Identification of a Putative Receptor for Subgroup A Feline Leukemia Virus on Feline T Cells

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Retrovirus infection is initiated by the binding of virus envelope glycoprotein to a receptor molecule present on cell membranes. To characterize a receptor for feline leukemia virus (FeLV), we extensively purified the viral envelope glycoprotein, gp70, from culture supernatants of FeLV-61E (subgroup A)-infected cells by immunoaffinity chromatography. Binding of purified 125I-labeled gp70 to the feline T-cell line 3201 was specific and saturable, and Scatchard analysis revealed a single class of receptor binding sites with an average number of 1.6 \times 10^6 receptors per cell and an apparent affinity constant (K_a) of 1.15 \times 10^9 M^{-1}. Cross-linking experiments identified a putative gp70-receptor complex of 135 to 140 kDa. Similarly, coprecipitation of 125I-labeled cell surface proteins with purified gp70 and a neutralizing but noninterfering anti-gp70 monoclonal antibody revealed a single cell surface protein of approximately 70 kDa. These results indicate that FeLV-A binds to feline T cells via a 70-kDa cell surface protein, its presumptive receptor.

Feline leukemia viruses (FeLV) are horizontally transmitted oncoretroviruses that are widespread in domestic cat populations and induce both suppressive and neoplastic diseases of the feline lymphoreticular system (19). The FeLV family consists of three known subgroups, A, B, and C, distinguished by virus and pseudotype interference (reflecting receptor specificity), neutralization, and host-range assays (25, 30, 31, 50). FeLV-A isolates generally grow only in feline cells, whereas FeLV-B and FeLV-C isolates have an extended host range that includes certain human, canine, and, in the case of FeLV-C, guinea pig cells (23-25, 51). Different subgroups of FeLV are associated with distinct diseases, the determinants for which have been mapped to the surface glycoprotein (SU) encoded by the envelope gene (13, 40, 47). FeLV-A is present in all natural infections, is noncytopathic, and is not acutely pathogenic, giving rise to lymphomas after a long latency (12, 23, 26, 41). In contrast, some molecularly cloned viruses derived from the FeLV-FAIDS isolate (21, 39) are T-cell cytopathic and cause fatal immunodeficiency disease in cats (43, 44), although they probably utilize the subgroup A receptor (30, 31). FeLV-C isolates are rare, utilize a distinct receptor, and induce aplastic anemia (7, 20, 22, 35, 47, 48). FeLV-B isolates readily arise via recombination between FeLV-A and endogenous FeLV-like elements (45, 57), are frequently found in natural infections, and are associated with proliferative disease induction (23, 26, 40). FeLV-B also shares a common cell surface receptor with gibbon ape leukemia virus and simian sarcoma-associated virus as initially concluded from syncytial cross-interference studies (55) and recently confirmed by superinfection interference experiments (59).

Normally, retrovirus infection results in the down-regulation or surface blockade of its cognate receptor, rendering the cell resistant to superinfection with viruses utilizing the same receptor (11, 14, 56, 65, 66). However, the mechanism of T-cell killing by FeLV-FAIDS involves massive superinfection of already infected susceptible cells (13), with FeLV-A isolates differing in their ability to block this superinfection (30). Some of the same viral genetic changes in the envelope gene responsible for this failure of superinfection resistance have been implicated in disease induction in vivo (13, 46a). Thus, virus-receptor interactions seem to be critically involved in the pathogenicity of FeLV.

