A Putative Thiamine Transport Protein Is a Receptor for Feline Leukemia Virus Subgroup A

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Feline leukemia virus (FeLV) is a horizontally transmitted virus that causes a variety of proliferative and immunosuppressive diseases in cats. There are four subgroups of FeLV, A, B, C, and T, each of which has a distinct receptor requirement. The receptors for all but the FeLV-A subgroup have been defined previously. Here, we report the identification of the cellular receptor for FeLV-A, which is the most transmissible form of FeLV. The receptor cDNA was isolated using a gene transfer approach, which involved introducing sequences from a feline cell line permissive to FeLV-A into a murine cell line that was not permissive. The feline cDNA identified by this method was approximately 3.5 kb, and included an open reading frame predicted to encode a protein of 490 amino acids. This feline cDNA conferred susceptibility to FeLV-A when reintroduced into nonpermissive cells, but it did not render these cells permissive to any other FeLV subgroup. Moreover, these cells specifically bound FeLV-A-pseudotyped virus particles, indicating that the cDNA encodes a binding receptor for FeLV-A. The feline cDNA shares ~93% amino acid sequence identity with the human thiamine transport protein 1 (THTR1). The human THTR1 receptor was also functional as a receptor for FeLV-A, albeit with reduced efficiency compared to the feline orthologue. On the basis of these data, which strongly suggest the feline protein is the orthologue of human THTR1, we have named the feline receptor feTHTR1. Identification of this receptor will allow more detailed studies of the early events in FeLV transmission and may provide insights into FeLV pathogenesis.

Retroviral infection is initiated by binding of the viral envelope glycoprotein surface unit (SU) to a specific cellular receptor. This specific SU-receptor interaction begins a series of events, including fusion of the viral membrane to the host cell membrane, that result in viral entry into the host cell. Therefore, the tropism of retroviruses is determined in part by whether the target cell expresses a surface receptor protein that is capable of binding the viral SU protein. Once cells are infected and the viral envelope protein is expressed in the cell, the viral receptor is typically downmodulated, thereby preventing successive rounds of infection of the same cell, a process referred to as superinfection interference (22, 69). The relative ability of a virus to cause superinfection interference of another virus has been used to define viruses that use the same receptor (22, 45).

Analysis of the superinfection interference properties of feline leukemia virus (FeLV) variants has led to the identification of four interference groups (3, 23, 56–58). These interference groups correspond to the FeLV subgroups A, B, C, and T, each of which has unique receptor requirements (3, 4, 23, 25, 41, 48, 56–58, 66, 67). FeLV is horizontally transmitted and causes both suppressive and neoplastic diseases in infected cats (19, 27). FeLV-A has been found in all naturally infected cats examined (24). It is in part for this reason that FeLV-A is considered the most transmissible form of FeLV (13, 26, 27). In support of this, FeLV-A has been found to be more infectious in experimental infection studies than the other FeLV

subgroups (26, 59). FeLV-A replicates in feline cells, but is more restricted in its replication in cells of other species (13, 19, 24–26, 52, 59). FeLV-A-pseudotyped viruses are capable of entering some human and dog cell types, although none of the nonfeline cells support infection to the same level as feline cell types (41, 42). This suggests that human and dog cells express a receptor for FeLV-A, but it may be suboptimal compared to the feline receptor orthologue.

The cellular receptors for FeLV types B, C, and T have been defined. FeLV-B uses the inorganic phosphate transporter Pit1 and the closely related Pit2; these receptors are also used by gibbon ape leukemia virus and certain murine leukemia viruses (4, 7, 28, 38, 39, 43, 67, 68). FeLV-B is capable of using both feline and human orthologues of Pit1 (43, 67), but can only use feline Pit2, not human Pit2, as a receptor (7, 39, 68). FeLV-T also uses Pit1 as a receptor, but it requires a second host protein called FeLIX for entry (3, 18, 53, 54). FeLIX, which is a secreted protein, is expressed primarily in lymphoid tissue, explaining the observation that FeLV-T exhibits a narrow tropism, limited to T cells, in vitro (34). The FeLV-C receptor (FLVCR) is a heme export protein (49), and both feline and human FLVCR proteins function as receptors for FeLV-C (48, 66). As predicted by interference studies, none of the currently identified viral receptors, Pit1, Pit2, or FLVCR, function as viral receptors for FeLV-A (41, 42, 56).

