth extracellular loop of Glvr1 (position 533 through 558) and Glvr2 (position 505 through 530) and a Glvr1 region A scrambled sequence are shown above the topological map. The nine amino acid residues making up region A are underlined in all three sequences. Identical residues are boldfaced. The positions of region A and the conserved *Pst*I site between Glvr1 and -2 are shown at the bottom. The *Pst*I site was used to make chimeras.

However, introduction of additional N- or C-terminal sequences from Glvr1 resulted in a functional receptor for FeLV-B. Region A of Glvr2, in contrast, was not found to be critical for the receptor function of Glvr2 for A-MLV infection, and it was not essential for conferring receptor specificity for A-MLV onto Glvr1. Rather, we found that different regions mapping to either the N terminus or the C terminus of Glvr2 were able to confer different degrees of receptor specificity for A-MLV onto Glvr1. These results are discussed in terms of possible modes of virus-receptor interactions. Surprisingly, though GALV/FeLV-B and A-MLV define two distinct interference groups, three of the chimeric genes encoded functional receptors for both GALV and A-MLV, and two of these chimeras also allowed infection with FeLV-B.

MATERIALS AND METHODS

Construction of expression plasmids. pOJ9 encodes human Glvr1 (GenBank accession L20859) in the expression plasmid pcDNA1 (Invitrogen) (4). In pOJ75, the human Glvr1 was transferred from pOJ9 (4) as a *Hin*dIII-*Xho*I fragment into pcDNA1A^{R}tkpA. pOJ74 was constructed by cloning a *Hin*dIII-*Sac*I fragment encoding Glvr2 (bases 184 to 2743 [GenBank numeration]) from the human cDNA clone pGlvr2-1 (18) (GenBank accession L20852) into pcDNA1A^{R}tkpA. pcDNA1A^{R}tkpA is a derivative of the expression vector pcDNA1 (Invitrogen) encoding the ampicillin resistance gene and in which the original splice and polyadenylation sequences were replaced with the herpes simplex virus type 1 thymidine kinase polyadenylation signal (18). The receptor genes are under transcriptional control of a cytomegalovirus promoter in pcDNA1A^{R}tkpA.

Chimeric receptors encoding mutated A regions (amino acid residues 550 through 558 in Glvr1 [5] and 522 through 530 in Glvr2 [18]) were obtained by in vitro mutagenesis as described elsewhere (7). For mutagenizing the codons encoding region A in Glvr1 (bases 2070 through 2096) (12), a 3′ *Pst*I fragment (base 1922 in Glvr1 to the *Pst*I site in the polylinker of pcDNA1) of pOJ9 was cloned into pUC118 to create pOJ31. pOJ36 and pOJ81 were derived from pOJ31 by mutagenizing Glvr1 region A to encode a scrambled region A sequence (pOJ36) or to encode the A region from Glvr2 (pOJ81), respectively (Fig. 1). pOJ80 was derived from pOJ74 by mutagenizing Glvr2 region A (bases 1807 through 1833) to the sequence of Glvr1 region A. In pOJ80, the *Pst*I restriction site at position 1652 in Glvr2 was removed without changing the amino acid sequence.

The recombinants were made by using a *Pst*I site conserved in Glvr1 (position 1922) and Glvr2 (position 1711) (Fig. 1). pOJ85 was made by a three-fragment ligation of two *Pst*I fragments from pOJ80 (a *Pst*I fragment harboring part of pcDNA1A^{R}tkpA [*Pst*I site in the ampicillin resistance gene] and the 5′ part of Glvr2 to position 1711 and another *Pst*I fragment harboring the remaining part of pcDNA1A^{R}tkpA) and the *Pst*I fragment from pOJ31 encoding the 3′ end of Glvr1. pOJ102, pOJ103, and pOJ104 were made by exchanging the 3′ *Pst*I fragment of Glvr1 in pOJ9 with the 3′ *Pst*I fragment from pOJ81 (pOJ102), pOJ80 (pOJ103), or pOJ74 (pOJ104); the coding sequences of all these recombinants were thereafter cloned into pcDNA1A^{R}tkpA by using the *Hin*dIII site upstream in the receptor sequence and a *Xho*I site in the polylinkers of the two vectors. pOJ85 was derived from pOJ80 by exchanging the 3′ *Pst*I fragment with the *Pst*I fragment of Glvr1 from pOJ31. pOJ105 and pOJ106 were obtained by three-fragment ligations of pcDNA1A^{R}tkpA digested with *Hin*dIII-*Eag*I (these sites are in the polylinker), the 5′ Glvr2 *Hin*dIII-*Pst*I fragment from pOJ80, and mutated *Pst*I-*Eag*I Glvr1 fragments derived from pOJ81 and pOJ36, respectively.