The nature of the cell surface components used by retroviruses as receptors remains undefined in most cases. However, genes coding for three retrovirus receptors have been identified. A receptor for human immunodeficiency virus is the cell surface protein CD4 (9, 29, 36), expression of which is required for infection of most cells (29). CD4 has also been shown to directly bind the human immunodeficiency virus SU protein (2, 34), and CD4-SU complexes can be immunoprecipitated with noninterfering anti-CD4 monoclonal antibodies (MAbs) (38). Genes encoding receptors for the ecotropic murine leukemia virus (MuLV) (1) and gibbon ape leukemia virus (42) have also been identified. Expression of these genes renders certain nonpermissive cells susceptible to infection and capable of binding viral surface glycoprotein (1, 28, 42, 64). Recently, Takeuchi et al. (59) have found that noninfectible mouse cells can become susceptible to infection with FeLV-B after they acquire the human gene for the gibbon ape leukemia virus receptor. In addition, several proteins have been identified on murine cell surfaces which bind MuLV (8, 10, 49, 62); however, the relationship of these to each other and their function as MuLV receptors remain to be determined.

We took a biochemical approach to the identification of a receptor for FeLV-A on the feline T-cell line 3201 (FeT). We purified the SU protein from a molecularly cloned representative of FeLV-A (clone 61E from the FeLV-FAIDS isolate [12, 21, 43]) virus to assess its binding affinity and to identify its putative receptor as a 70-kDa protein by coprecipitation of cross-linked and non-cross-linked SU-receptor complexes.

MATERIALS AND METHODS

Cells and virus. The feline T-lymphocyte cell line 3201 (referred to here as FeT) (54) was a gift from W. D. Hardy, Jr., and E. E. Zuckerman and was grown at 37°C in 5% CO₂.
in 1:1 L15-RPMI medium (GIBCO) supplemented with 20% fetal calf serum, penicillin, streptomycin, and l-glutamine. The human T-lymphoma cell line HuT78 (17) and the feline embryo fibroblast (FeF) cell line AH927 (originated by W. Nelson-Rees and provided by S. Rasheed) were cultured in RPMI 1640 medium and Dulbecco modified Eagle medium, respectively, both with 10% fetal calf serum and supplemented as described above. Molecularly cloned subgroup A FeLV (isolate 61E) (12, 43) was transfected into FeF cells and grown in large volumes in Spinner flasks at the Massachusetts Institute of Technology cell culture facility. Ten-liter batches of culture supernatant were harvested, clarified by centrifugation (10,000 × g for 10 min), and concentrated to about 300 ml by ultrafiltration (Amicon). The concentrated virus preparation was stored in 1-ml aliquots in liquid nitrogen.

**MAB preparation.** A mouse hybridoma cell line that secretes the MAAb C11D8 was a gift from C. K. Grant and was grown in RPMI 1640 medium with 10% fetal calf serum supplemented as described above. It is a broadly neutralizing antibody that recognizes a linear epitope in a constant region of FeLV envelope protein (15, 18). MAAb was purified from 500 ml of culture supernatant by filtration (0.45-μm pore) and then protein A-Sepharose chromatography (2 ml) at a flow rate of 0.5 ml/min at 4°C (3). The column was washed with phosphate-buffered saline (PBS), and bound antibody was eluted with 50 mM glycine-0.15 M NaCl (pH 2.5), neutralized with 0.1 volume of 1 M Tris (pH 9.0), and desalted by dialysis against PBS. Finally, antibody was concentrated by ultrafiltration (Amicon) and stored at 80°C.

**Purification of envelope glycoprotein (gp70).** Envelope glycoprotein (gp70) was purified from FeLV-61E-A by immunoaffinity chromatography (3) with C11D8. Concentrated virus was disrupted with TSA buffer (0.01 M Tris [pH 8.0], 0.14 M NaCl, 0.0025% sodium azide, 0.1% Triton X-100, 0.1% deoxycholate, 1 mM phenylmethylsulfonyl fluoride) at 4°C for 2 h, and insoluble material was removed by centrifugation (4,000 × g for 10 min). C11D8 was coupled to cyanogen bromide-activated Sepharose 4B (Pharmacia) according to the manufacturer’s instructions (2.5 mg of MAAb per ml of Sepharose). Detergent-solubilized virus was mixed with 2 ml of antibody-Sepharose (preequilibrated with TSA buffer) for 3 h at 4°C and then packed into a chromatography column. The column was washed with 10 volumes of TSA and 5 volumes of sodium phosphate buffer (50 mM sodium phosphate [pH 6.3], 0.1% Triton X-100, 0.5 M NaCl) to remove nonspecifically bound material. Bound gp70 was eluted with 5 volumes of 50 mM glycine-0.15 M NaCl (pH 2.5). Eluted gp70 was neutralized with 0.1 volume of 1 M Tris (pH 9.0), desalted by dialysis against 5 mM ammonium bicarbonate at 4°C for 24 h, and then concentrated by lyophilization and stored at −80°C.