Many retroviral receptors have been identified using a gene transfer approach (2, 6, 48, 50, 64). This approach relies on the introduction of coding sequences from a susceptible cell type into a nonsusceptible cell, often using retroviral transduction of a cDNA library. The success of this approach relies heavily on the identification of a cell line that is not permissive to the virus of interest due to a block at entry, rather than a block at

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other stages in the virus life cycle. Previous studies have demonstrated that *Mus dunni* tail fibroblast (MDTF) cells are resistant to FeLV-A infection, but they can support productive infection by FeLV-B, -C, or -T if engineered to express the appropriate cognate receptor (3, 63). This suggests that there are no postentry restrictions to FeLV infection in MDTF cells.

Here we describe the identification of the FeLV-A receptor using gene transfer of feline cDNA into MDTF cells. Because FeLV-A represents the transmissible form of FeLV, the identification of its cellular receptor may provide important clues regarding the earliest target cells for FeLV infection. Moreover, FeLV provides a valuable model to study the process of retroviral evolution in the host because the other FeLV subgroups can arise from FeLV-A in infected cats (8, 53). This evolutionary process may reflect adaptation of the virus to find new target cells in the host during persistent infection, a process that is also thought to occur in other retroviral infections, including human immunodeficiency virus infection of humans (10, 45, 60, 61).

MATERIALS AND METHODS

Cell culture. AH927 feline fibroblasts, *Mus dunni* tail fibroblast cells (33), and 293T human embryonic kidney cells were maintained in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U penicillin per ml, 100 μ g streptomycin per ml, 0.25 μ g amphotericin B per ml (referred to as complete DMEM). When indicated, G418 (geneticin, Gibco-BRL) was added at 0.6 mg/ml, or histidinol (Gibco-BRL) was added at 1 nM concentration in histidine-free DMEM (Gibco-BRL). All MDTF cell lines expressing feline receptor proteins (MDTF-feTHTR1, MDTF-huTHTR1, and MDTF-fePit1, MDTF-FLVCR) were maintained in complete DMEM with 0.6 mg/ml G418.

Plasmids and constructs. The retroviral vector LAPSN expresses the human alkaline phosphatase (AP) and neomycin phosphatase (*neo*) genes, LXSHD expresses the histidinol dehydrogenase (*hisD*) gene, and both have been described previously (38, 62). The retroviral vector pMX used for feline cDNA library expression, is derived from pBabe-puro and does not express a selectable marker (31).

Preparation of viral supernatants. To generate pseudotyped viruses, cells were transfected with equal amounts of the appropriate FeLV envelope expression construct (41), an FeLV *gag-pol* expression construct (61E-FeLV- $\Delta\Psi$ -*gag-pol*) (63), and murine leukemia virus-based vector genomes encoding various marker genes: *his*, LXSHD (38), *neo* and alkaline phosphatase, LAPSN (38); β -galactosidase, pRT43.2Tnls β gal1 (30); or the green fluorescent protein (GFP), pRT43.2GFPbn1 (a gift from M. Eiden). Pseudotyped viruses are referred to here by the subgroup of their envelope protein and the marker gene carried on the vector genome: e.g., FeLV-A carrying the GFP reporter gene is called FeLV-A/GFP.

Single-cycle infection assay. Target cells were plated at 2.0×10^4 cells/well in 24-well dishes the day before infection. The next day, culture media were replaced with media containing 4 μ g/ml Polybrene. Cells were infected with viral pseudotypes carrying the gene for β -galactosidase, GFP, alkaline phosphatase, or *His*. In some cases, 100 μ l of conditioned-medium FeLV was added to cells prior to infection as described previously (3, 34). At 48 h postinfection, cells were either stained for β -galactosidase expression (30) or examined by fluorescence microscopy for GFP expression or transferred to drug selection media for neomycin resistance and histidine selection.