Cell culture. CHO-K1 (ATCC CCL 61) cells were grown in α-modified minimal essential medium (α-MEM) supplemented with 10% fetal bovine serum. D17 (ATCC CCL 183) and NIH 3T3 cells were grown in Dulbecco's MEM supplemented with 10% fetal bovine serum and 10% calf serum, respectively. GALV (SEATO) pseudotypes of the LacZ-encoding vector G1BgSvN (8) were derived from the producer cell line PG13GBN (8, 9) (ATCC CRL 10686), which was grown in Dulbecco's MEM–10% calf serum. The LacZ-encoding vector BAG (13), pseudotyped with the envelope of the A-MLV 4070A, was obtained from the producer cell line PAP3 (10, 13), which was cultivated in Dulbecco's MEM–10% calf serum. When used as producer, the PAP3 cells were grown in α-MEM–10% fetal bovine serum. FeLV-B pseudotypes of the LacZ-encoding vector LNPOZ (1) were obtained by infecting a D17 clone (unpublished data) harboring the expression plasmid pLNPOZ (1) with viruses derived from a molecular clone of FeLV-B (Gardner-Arnstein-B). The D17 cell line producing FeLV-B pseudotypes was grown in Dulbecco's MEM–10% fetal bovine serum.

Transient transfection and infection assay. NIH 3T3 or CHO-K1 cells were seeded at 4×10^4/60-mm-diameter dish. Next day, the cells were transfected by the calcium phosphate-DNA precipitation method (3). Each precipitate contained 10 μg of a CsCl-purified expression plasmid and 5 μg of the CsCl-purified pUC19 plasmid as carrier in 1 ml. From each precipitate, aliquots of 200 μl, corresponding to 2 μg of the expression plasmid, were added to two 60-mm-diameter dishes with NIH 3T3 cells and one 60-mm-diameter dish with CHO-K1 cells. Three independent precipitates were made per construct to be tested. Forty-eight hours after transfection, the cells were challenged with 1 to 1.5 ml of filtered supernatant (0.45-μm pore size) from PG13GBN (NIH 3T3), the FeLV-B producer (NIH 3T3), or PAP3 (CHO-K1) in the presence of Polybrene (8 μg/ml). Thus, all three virus pseudotypes were tested for their ability to infect cells transfected with the same three independent precipitates of a given construct. Four hours later, fresh media were added, and the cells were incubated for another 2 days, after which time they were fixed in 0.05% glutaraldehyde and assayed for β-galactosidase activity with X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) as a substrate (17). Plates were examined under the light microscope for positively stained (blue) cells, and the number of blue cells per plate was counted. The experiment was performed at least twice with independent preparations of plasmid DNA.

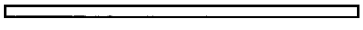
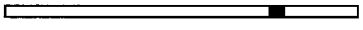
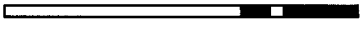
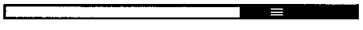
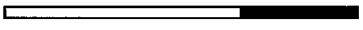
Virus titer. The titers of the virus pseudotypes were determined on NIH 3T3 cells for PAP3 supernatant and on D17 cells for PG13GBN supernatant and FeLV-B pseudotypes. Cells were exposed to serial dilutions of producer cell supernatant for 4 h in medium containing 8 μg of Polybrene per ml, fresh media were added, and 48 h after infection the cells were assayed for the presence of β-galactosidase-expressing colonies as described above. PAP3 and PG13GBN both gave titers of 10^5 CFU/ml. The titer of FeLV-B pseudotypes was 3×10^3 CFU/ml.

RESULTS

Expression plasmids encoding Glvr1 and -2 and seven hybrids of these were constructed and tested for the ability to confer permissivity for GALV, FeLV-B, and A-MLV infection. A stretch of nine amino acids (residues 550 through 558) in the putative fourth extracellular loop of Glvr1 has previously been shown to be critical for receptor function of Glvr1 for GALV and FeLV-B (5, 15). Only two of the nine amino acids in the corresponding region A in Glvr2 (residues 522 through 530) are conserved in Glvr1 region A (Fig. 1). Therefore, two of the hybrids (pOJ80 and pOJ102) were designed to test if simple exchange of region A was sufficient to alter receptor specificity. Four other hybrids (pOJ85, pOJ103, pOJ104, and pOJ105) tested the effect of additional or alternate receptor regions on specificity, while pOJ106 tested whether region A was critical for infection.