The purity and integrity of gp70 was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) by the method of Laemmli (33). After electrophoresis, the gel was fixed in 50% methanol in water and then silver stained (Stratagene). Purified gp70 was labeled with 125I by the Enzymobead lactoperoxidase reaction (Bio-Rad) according to the manufacturer’s instructions. Unincorporated iodine was removed by two successive rounds of size exclusion chromatography (Bio-Rad Bio-Gel P-6-DG column). An aliquot of labeled gp70 was precipitated with 10% trichloroacetic acid at 4°C for 1 h and centrifuged, and the radioactivity of the pellet was counted to determine the trichloroacetic acid-precipitable radioactivity. An aliquot of gp70 was also analyzed by SDS-PAGE on a 10% gel. After electrophoresis, the gel was fixed with 30% methanol-10% acetic acid for 30 min, washed in water for 15 min, dried, and then exposed to X-ray film (Kodak) with an intensifying screen (DuPont).

**gp70 cell binding assay.** 125I-labeled gp70 binding to FeT cells was measured with cells harvested in the log phase by the method of Johnson and Rosner (27). Cells were washed twice in reaction buffer (PBS [pH 7.4] containing 55 mM glucose, 1 mM CaCl2, 1 mg of bovine serum albumin (BSA) per ml, and 1% nonfat dry milk) and then resuspended in 0.3 ml of reaction buffer containing 5 × 106 cells. 125I-labeled gp70 (2.5 to 160 ng, 2 × 106 cpm/ng) in 0.1 ml of reaction buffer was added, and the cells were incubated at room temperature (24°C) for 2 h with rotation. At the end of incubation, the cells were collected on filter discs presoaked in 100% fetal calf serum for 1 h to prevent nonspecific adsorption (cellulose acetate; 0.45-μm pore size; 25-mm diameter; Millipore) and washed with 20 ml of ice-cold reaction buffer. Filters were dried, and bound radioactivity was determined in a gamma scintillation counter. Nonspecific binding to FeT cells was estimated from the radioactivity bound to HuT78, and specific binding to FeT cells was calculated by subtracting the amount bound to HuT78 cells.

**Cross-linking studies.** After incubation of FeT cells with 125I-labeled gp70 in 1 ml of reaction buffer as described above, cells were harvested by centrifugation at 400 × g for 10 min, and unbound gp70 present in the supernatant was removed. Cells were then resuspended in 1 ml of PBS (pH 8.3) containing 1 mM MgCl2. Chemical cross-linking was performed by the method of Brenner et al. (6). The cross-linker disuccinimidyl suberate (DSS) or dithiobisuccinimidyl propionate (DSP) (Pierce Chemical Co.) was dissolved at 10 mg/ml in dimethyl sulfoxide and added to the cell suspensions to obtain a final concentration of 100 μg/ml. Cells were mixed at 4°C for 25 to 30 min by rotation and then centrifuged at 400 × g for 10 min. The cell pellet was extracted in a solution of 300 mM NaCl, 50 mM Tris, and 0.5% Nonidet P-40 (pH 7.4) (100 μl/106 cells) for 1 h at 4°C, nuclei were removed by centrifugation (400 × g for 10 min), and the supernatant was analyzed by SDS-PAGE under nonreducing and reducing conditions (33).