Retroviral cDNA library screen. The construction of the cDNA library from a feline fibroblast cell line (AH927) was performed using methods similar to those described previously for the construction of a feline T-cell cDNA library (3, 31). Briefly, mRNA was isolated from AH927 cells using Fast Track (Invitrogen, Carlsbad, CA), and used as a template for cDNA synthesis using Super Script II murine leukemia virus reverse transcriptase enzyme (Gibco-BRL). Following ligation of the cDNA to BstXI adapters with the sequence 5'-CTTTCAGCA CA-3' (Invitrogen, Carlsbad, CA.), the cDNA was ligated to gel-purified, BstXI-digested pMX DNA (31). The ligation was transformed into Ultra-competent cells, and success of ligation was confirmed by digestion with BamHI and Gene-Checker (Invitrogen, Carlsbad, CA) was used to define whether various cDNAs were represented in the library and whether they were of sufficient length.

Viral particles packaging the AH927 cDNA library in the pMX vector were generated by cotransfection of two 10-cm dishes of HEK293T cells. Each received 10 μ g of library cDNA along with 10 μ g each of 61E-FeLV- $\Delta\Psi$ -*gag-pol* and the murine leukemia virus amphotropic murine leukemia virus envelope (SV-A-MLV-env) (37). Virus was harvested 48 h posttransfection and filtered through 0.22- μ m filters (syringe filters, Millipore, Billerica, MA). Ten cultures of MDTF cells that had been seeded the previous day at 2×10^5 cells per 10-cm plate received 2 ml of the harvested supernatant. The next day the cultures were split 1:2. About 24 h later, the 12 cultures were infected at a multiplicity of infection of ~ 1.0 with FeLV-A/*neo* in a 10-ml volume with 4 μ g per ml Polybrene. After 24 h, the cultures were placed under selection in G418. After 10 to 14 days of selection single-cell clones were visible, and a subset of these clones was selected for further study. Viral receptor activity was tested by single-cycle infection assay using FeLV-A/GFP as described above.

To rescue the transduced pMX cDNA, primary MDTF cell clones were cotransfected with plasmids encoding 61E-FeLV- $\Delta\Psi$ -*gag-pol* and SV-A-MLV-env. The resulting virus would be expected to package the pMX vector containing the AH927 cDNA expressed in the cell line. The virus was collected, filtered through a 0.2- μ m filter, and used to infect MDTF cells seeded the previous day at 2×10^5 cells per 10-cm dish. Two days later, the cells were challenged with two FeLV-A-pseudotyped vector FeLV-A/*his*. After 10 to 14 days in selection with histidinol, single-cell clones were isolated and expanded. These single-cell clones are referred to as secondary clones and represent cells derived from rescued virus from the primary clones identified in the original screen. Secondary clones were screened for receptor activity by single-cycle infection assay using FeLV-A/GFP, as described above.

Receptor cDNA cloning. Genomic DNA from FeLV-A-susceptible secondary cell clones was isolated and used as the template for PCR amplification. Amplification primers that anneal to the sequence of the pMX vector (pMX#11, 5'-GTGGACCATCTCTAGACTGC-3', and pMX#14, 5'-GAAAATAAAAT AGCAGCTGGTGACACG-3') were used (48). Initial attempts to amplify a specific product using previously described PCR conditions were unsuccessful (48). Successful amplification was achieved using the Fail Safe kit (Epicenter) and cycle parameters of 35 cycles at 95°C for 1 min and 68°C for 5 min, with a final extension at 72°C for 5 min. The reactions were analyzed by gel electrophoresis. Of the 12 premixed buffers supplied with the Epicenter Fail Safe kit, only one buffer system, buffer H, produced a detectable PCR product of approximately 3.5 kb. The 3.5-kb PCR product was isolated following gel electrophoresis, and cloned into the mammalian expression vector pcDNA3.1/V5-His (TOPO TA cloning kit, Invitrogen, Carlsbad, CA). The feline receptor cDNA insert was then subcloned into a retroviral vector genome by digestion with BamHI, followed by gel electrophoresis. The isolated 3.5-kb fragment was ligated into BamHI-digested pLXSN to generate the L(feTHTR1)SN vector.

To generate the L(huTHTR1)SN vector, the human THTR1 cDNA (5) was introduced into pLXSN in a similar manner.

