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Feline Leukemia Virus Subgroup B Uses the Same Cell Surface Receptor as Gibbon Ape Leukemia Virus

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Pseudotypes of gibbon ape leukemia virus/simian sarcoma-associated virus (GALV/SSAV) and feline leukemia virus subgroup B (FeLV-B) have been constructed by rescuing a Moloney murine leukemia virus vector genome with wild-type GALV/SSAV or FeLV-B. The resulting recombinant viruses utilized core and envelope proteins from the wild-type virus and conferred resistance to growth in L-histidinol upon infected cells by virtue of the *HisD* gene encoded by the vector genome. They displayed the host range specificity of the rescuing viruses and could be neutralized by virus-specific antisera. Receptor cross-interference was observed when the GALV/SSAV or FeLV-B pseudotypes were used to superinfect cells productively infected with either GALV/SSAV or FeLV-B. Although murine cells are resistant to FeLV-B infection, murine cells expressing the human gene for the GALV/SSAV receptor became susceptible to FeLV-B infection. Therefore GALV/SSAV and FeLV-B utilize the same cell surface receptor.

The receptor utilized by a pathogenic retrovirus can be an important determinant of specific disease. This is exemplified by the feline leukemia virus subgroups A, B, and C (FeLV-A, FeLV-B, and FeLV-C), which interact with distinct host cell receptors (11, 29) and induce diseases as diverse as leukemia, aplastic anemia, and severe immunodeficiency (9, 26–28). All field isolates of FeLV contain FeLV-A, either alone or in a mixture of subgroups (10). FeLV-C isolates are associated with feline aplastic anemia (9, 27), whereas FeLV-B isolates may be specifically associated with higher rates of leukemia (10, 28). Subgroup B isolates of FeLV have probably arisen as a result of recombination between weakly pathogenic subgroup A viruses and endogenous envelope sequences (6, 34), in a similar manner to the generation of the murine mink cell focus-forming viruses (17). It is possible that FeLV-B causes accelerated leukemogenesis as a result of an expanded cellular tropism produced by these recombination events which allow FeLV-B to recognize a novel cellular receptor. Demonstration of increased leukemogenicity correlating to an expanded host cell range of an *env* recombinant-derived viral subgroup has been reported for the 10A1 strain of murine leukemia virus (MLV) relative to the amphotropic murine leukemia virus (25).

Hitherto, only three retroviral receptors have been identified, those for human and simian immunodeficiency viruses (4, 18, 30), the Moloney MLV ecotropic strain (2), and gibbon ape leukemia virus (GALV) (24). In a previous study (31) we demonstrated syncytial cross-interference between GALV, simian sarcoma-associated virus (SSAV), and FeLV-B. Such interference is postulated to occur when cells expressing the viral envelope glycoproteins of one virus prevent fusion induced by another virus by competitively blocking a shared receptor (31, 33). SSAV is highly homologous to GALV (5) and probably represents a single zoonotic case of infection of a woolly monkey by GALV. GALV has been isolated from animals with lymphosarcoma or granulocytic leukemia and has been shown to lead to myeloid leukemias upon inoculation of juvenile gibbons (14–16). The indication from syncytial cross-interference that FeLV-B and GALV/SSAV shared a cell surface receptor was plausible, since the receptor for GALV, like that for FeLV-B, has a broad species and tissue distribution (24, 37). We were unable, however, to demonstrate any interference with infection between GALV and FeLV-B, since suitable high-titer pseudotype stocks were not available (31).

To investigate the relationship between the FeLV-B and GALV/SSAV receptors, pseudotype stocks of FeLV-B (11) or the SSAV and SF strains of GALV (15, 36) carrying a dominant selectable marker gene were prepared as will be described in detail elsewhere (35). Briefly, pseudotypes were prepared by infecting 5×10^4 HOS(HisD) cells (HOS cells stably transfected with an MLV vector in which an internal murine *Thy-1* promoter drives expression of *HisD* [35]) with 5 ml of 0.45-μm filtered supernatants from SSAV- or FeLV-B-producing cells (31). The cells were washed after 24 h and grown for 4 to 8 days, after which medium was harvested as a source of pseudotype. The MLV-A pseudotype was prepared by harvesting medium from an amphotropic packaging cell line stably transfected with the same MLV vector. Pseudotype titers were determined by seeding target cells at 5×10^4 to 8×10^4 cells per ml and exposing them to dilutions of the filtered pseudotype stocks. At 2 days after infection, aliquots of cells were transferred to selective medium lacking histidine and containing 0.5 to 2 mM L-histidinol. Resistant colonies were counted after 9 to 15 days. Replication-competent virus was used to rescue a *HisD* (8)-containing Moloney MLV vector from human HOS cells. Table 1 shows the host range of such pseudotypes in which the *HisD* vector genome is packaged by the capsid and envelope proteins of the input virus. Recombinant virus harvested from a clone of the amphotropic packaging cell line PA317 (22) transfected with the *HisD* genome was also used to infect the same range