For immunoprecipitation, 100 μl of cross-linked cell lysate was diluted with 400 μl of dilution buffer (190 mM NaCl, 60 mM Tris-Cl [pH 7.4], 6 mM EDTA, 1% Nonidet P-40, 10 U of the protease inhibitor aprotinin per ml) and preabsorbed by incubation with 100 μl of nonimmune mouse immunoglobulin G (10 μg) bound to fixed Staphylococcus aureus cells (Calbiochem) at 4°C for 2 h. S. aureus cells were removed by centrifugation (10,000 × g for 10 min), and this precleared lysate was immunoprecipitated with 100 μl of C11D8 (20 μg)-bound protein A-Sepharose beads for 3 to 4 h at 4°C with rotation (as described above). Sepharose beads were pelleted by centrifugation, and the supernatant was aspirated. Beads were washed four times in wash buffer (0.1% Nonidet P-40, 0.02% SDS, 150 mM NaCl, 50 mM Tris-Cl [pH 7.4], 5 mM EDTA, 10 U of aprotinin per ml). A final wash without detergent was performed, and the supernatant was removed completely. The beads were then boiled in 100 μl of electrophoresis sample buffer (60 mM Tris [pH 6.8], 10% glycerol, 2% SDS, 5% β-mercaptoethanol, 1 μg of bromophenol blue per ml) for 2 to 3 min and analyzed by SDS-PAGE under reducing conditions.

**Coprecipitation of putative receptor with gp70.** (i) Intact cells. Coprecipitation of receptor with gp70 by using C11D8 was performed by the method of McDougal et al. (38). Cell
surfaces (FeT or HuT78) were labeled with $^{125}$I by the glucose oxidase-lactoperoxidase technique (61). Briefly, exponential-phase cells were washed twice in PBS and once in PBS containing 1 mM potassium iodide. Cells (4 x 10$^7$) were then resuspended in 500 μl of PBS containing 10 mM glucose, and 25 μl of lactoperoxidase (2 mg/ml), 100 μl of glucose oxidase (15 U), and Na$^{125}$I (1 mCi, carrier free) were added sequentially. The reaction was continued at 24°C with occasional mixing and stopped after 30 min by dilution in 50 volumes of cold PBS containing 5 mM potassium iodide and 0.02% sodium azide and centrifugation (400 x g for 10 min). The $^{125}$I-labeled cells were finally resuspended in reaction buffer (PBS containing 1 mM CaCl$_2$, 55 mM glucose, and 1 mg of BSA per ml) at a cell concentration of 3 x 10$^7$/ml. Cells (1 ml) were incubated with unlabeled gp70 (10 μg) for 2 h at room temperature with rotation, unbound gp70 was removed by washing with reaction buffer, and the cells were lysed with 1 ml of lysis buffer (0.02 M Tris [pH 8.0], 0.12 M NaCl, 0.2 mM phenylmethylsulfonyl fluoride, 5 μg of aprotinin per ml, 0.2 mM EGTA, 0.2% sodium deoxycholate, 0.5% Nonidet P-40) for 1 h at 4°C. Nuclei were removed by centrifugation at 3,000 x g for 20 min, and the supernatant was preabsorbed by mixing with 100 μl of nonimmune mouse immunoglobulin G (10 μg) bound to Protein A-Sepharose for 3 h at 4°C. Preabsorbed beads were washed once with lysis buffer, once with lysis buffer containing 0.5 M NaCl, and once with lysis buffer containing 0.1% SDS. Beads were then mixed with 100 μl of electrophoresis sample buffer and heated at 65°C for 30 min. After centrifugation, the supernatant was analyzed by electrophoresis on an SDS-7.5% acrylamide gel with a 3% stacking gel. The gel was then fixed, dried, and autoradiographed.