Sequencing and amino acid alignments. Primers that recognized sequences in the pcDNA 3.1 vector, just 5' and 3' of the insert, were used to initiate nucleotide sequence analysis. Subsequent primers were designed as insert sequences were obtained. The 3.5-kb sequence was compared to the NCBI database using the BLAST Search program. The predicted amino acid sequences were aligned to the human THTR1, protein accession number NM_06996, using BioEdit.

Generating cell lines expressing feline and human THTR1. Viral particles packaging L(feTHTR1)SN and L(huTHTR1)SN vector genomes were generated in HEK293T cells using CaPO₄ transient transfection (Stratagene); 10 μ g of each plasmid, SV-A-MLV-env, FeLV- $\Delta\Psi$ *gag-pol*, and either pL(feTHTR1)SN or pL(huTHTR1)SN, was cotransfected into HEK293T cells. The following day cells were washed with phosphate-buffered saline and 8 ml of medium was added. Two days posttransfection, supernatant was collected and filtered through a 0.22- μ m filter, and 1 to 2 ml was used to infect MDTF cells seeded in 6-cm dishes at 2×10^5 cell/plate. Polybrene, at a concentration of 4 μ g/ μ l, was added to the infection. At 24 h postinfection, cells were split into 10-cm dishes and maintained in complete DMEM containing G418 at a concentration of 0.6 mg/ml. The medium was replaced every 2 days with complete DMEM containing G418. After 10 to 14 days in selection, single-cell colonies became visible. These single-cell colonies were isolated and expanded.

Flow cytometry-based binding assay. The details of the flow cytometry assay for FeLV binding have been described previously (34). Briefly, cells were incubated in conditioned medium containing FeLV-A-pseudotyped virus at a density of 2×10^5 cells/ml on a rocking platform. The cells were then washed and resuspended in 200 μ l of Hanks' balanced salt solution containing anti-FeLV gp70 monoclonal antibody (C11D8, Custom Monoclonal Antibodies, Sacramento, CA), incubated at 4°C for 1.5 h, after which cells were pelleted, washed, and resuspended in 150 μ l of a 1:1,000 dilution of R-phycoerythrin conjugated

goat anti-mouse antibody (DAKO, Carpinteria, CA) and incubated at 4°C for 45 min. The cells are analyzed by fluorescence-activated cell sorter (Becton Dickinson FACS Calibur Cytometer).

RNA isolation and Northern blot analysis. Feline tissue from an uninfected cat was used to extract total RNA from liver, spleen, small intestine, kidney, lymph node, lung, muscle, monocytes and T cells; 10 µg of total RNA from each sample was mixed with 1.3 volumes of formaldehyde/morpholinepropanesulfonic acid (MOPS) gel loading buffer (Ambion, Austin, TX) and electrophoresed on a 1% agarose/formaldehyde/MOPS gel. Gels were transferred to nylon membranes (hybond-XL, Amersham) by capillary using 20× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) (Mediatech). The probe for feTHTR1 was made from a gel-isolated PCR-generated fragment from the L(feTHTR1)SN corresponding to a ~600-bp portion of the coding region of the cDNA.

A ³²P-labeled DNA probe was generated using Klenow random labeling kit (Roche), purified by spin column and phenol-chloroform extraction. Hybridization was carried out overnight at 68°C in 5% sodium dodecyl sulfate, 0.5 M Na₂HPO₄, 1 mM EDTA, 100 µg/ml salmon sperm DNA, and 10⁶ cpm of probe per ml of buffer. Membranes were washed twice at room temperature with 2× SSC–0.1% sodium dodecyl sulfate (SDS), twice with 1× SSC–0.1% SDS, and followed by one wash at 58°C with 0.1× SSC–0.1% SDS. Membranes were then exposed to film with intensifying plates at –80°C.

Nucleotide sequence accession number. The feline THTR1 sequence has been submitted to GenBank under accession number DQ391281.