The ability of these plasmids to confer permissivity was investigated by transfecting them into nonpermissive cells and assaying the susceptibility of these cells to infection with virus

TABLE 1. Permissivity for infection in cells transiently expressing Glvr1 or -2 or chimeras

Plasmid[a]	Diagram[b]	% Permissivity[c]		
		GALV	FeLV-B	A-MLV
pOJ75		100 ± 15	100 ± 21	0.9 ± 0.1
pOJ74		0.06 ± 0.01	<0.2[d]	100 ± 5
pOJ80		91 ± 12	<0.2	130 ± 1
pOJ102		0.08 ± 0.06	<0.2	8 ± 0.8
pOJ103		108 ± 15	21 ± 12	9 ± 1
pOJ105		0.04 ± 0.02	<0.2	129 ± 7
pOJ106		0.06 ± 0.04	<0.2	93 ± 9
pOJ85		78 ± 8	52 ± 4	93 ± 3
pOJ104		0.03 ± 0.01	<0.2	50 ± 1
pcDNA1A^{R}tkpA		0.03 ± 0.02	<0.2	<0.003

[a] Receptor sequences were cloned into pcDNA1A^{R}tkpA. pOJ75, Glvr1; pOJ74, Glvr2.
[b] Striped bar in pOJ106, a scrambled Glvr1 region A sequence.
[c] See Materials and Methods. The data are averages of three independent transfections; however, the three different virus pseudotypes were tested on the same three precipitates of a given construct. The data are the standardized numbers ± the standard deviations of the actual means. The number of blue cells per 60-mm-diameter dish transfected with the wild-type receptor for a given virus was assigned a value of 100% (15,962, 159, and 11,268 blue cells per dish for GALV, FeLV-B, and A-MLV, respectively). The experiment was repeated with an independent preparation of plasmid DNA, and similar results were obtained.
[d] Based on one blue cell per three 60-mm-diameter dishes.

pseudotypes of LacZ-encoding vectors. The results obtained are shown in Table 1.

Region A from Glvr1 is sufficient to allow Glvr2 to function as a receptor for GALV. NIH 3T3 cells transfected with empty vector alone showed a background level of infection with GALV which was 0.03% of the level obtained with the Glvr1-encoding plasmid (pOJ75) (Table 1). Glvr2 (pOJ74) gave infectivity comparable to background levels and therefore could not support infection with GALV, in agreement with earlier observations (11, 18). Replacement of the nine-amino-acid sequence of Glvr2 region A with the Glvr1-specific sequence (pOJ80) was sufficient to confer receptor specificity for GALV onto Glvr2; moreover, the same infectibility was obtained with pOJ80 as with Glvr1 (pOJ75). Introduction of further Glvr1 sequences besides region A had no major influence on receptor function for GALV in that pOJ85 and pOJ103 gave infectibilities comparable to that of pOJ80.

All chimeras encoding the Glvr2 form of region A (pOJ102, pOJ104, and pOJ105) were unable to confer infectibility. Moreover, scrambling of the Glvr1 region A amino acid sequence in pOJ85 (pOJ106) also abolished receptor function. Therefore, Glvr1 region A is indispensable for receptor function for GALV, in agreement with previous results (5, 15).

FeLV-B is dependent on N- or C-terminal sequences from Glvr1 in addition to region A for infectivity. No infected NIH 3T3 cells were observed in cells transfected with empty vector or with the Glvr2-expressing construct (pOJ74) and challenged with FeLV-B (Table 1). In contrast to the results obtained with GALV, replacement of Glvr2 region A with Glvr1 region A (pOJ80) was insufficient to confer receptor specificity for FeLV-B. However, when the 5′ *Pst*I fragment of Glvr1 was introduced into Glvr2 in addition to Glvr1 region A-encoding sequences, the chimera (pOJ103) allowed infection with FeLV-B at levels of 21% of the level for the wild type. A chimera (pOJ85) in which the entire 3′ *Pst*I fragment was derived from Glvr1, including region A-encoding sequences, also supported infection with FeLV-B, at levels of 52% of the level for the wild type.

All chimeras encoding the Glvr2 form of region A (pOJ102, pOJ104, and pOJ105) were unable to confer infectibility with FeLV-B. Moreover, scrambling of the Glvr1 region A amino acid sequence in pOJ85 (pOJ106) also abolished receptor function. Therefore, Glvr1 region A is indispensable for receptor function for FeLV-B, in agreement with previous results (15).