(ii) Cell membranes. After $^{125}$I labeling, plasma membranes were isolated by sucrose density gradient centrifugation by the method of Maeda et al. (37). FeT or HuT78 cells were washed twice with 10 mM Tris-saline (pH 7.5), resuspended in homogenization buffer (10 mM sodium phosphate [pH 7.4] containing 1 mM MgCl$_2$, 30 mM NaCl, 0.005 mM phenylmethylsulfonyl fluoride, and 0.02% sodium azide) at a cell concentration of 3 x 10$^7$/ml, and then homogenized in a loose-fitting Dounce homogenizer, using 20 to 25 strokes for cell disruption (monitored under a light microscope). About 25 ml of the homogenate was layered over 10 ml of 41% sucrose in homogenization buffer and centrifuged at 95,000 x g for 1 h in a Beckman SW27 swinging-bucket rotor. A white interface band of enriched membrane was collected, sucrose was diluted by adding a two- to threefold excess of homogenization buffer, and membranes were pelleted by centrifugation at 95,000 x g for 20 min. Membranes were washed twice and used immediately or stored at -80°C until used. Protein concentration was determined by the method of Bradford (5) with bovine gamma globulin as the standard. Coprecipitation of labeled cell membrane with gp70 was then performed as described above.

RESULTS

Purification and binding of FeLV envelope glycoprotein (gp70) to FeT cells. The gp70 used in the present study was purified from a detergent-solubilized concentrated virus preparation by immunoaffinity chromatography with the anti-gp70 MAb, C11D8. A single major band of protein of 70 kDa was observed after electrophoresis and silver staining (Fig. 1A, lane 2). After $^{125}$I labeling, a major band of 70 kDa was found along with two minor bands (46 and 32 kDa) (Fig. 1B). About 80% of the radioactivity from $^{125}$I-labeled gp70 could be precipitated with 10% trichloroacetic acid, and the final specific radioactivity of iodinated gp70 was 1.5 x 10$^{-4}$ to 2 x 10$^{-5}$ cpm/ng of protein.

Binding of $^{125}$I-labeled gp70 to cells grown in the log phase was measured, and saturation of cellular binding sites was achieved by using 5 x 10$^7$ cells and increasing concentrations of $^{125}$I-labeled gp70. The apparent saturation of receptor sites on FeT cells was achieved by the addition of approximately 80 ng of gp70, with the binding of 1 ng of gp70 or approximately 1.4 x 10$^{-14}$ mol (Fig. 2A). Under the same conditions, a small linearly increasing amount of gp70 was found to bind HuT78 cells, which we considered to be nonspecific. The amount of gp70 bound to HuT78 cells was subtracted from the amount bound to FeT cells to determine the specific binding to FeT cells (Fig. 2B, inset). The specificity of gp70 binding to FeT cells was further demonstrated through viral interference. The saturation level of gp70 binding was reduced to 44% when FeT cells chronically infected with FeLV-A were used (data not shown).

Scatchard analysis (52) of saturation binding curves shown in Fig. 2B yielded linear plots, indicative of the presence of a single class of receptor sites. Two separate binding studies yielded similar curves, with apparent affinity constants ($K_a$) of 1.2 x 10$^9$ and 1.1 x 10$^9$ M$^{-1}$ in the two experiments (average $K_a = 1.15$ x 10$^9$ M$^{-1}$). The average number of available virus-binding sites per cell predicted by the intercepts on the horizontal axis of the Scatchard plot was 1.6 x 10$^9$. Thus, FeT cells possess a moderate number of single high-affinity binding sites per cell (Table 1).