RESULTS

Identification of a cDNA that renders cells permissive to FeLV-A. To identify a feline cDNA that permits entry by FeLV-A, nonpermissive MDTF cells were transduced with a cDNA library from permissive feline cells (AH927 cells). These MDTF cells were infected with a FeLV-A/neo, and infected cells were identified by their ability to grow in G418. Using this method, approximately 500 drug-resistant colonies were detected among cultures transduced with the feline cDNA library; one colony was detected in three cell cultures transduced with the pMX vector alone carried out in parallel. Of the numerous primary colonies generated, 10 were isolated, expanded, and retested for receptor activity by single-cycle infection assay using FeLV-A/GFP. All 10 primary clones were permissive to FeLV-A-pseudotyped virus, whereas MDTF cells, and MDTF cells transduced with an empty vector, were not infectible by FeLV-A (data not shown). These data suggest that the feline cDNA in the primary cell clones isolated from this screen renders MDTF cells permissive to FeLV-A infection.

Our initial attempts to identify a feline cDNA among the 10 primary cell clones using PCR were not successful. In most primary cell clones, multiple PCR products were amplified using primers specific to the pMX vector that was used to introduce the feline cDNA library, and these products were not seen in PCRs of MDTF cells that were not transduced. However, none of the PCR products were common to different susceptible primary cell clones, and none of the PCR-amplified cDNAs conferred susceptibility when transferred to MDTF cells (data not shown). Because most of the primary cell clones contained multiple feline cDNAs, the pMX retroviral genomes in these cell clones were rescued into retroviral particles, and transferred into MDTF cells at low multiplicity of infection. This was done for three different primary cell clones, and the resulting transduced cells were infected with FeLV-A/his. Susceptible cells were identified by their ability to grow in the presence of histidinol-containing medium.

In total, 180 secondary cell clones were obtained under drug selection. A subset of these ($n = 20$) were infected by FeLV-

A/GFP and GFP-positive cells were detected in all cases, whereas in the mock-transduced MDTFs no GFP-positive cells were seen. These findings indicate that a feline cDNA in both the original primary cell clones and the secondary cell clones generated by vector genome rescue encoded a protein that rendered MDTF cells permissive to FeLV-A.

The feline cDNA was amplified from the genomic DNA of three secondary clones that were permissive to FeLV-A/GFP. A PCR product of 3.5 kb was consistently amplified from each clone. This 3.5-kb PCR product is consistent with the size of other retroviral cellular receptors in this family, and was isolated for further study (43, 64, 66, 67).

Expression of the 3.5-kb feline cDNA in MDTF cells confers susceptibility to FeLV-A infection. To address whether the 3.5-kb feline cDNA, which we have named feTHTR1, rendered cells permissive to FeLV-A, we generated a retroviral expression vector containing the cDNA, introduced it into MDTF cells, and challenged the cells for infectivity to FeLV-A-, -B-, -C-, and -T-pseudotyped vectors carrying a β-galactosidase reporter gene. MDTF cells expressing the feTHTR1 cDNA (MDTF-feTHTR1) were susceptible to FeLV-A transduction, with the titers of virus for these cells ($\sim 2.8 \times 10^5$ focus-forming units [FFU]/ml) reaching levels that were similar to those observed for AH927 ($\sim 5 \times 10^5$ FFU/ml) tested in parallel (Fig. 1). MDTF cells that received an empty vector or no vector remained resistant to FeLV-A infection (< 10 FFU/ml). In addition, MDTF-feTHTR1 cells were not susceptible to FeLV-B, -C, or -T when infected at doses of 10⁴ or greater, as judged by infection of AH927 cells and infection of MDTF cells engineered to express their cognate receptor (Pit1, FLVCR, or Pit1 plus FeLIX, respectively; Fig. 1). These data demonstrate that the 3.5-kb feline cDNA (feTHTR1) encodes a receptor that is specific for FeLV-A-pseudotyped virus infection; this receptor does not support entry of viruses from other FeLV subgroups.

Characterization of feline receptor cDNA reveals significant homology to the human high-affinity thiamine transport 1 protein. Analysis of the structure of the feline cDNA revealed a large open reading frame of 1,470 bp flanked by untranslated regions at the 5' and 3' ends of 187 bp and 1.7 kb, respectively. The open reading frame is predicted to encode a polypeptide of 490 amino acids that shares approximately 93% homology with the human (*Homo sapiens*) high-affinity thiamine transporter 1 (huTHTR1; SLC19A2). FeTHTR1 also shows homology to other thiamine transporter proteins, ranging from ~85 to ~75% homology to the *Mus musculus* and *Rattus norvegicus* orthologues.