Receptor specificity of Glvr2 for A-MLV cannot be mapped to a specific region of Glvr2. No infected cells were detected in CHO-K1 cells transfected with the empty vector (Table 1). CHO-K1 cells transfected with the Glvr1-expressing plasmid (pOJ75) gave 0.9% of the infection level achieved with the Glvr2-expressing plasmid (pOJ74), that is, at least 300 times above background. Thus, A-MLV can use Glvr1 as a receptor, albeit with low efficiency.

Introduction of the Glvr2 form of region A into Glvr1 (pOJ102) increased infectibility with A-MLV to 8% of that obtained in Glvr2-expressing (pOJ74) cells. However, cells expressing pOJ103, which encodes the 3′ *Pst*I fragment from Glvr2 but with Glvr1 region A showed a similar increase in infectibility, that is, 9% of that in cells expressing Glvr2 (pOJ74). The presence of the entire 3′ *Pst*I fragment from Glvr2 (pOJ104) resulted in an infectibility of 50% of that for the wild type. Introduction of the 5′ *Pst*I fragment from Glvr2 (pOJ85) resulted in wild-type levels of infectivity for A-MLV. Conversion of Glvr1 region A to the Glvr2 form in pOJ85 (pOJ105) increased infectibility above the wild-type level. In summary, though Glvr2 region A can confer receptor specificity for A-MLV onto Glvr1, it cannot do so more efficiently than other Glvr2 sequences. Furthermore, introduction of Glvr1 region A into Glvr2 (pOJ80) increased the infectibility compared with Glvr2 rather than impairing it. It could be argued that pOJ80, pOJ85, and pOJ103 functioned as receptors for A-MLV because Glvr1 region A can substitute for Glvr2 re-

gion A. However, comparison between the results obtained with the constructs pOJ85 and pOJ106 indicates that this is highly unlikely in that scrambling of the Glvr1 region A sequence in pOJ85 (pOJ106) did not decrease the infection level. Therefore, Glvr2 region A is only a minor determinant of A-MLV receptor specificity, and it is dispensable for Glvr2 receptor function. In general, the receptor specificity could not be mapped to a single domain; sequences in the amino and the carboxy termini of Glvr2 can confer receptor specificity for A-MLV onto Glvr1.

Chimeras between Glvr1 and -2 can function as receptors for viruses from different interference groups. All chimeras encoding the Glvr1 form of region A (pOJ80, pOJ85, and pOJ103) functioned as receptors for both GALV and A-MLV. pOJ80 and pOJ85 gave infectibilities comparable to those obtained with the wild-type receptors for the individual viruses. Moreover, chimeras encoding the Glvr1 3′ *Pst*I fragment (pOJ85) or the Glvr1 5′ *Pst*I fragment and the Glvr1 form of region A (pOJ103) functioned as receptors for A-MLV, GALV, and FeLV-B, with pOJ85 being a nearly fully functional receptor for all three viruses.

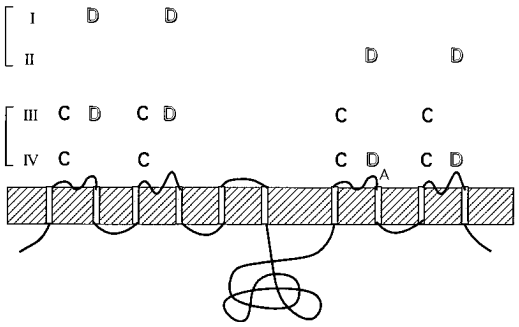


FIG. 2. Schematic illustration of a suggested model for A-MLV–receptor interaction. Speculative topological map of Glvr1, Glvr2, and chimeras. The position of region A (A) is indicated. D and C, regions of the putative extracellular loops divergent or conserved, respectively, between Glvr1 and -2. The majority of these amino acids are also found conserved between the first and fourth extracellular loops and between the second and fifth extracellular loops in both receptors. I and II, possibility that infection by A-MLV is dependent on only divergent regions and that these can be supplemented by either the N terminus, here illustrated as the first and second putative extracellular loops, or the C terminus. III and IV, possibility that, besides the divergent receptor regions which determine the receptor specificity for A-MLV, the virus also is dependent on one or more of the regions conserved between Glvr1 and -2 for infection.

DISCUSSION

Glvr1 is the human receptor for GALV and FeLV-B, while Glvr2 is the human receptor for A-MLV. The proteins are divergent in 38% of their amino acids, and it must be presumed that some of these differences control their viral specificities. The proteins are proposed to have 10 transmembrane domains and five extracellular loops (Fig. 1) (4, 18), with many of the amino acid differences being clustered in these putative loops. In particular, the predicted second and fourth loops are highly divergent towards their C termini. It was previously shown that the C-terminal part of the putative fourth loop (here termed region A) is essential for infection by GALV and FeLV-B (5, 15). There is sequence homology between the N-terminal parts of the putative first and fourth loops and also of the putative second and fifth loops (4, 18). These N-terminal sequences are also highly conserved between Glvr1 and -2 (18). In this work, chimeras were made between the two genes to further investigate the role of Glvr1 region A in defining receptor specificity for GALV and FeLV-B and to map which regions of Glvr2 control receptor specificity for A-MLV (Fig. 1; Table 1).