Identification of putative gp70-receptor complex. Affinity cross-linking has been used to investigate the interaction of various peptide hormones, growth factors, and their receptors (32, 46, 53, 58, 67) as well as the interaction of human immunodeficiency virus and CD4$^+$ T cells (38). In this study, we used the homobifunctional cross-linking reagents DSS (noncleavable) and DSP (cleavable under reducing conditions) to detect membrane components that interact with gp70. FeT cells and HuT78 cells were incubated with $^{125}$I-labeled gp70, and then DSS or DSP was added to covalently...
cross-link gp70 to the surface of intact cells. Detergent lysates of treated cells were prepared and directly analyzed by electrophoresis on a 6% acrylamide gel under nonreducing conditions (Fig. 3). One major cross-linked band of 135 to 140 kDa was found in addition to gp70 (lanes c and d). In contrast, no such cross-linked band was visible in HuT78 cells, which binding studies had suggested were receptor negative (lane e). Further evidence of specific binding to FeT cells was obtained by examining cross-linked products after preincubation with an excess of unlabeled gp70 and after examination of cells chronically infected with FeLV-A. No cross-linked bands were visible when FeT cells were incubated with a 600-fold excess of unlabeled gp70 (Fig. 3, lane b) or virus (lane a) for 1 h at room temperature before the addition of labeled gp70. In addition, the intensity of the 135- to 140-kDa cross-linked band was decreased by about 50% in chronically FeLV-A-infected cells (Fig. 3, lane f) compared with uninfected cells, consistent with the reduced availability of receptor described above. To address the possibility that the 135- to 140-kDa cross-linked band resulted from dimerization of gp70, we performed the experiment under the same conditions using ^125I-labeled gp70 and incubation in the absence of cells. Under these conditions, no cross-linked band was detected (Fig. 3, lane g).

When examined under reducing conditions, the DSS-cross-linked band was retained, whereas the DSP-cross-linked band disappeared, indicating dissociation of the gp70-receptor complex. These experiments indicate that gp70 binds to a protein on the FeT cell surface having a molecular mass of approximately 65 to 70 kDa.

Immunoprecipitation of DSS-crosslinked ^125I-gp70 and cell lysates with the anti-gp70 MAb, C11D8, also revealed a 135- to 140-kDa band (Fig. 4, lane b) in addition to gp70. In contrast, parallel immunoprecipitation of HuT78 cell lysates failed to reveal any bands above the size of gp70 (Fig. 4, lane c). Because of the large quantity of BSA present in these binding experiments, we included antibody to BSA as an additional control in immunoprecipitations. No radiolabeled bands were visible when anti-BSA antibody was used (Fig. 4, lane d), indicating that the 135- to 140-kDa band did not result from cross-linking of ^125I-labeled gp70 to BSA. In addition, nonimmune mouse immunoglobulin G2b (same subclass as C11D8) did not precipitate a detectable band (Fig. 4, lane e). Last, the detectable level of the cross-linked band was reduced in virus-infected cells (Fig. 4, lane f). Thus, the 135- to 140-kDa band appears to result from formation of a specific complex between gp70 and its putative receptor.

Coproccipitation of 70-kDa cell surface molecule with gp70. To directly identify the cell surface molecule that binds gp70, cells were surface labeled with ^125I by the glucose oxidase-lactoperoxidase technique (as described in Materials and Methods). Subsequently, these labeled cells were incubated with or without unlabeled gp70 for 2 h in binding buffer, unbound gp70 was removed by washing, and a detergent lysate of the cells was immunoprecipitated with the anti-gp70 MAb, C11D8 (Fig. 5). A single band with a molecular mass of approximately 70 kDa was found to coprecipitate from FeT cells in the presence of gp70 (Fig. 5, lane b) but not in the absence of gp70 (Fig. 5, lane c). Incubation of cells with 10 mM EDTA prevented the coprecipitation of the 70-kDa cell membrane protein (Fig. 5, lane d). Also, no such band with a similar molecular mass was coprecipitated from HuT78 cells either in the presence (Fig. 5, lane e) or in the absence (Fig. 5, lane f) of gp70. To rule out the possibility that some free ^125I may have been trapped on the cell or carried over in the reaction mixture, resulting in transfer of label to gp70, we prepared membranes from labeled cells on sucrose density gradients. The labeled membranes were then incubated with unlabeled gp70 and immunoprecipitated with C11D8 (Fig. 6). The results were identical to that seen with intact cells: a 70-kDa protein band was coprecipitated in the presence (Fig. 6, lane b) but not in the absence (Fig. 6, lane c) of gp70.
TABLE 1. Characteristics of several virus-cell receptor parameters