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TABLE 1. Host range of SSAV and FeLV-B pseudotypes

Species	Target cell	Titer of pseudotype (HisD CFU/ml)		
		SSAV	FeLV-B	MLV-A
Human	HOS	2,280	4,800	9,600
	HeLa	3,200	3,300	3,600
Mouse	NIH 3T3	0	0	10,000
Rat	XC	3,300	750	27,000
	HSN	5,700	0	8,300
	NRK	2,100	0	33,000
Mink	CCL 64	1,200	0	21,000
Hamster	BHK	0	0	0
	CHO	0	0	0
Rabbit	Sirc	850	0	13,400

of mammalian cell lines as a positive control for HisD plating.

The species specificity of infection by the SSAV and FeLV-B pseudotypes was broadly similar (Table 1). Notable exceptions were two rat cell lines, a mink cell line, and a rabbit cell line, which were readily infected by SSAV but not by FeLV-B. This lack of FeLV-B infection could potentially be due either to a lack of receptor expression in the nonsusceptible cells, with the implication that the GALV and FeLV-B receptors are different in these species, or to a virus-specific postreceptor block in replication for the pseudotype carrying the FeLV-B core proteins. Unlike experiments in which a GALV envelope protein was expressed with MLV core proteins (23), infection of Syrian (BHK) and Chinese (CHO) hamster cells by a pseudotype carrying SSAV envelope and core was not observed (Table 1). This suggests a postreceptor block in GALV replication in hamster cells.

HisD(SSAV) and HisD(FeLV-B) pseudotypes were further characterized by using anti-serum raised against GALV or FeLV-B. Anti-FeLV-B and anti-GALV goat serum specifically neutralized FeLV-B and GALV pseudotype particles, respectively, whereas neither type of serum was able to neutralize the heterologous pseudotype (Fig. 1). Therefore, these virus pseudotypes faithfully represented the properties of the rescuing helper viruses.

To investigate the receptor specificity of the HisD pseudotypes, we plated SSAV and FeLV-B pseudotypes on cell lines already productively infected with wild-type virus. In these experiments, interference with infection was observed when SSAV or FeLV-B pseudotypes were used to infect either human HOS cells or canine S^+L^- cells productively infected with either SSAV or FeLV-B (Fig. 2). Identical cross-interference was observed for vesicular stomatitis virus pseudotypes (Fig. 2B). The plating of MLV amphotropic pseudotypes was unaffected by the production of either GALV or FeLV-B from the target cells. The partial nature of the interference observed on HOS cells infected with SSAV, when challenged by FeLV-B (Fig. 2A), might be explained by a higher affinity of FeLV-B for the receptor. We observed a similar effect in SSAV-infected HeLa cells, and we have previously documented that SSAV-infected rat XC cells are not resistant to infection by vesicular stomatitis virus-(FeLV-B) pseudotypes (31).

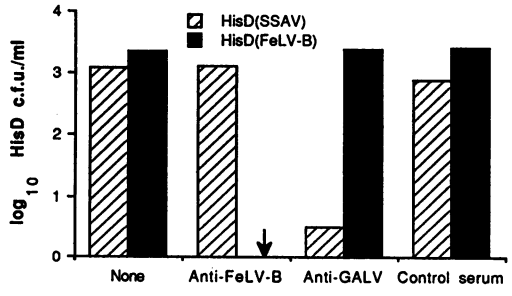

FIG. 1. Specific antibody blocking of pseudotype infection. The titers of HisD(SSAV) and HisD(FeLV-B) pseudotypes on HOS cells were determined as described in the text. The pseudotypes were incubated prior to infection with 5% anti-FeLV-B, anti-GALV, or control goat serum for 1 h at 37°C.