All three viruses were found to differ in their requirements for specific receptor domains or specific sequences of the same receptor domains. Glvr1 region A, when introduced into Glvr2, allowed the chimera to function as a receptor for GALV. The simplest interpretation of this result is that the GALV envelope glycoprotein binds region A during the process of infection. The virus may interact with other regions of the receptor, e.g., those conserved between Glvr1 and -2; this interaction would not be revealed by the chimeras constructed. Other interpretations of the data—in which the regions identified as necessary for infection do not take part in the binding but, e.g., change the conformation of the receptor in a way that creates an envelope-binding site in another domain in the receptor or in another, as yet unidentified accessory protein present on the cell surface—are possible. Alternatively, the identified regions may be involved in postbinding steps required for viral internalization.

Region A was also found to be essential for infection by FeLV-B. However, this region alone could not confer receptor specificity for FeLV-B onto Glvr2. Chimeras encoding Glvr1 region A plus additional N- or C-terminal sequences from Glvr1 allowed infection by FeLV-B. The simplest interpretation of this is that FeLV-B envelope glycoprotein will initiate infection following binding to region A and at least one other Glvr1-specific sequence in either the N terminus or the C terminus.

The observation that Glvr1 region A is critical for infection by GALV and FeLV-B and the divergence between the A regions of Glvr1 and Glvr2 suggested that region A in Glvr2 might be equally critical for infection by A-MLV. However, while introduction of Glvr2 region A into Glvr1 did increase infectibility by A-MLV to 8% of that obtained with Glvr2, a similar result was found with a chimera encoding the C-terminal third of Glvr2 but with region A mutated to the Glvr1 form. The presence of the entire C-terminal third of Glvr2 in Glvr1 increased infectivity to close to the wild-type level. The converse chimera, consisting of the N-terminal two-thirds of Glvr2 and the C-terminal third of Glvr1, showed wild-type levels of infectivity. Finally, a hybrid in which the sequence of region A was scrambled continued to function as a receptor for A-MLV. These results indicate that the regions of Glvr2 controlling specificity for A-MLV cannot be mapped to a single critical domain. In contrast, contact with Glvr2-specific sequences in either the N- or the C terminus gives infection. As an example, Fig. 2 illustrates that infection by A-MLV could follow from binding to the regions of the putative first and second loops or of the putative fourth and fifth loops diverging between Glvr1 and -2. The figure also shows that binding to the regions conserved between Glvr1 and -2 may occur; this will, however, not be revealed by the chimeras. It is possible that the conserved regions serve some function in infection by A-MLV, because Glvr1 raises infection levels by A-MLV at least 300-fold above background. Alternatively, A-MLV interacts with the domains differing between Glvr1 and -2 with low affinity. The fact that changing different Glvr1 sequences to the Glvr2 form has an increasing effect on A-MLV infection suggests that no single region added is critical but that each merely improves the affinity between A-MLV envelope glycoprotein and the receptor.

Whether the regions identified as critical for infection actually directly bind the viral envelope might be tested by introducing these regions in nonrelated proteins and investigating whether the viruses bind to the chimeric proteins.

Surprisingly, three of the chimeras could act as receptors for

both GALV and A-MLV, and two of these also functioned as receptors for FeLV-B. This observation was unexpected, since GALV/FeLV-B and A-MLV are members of different interference groups (14). However, it demonstrates that the characteristics which define receptor specificity for these viruses are separate entities which can be combined in a single protein. The observation is consistent with the possibility that these viruses arose from a common ancestor by divergent evolution and that the ancestral virus used a member of the Glvr family as receptor. This would suggest that species in which GALV/FeLV-B and A-MLV utilize the same receptor for infection may still exist. An example of this may be the interference between GALV and A-MLV in Chinese hamster E36 cells (2). Chronic infection of these cells with GALV renders them resistant to superinfection with A-MLV, while A-MLV does not interfere with GALV infection. This is consistent with the possibility that GALV can use two different receptors in E36 cells, one of which is the receptor used by A-MLV. While we have not performed interference analysis on the chimeras analyzed here, it can be predicted that GALV, FeLV-B, and A-MLV will show reciprocal interference when chimeras which allow infection of the two viruses tested are used.

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