<table>
<thead>
<tr>
<th>Virus*</th>
<th>Cell</th>
<th>Dissociation constant ($K_d$) (nM), association constant ($K_a$)</th>
<th>No. of binding sites/cell</th>
<th>Reference</th>
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</thead>
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<td>0.87, 1.15</td>
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<tr>
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<td>Murine L</td>
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<td>Mo-MuLV</td>
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<td>0.83, 1.2</td>
<td>$1.3 \times 10^4$</td>
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<tr>
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<td>NIH 3T3</td>
<td>0.12, 8.3</td>
<td>$4 \times 10^4$</td>
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<tr>
<td>HIV</td>
<td>CEM</td>
<td>4, 0.25</td>
<td>$1.7 \times 10^4$</td>
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<tr>
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<td>$5.26 \times 10^4$</td>
<td>60</td>
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<tr>
<td>Reovirus</td>
<td>Rat endothelial</td>
<td>0.5, 2.0</td>
<td>$6.8 \times 10^4$</td>
<td>63</td>
</tr>
</tbody>
</table>

* HIV, human immunodeficiency virus; HCMV, human cytomegalovirus virus; Mo, Moloney; Ra, Rauscher.

**DISCUSSION**

We describe here (i) the purification of FeLV-A gp70 by immunoaffinity chromatography; (ii) specific and saturable binding of gp70 to FeT cells; (iii) immunoprecipitation of cross-linked gp70-receptor complex; and (iv) coprecipitation of a gp70-binding protein from the surface of FeT cells. The method of purification of gp70 was important because the requirements for binding of gp70 to its receptor are more stringent than those required for antigenicity. For example, it has been reported that MuLV envelope glycoprotein prepared by the guanidine hydrochloride-agarose method (16) was precipitable with a specific antiserum to gp71, but neither bound to murine cells nor competed for the binding sites when concentrations as high as 18 μg/ml were used (10). In contrast, the gp70 purified here by immunoaffinity chromatography from detergent-solubilized FeLV is almost homogeneous and retains its capacity to bind to feline cells. The specific and saturable binding of radiolabeled gp70 to feline T cells but not to human T cells is consistent with the host range of FeLV-61E-A, which is restricted to growth in feline cells (47). The characteristics of gp70 binding to FeT cells are comparable with those of several other virus-receptor interactions (Table 1) and indicate that FeT cells possess a moderate number of relatively high-affinity gp70-binding sites.

From the molecular mass of gp70-receptor complex (135 to 140 kDa), formed by chemical cross-linking and immunoprecipitated with an anti-gp70 MAb, the molecular mass of putative receptor was calculated to be 65 to 70 kDa. This is confirmed by the coprecipitation with gp70 of a single protein molecule of approximately 70 kDa from intact cells or purified cell membranes by using an anti-gp70 MAb. The partial interference of gp70 binding to chronically FeLV-A-infected FeT cells (data not shown) as well as a lowered efficiency of gp70-receptor complex formation suggests that receptors on infected cell surfaces are only partially occupied or blocked by viral glycoprotein and/or the receptors are not completely down-regulated. Consistent with these
The failure of the neutralizing MAb C11D8 (which binds to a constant region near the center of gp70) (15) to prevent receptor binding suggests that sequences involving the C11D8 epitope are involved in a postattachment phase of viral entry. The region of gp70 imparting A and C subgroup specificity, in contrast, is found near the N terminus of gp70 (7). The characterization of a cellular receptor for FeLV and subsequent glycoprotein-receptor interactions may therefore lead to further understanding of the mechanisms of FeLV pathogenesis.

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