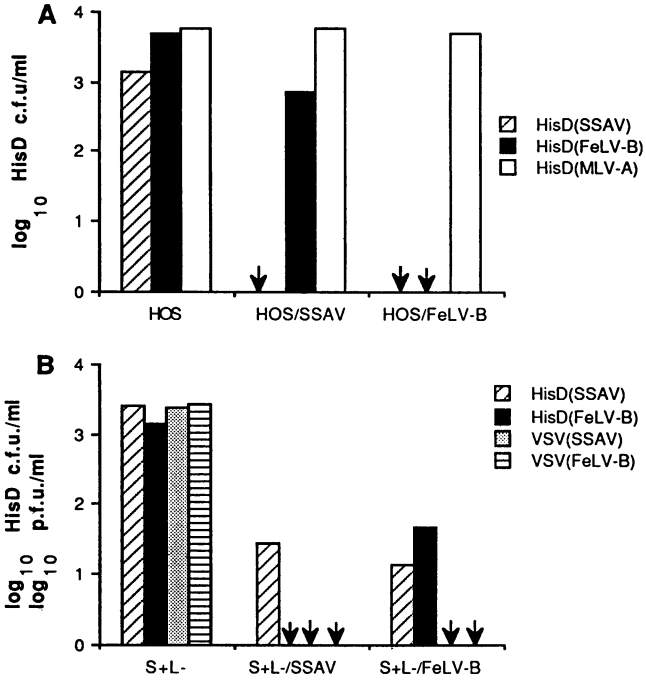

FIG. 2. Cross-interference between SSAV and FeLV-B. (A) The titers of HisD(SSAV), HisD(FeLV-B), and HisD(MLV-A) pseudotypes on HOS cells or HOS cells productively preinfected with SSAV or FeLV-B as indicated were determined as described in the text. (B) The titers of HisD or vesicular stomatitis virus (VSV) pseudotypes of SSAV or FeLV-B were determined as described in the text and reference 31, respectively. The target cells were canine S^+L^- cells productively preinfected with SSAV or FeLV-B.

The receptor interference observed on two host species to the plating of GALV and FeLV-B pseudotypes indicated that these two viruses may recognize the same cellular receptor. Therefore, HisD(SSAV), HisD(GALV) (15), and HisD(FeLV-B) pseudotypes were used to infect two murine NIH 3T3 cell lines, GRT-5 and JO2-5, which had been stably transfected with the human genomic sequences for the GALV receptor gene, or the equivalent cDNA, respectively (24). Although neither pseudotype could infect the parental NIH 3T3 cell line, both GALV/SSAV and FeLV-B pseudotypes were able to infect both GRT-5 and JO2-5 cells (Table 2). The titer of HisD (in CFU per milliliter) was 10- to 100-fold lower on the murine cell lines than on human cells, as was previously reported for cells transfected with the GALV receptor gene and infected with other recombinant GALV viral stocks (24). This may imply poor expression or

TABLE 2. Infection of murine cells expressing the human GALV receptor

Target cell line	Titer of pseudotype (HisD CFU/ml)		
	SSAV	GALV	FeLV-B
HeLa	2,500	2,500	7,500
HOS	950	1,600	5,000
BHK	0	NT[a]	0
NIH 3T3	0	0	0
GRT-5	90	195	190
JO2-5	55	25	80

[a] NT, not tested.

function of the human receptor in mouse cells or a common postreceptor restriction of infection. Nonetheless, it is clear that transfer of the human gene encoding the cellular receptor for GALV to murine cells normally refractory to infection by FeLV-B was sufficient to confer susceptibility to infection by HisD(FeLV-B) pseudotypes.

These data demonstrate that a feline and a primate virus share the same cellular receptor. The lack of a significant region of homology between the amino-terminal portions of the FeLV-B and GALV viral envelope proteins (5, 6) means that the envelope determinant which specifies receptor interaction cannot be readily identified. It appears that some murine viruses may also share the GALV/FeLV-B receptor, since endogenous xenotropic viruses, antigenically related to GALV, have been isolated from two species of Asian mouse, *Mus caroli* (21) and *M. cervicolor* (3). The former has been shown to cross-interfere with GALV (21). Also, the recent mapping of the murine homolog of the gene encoding the GALV receptor to chromosome 2 suggests that M813, a distinct ecotropic virus isolated from *M. cervicolor*, may utilize the product of this gene, since the *Rec-2* locus which encodes its receptor maps to chromosome 2 (1, 13). This parallels the observation that monkey D-type viruses, the endogenous feline C-type virus RD114, and baboon endogenous virus also share a common receptor (31, 32). Indeed, an analysis of 25 retroviruses plating on human cells identified only eight distinct receptors defined by cross-interference patterns (31). The recognition of a common receptor by GALV and FeLV-B may have arisen either from divergent evolution of an ancestral virus with conservation of receptor usage or from convergent evolution of viral envelopes adopting the use of the same receptor.

It therefore appears that a limited set of cell surface proteins are appropriate to act as retroviral receptors. The GALV receptor is a multiple membrane-spanning domain protein, homologous to the phosphate transporter of *Neurospora crassa* (12). Its suitability as a virus receptor might be due to its widespread distribution, or to its ability to allow virus internalization after binding, either because of its close proximity to the cell membrane or because of an interaction between the envelope glycoprotein of the virus and transmembrane domains of the receptor. Alternatively, particular viral envelope-receptor interactions might have come about because they result in enhanced proliferation of the infected cell, thus favoring the spread of the virus. As examples, human T-cell leukemia virus type I (HTLV-I) virions directly activate resting T lymphocytes (7), and Friend spleen focus-forming virus (20) and other murine mink cell focus-forming virus (19) envelope proteins directly trigger cytokine receptors. This hypothesis is attractive because the FeLV-B envelope, in contrast to FeLV-A and FeLV-C, tends to be directly associated with leukemogenesis (10, 28). It is therefore possible that a cell proliferative advantage conferred by GALV/SSAV or FeLV-B envelope-receptor interaction is a process favored in evolution.

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