Figure 2 shows an alignment of the predicted amino acid sequences of the polypeptide encoded by the feline cDNA and huTHTR1. There are 33 amino acid differences between the two proteins (Fig. 2). Additionally, there are two deletions, one of two amino acids and one of five amino acids, clustered in two areas that differentiated the feline from the human protein. These findings suggest that the feline cDNA that confers FeLV subgroup A entry is the feline orthologue of huTHTR1.

huTHTR1 protein can also render MDTF cells permissive to FeLV-A. Given the strong sequence homology we observed between the feline and human THTR1 proteins, and the observation that FeLV-A is capable of infecting certain human

FIG. 1. Infection of selected cell lines by FeLV viral pseudotypes carrying a vector encoding β-galactosidase. The x axis indicates the cell line tested. The y axis indicates the results of a single-cycle infection as measured by log₁₀ of β-galactosidase FFU per milliliter. The coding for the viruses tested, which included FeLV-A, FeLV-B, FeLV-C, and FeLV-T for each cell line, is indicated to the right of the graph. In the case of FeLV-T infections, 100 μl of conditioned medium containing the cofactor FeLIX was included. Data were obtained from three independent infections done in triplicate and are the averages of the nine infections. Errors bars are based on the standard deviation for all nine results as well. A zero value indicates that no β-galactosidase-positive cells were detected in any of the triplicate infections using up to 100 μl of viral supernatant.

cell lines (41, 42, 63), we hypothesized that the human THTR1 protein may act as a viral receptor for FeLV-A. To test this hypothesis, we generated MDTF cells expressing the human THTR1 cDNA (MDTF-huTHTR1) and infected them using FeLV-A viral pseudotypes. The titer of the FeLV-A-pseudotyped virus on MDTF-huTHTR1 cells was 1.5×10^4 FFU/ml, which was ~10- to 30-fold lower than the titer of this virus on AH927 cells or MDTF-feTHTR1 cells, which were 4.0×10^5 and 2.0×10^5 FFU/ml, respectively (Fig. 3). As a comparison, human embryonic kidney cells (HEK293T) were examined. HEK293T cells have previously been shown to be infectible by FeLV-A, but less efficiently than feline cells (41). The infectivity of FeLV-A-pseudotyped viral particles was 4.0×10^3 for HEK293T, somewhat lower than huTHTR1 cells

(Fig. 3). Thus, introduction of huTHTR1 into MDTF cells renders these cells permissive to FeLV-A infection, but less so than MDTF-feTHTR1 or AH927 cells.

Analysis of FeLV-A binding to feTHTR1- and huTHTR1-expressing cells. To determine whether the THTR1 proteins can bind the FeLV-A envelope protein, we examined binding of FeLV-pseudotyped particles to MDTF-feTHTR1 or MDTF-huTHTR1 cells. For this purpose, we used a monoclonal antibody directed to the SU protein to detect bound virus using flow cytometry (Fig. 4). There was no observable shift in fluorescence when FeLV-A-pseudotyped viral particles were incubated with MDTF cells expressing an empty vector, pLXSN, whereas there was a clear shift in fluorescence when FeLV-A was added to MDTF cells expressing-feTHTR1 (Fig. 4A). This

FIG. 2. Alignment of the predicted amino acid sequences of feTHTR1 and huTHTR1 in single-letter amino acid code. The alignment was generated using Bioedit. Dots indicate conserved residues, and positions where there are sequence differences between the feline and human orthologues are shown as letters. Gray boxes are predicted transmembrane domains based on the huTHTR1 topology plot based on previous studies (44).

FIG. 3. FeLV-A infection of cells expressing the human versus the feline THTR1 gene. AH927, HEK293T, MDTF-feTHTR1, MDTF-huTHTR1, and MDTF cells were infected with FeLV-A/β-gal. The x axis indicates the cell line tested. The y axis indicates the results of a single-cycle infection as measured by log₁₀ of β-galactosidase FFU per milliliter. Data were obtained from two independent infections done in duplicate. The results are reported as the averages of all four infections with error bars as the standard deviation. A zero value indicates that no β-galactosidase-positive cells were detected in any of the infections using up to 100 μl of viral supernatant.

binding was comparable to that seen with feline fibroblast AH927 cells (Fig. 4C). However, the shift in fluorescence was weaker when FeLV-A was incubated with MDTF-huTHTR1 cells than with cells incubated with antibody but no virus, however, this shift was reproducible in different experiments (Fig. 4B).

We did not detect any shift in fluorescence when FeLV pseudotypes that had the envelope proteins of other FeLV subgroups were incubated with MDTF-feTHTR1 or MDTF-huTHTR1 cells compared to fluorescence in MDTF cells (data not shown). The data from these experiments suggest that feTHTR1 can specifically bind to FeLV-A envelope protein; binding between huTHTR1 and FeLV-A SU was relatively weak and thus more difficult to detect by the methods used here.

Pattern of feTHTR1 expression. Total RNA was extracted from various feline tissue and probed with a partial feTHTR1 cDNA sequence (Fig. 5). The feTHTR1-specific signal (~3.2 kb) was widely distributed in feline tissue. Among the tissues studied, the most abundant expression was found in kidney, small intestine, and liver tissue. Lower levels of expression were found in the spleen, lymph node, and muscle tissue. Purified monocytes and T cells were also examined and found to express the highest levels of feTHTR1. β-Actin expression was similar for the various tissues, indicating that equivalent amounts of RNA were loaded for each sample.

DISCUSSION

We have identified a feline cDNA that confers susceptibility to FeLV-A infection. We showed by several criteria that a 3.5-kb feline cDNA that was isolated by a gene transfer strategy encodes the FeLV-A cellular receptor. Expression of this feline cDNA in normally nonpermissive MDTF cells resulted in specific binding of FeLV-A envelope protein and rendered the cells susceptible to FeLV-A infection. Analysis of the predicted polyprotein sequence encoded by this feline cDNA re-

FIG. 4. Flow cytometric analyses of FeLV-A binding to MDTF-feTHTR1, MDTF-huTHTR1, and AH927 cells. Binding was detected by staining cells with monoclonal antibody C11D8, which recognizes a conserved epitope in SU of all FeLV subgroups (17). The x axis shows fluorescence intensity (log scale) and the y axis shows cell number. Data shown are representative of independent experiments. (A) Binding of FeLV-A to MDTF-feTHTR1 cells. Areas outlined in light gray are MDTF cells exposed to FeLV-A followed by incubation with fluorescent antibody. The heavy black line profiles the results with MDTF-feTHTR1 cells incubated with FeLV-A-pseudotyped virus and C11D8 antibody. The dark-gray-filled histogram shows the results of MDTF-feTHTR1 cells exposed to C11D8 antibody only (no virus). (B) Binding of FeLV-A to MDTF-huTHTR1 cells. The coding for this panel is as described for panel A, except MDTF-huTHTR1 cells were used. (C) Binding of FeLV-A to feline fibroblast AH927 cells. The dark-gray-filled histogram shows the results of AH927 cells incubated with antibody only and no virus, representing background fluorescence. The heavy black line shows the results with AH927 cells and virus pseudotyped with FeLV-A envelope and C11D8 antibody.

vealed a high amino acid identity (~93%) to the human thiamine transport protein huTHTR1. Indeed, the human thiamine transport protein rendered cells permissive to FeLV-A, further indicating that the feline receptor is the orthologue of huTHTR1. Thus, the feline receptor is most likely a thiamine transport pro-

FIG. 5. Northern blot analyses of RNA from feline tissue. Total RNA from an adult cat was run on a 1% formaldehyde-agarose gel and the blot was probed with sequences specific for feTHTR1 (top panel) or β -actin (bottom panel). The positions of molecular size markers are designated on the left.

tein in feline cells, and we have therefore called this receptor feTHTR1.

In general, the receptors for many of the gammaretroviruses are multiple transmembrane proteins that transport small molecules. Some examples include mCAT, the cationic amino acid transporter utilized by ecotropic murine leukemia viruses (29); Pit1 and Pit2, the sodium-dependent phosphate symporters utilized by murine leukemia viruses, gibbon ape leukemia virus, and some FeLVs (28, 67, 68); and FLVCR, a heme export protein used by FeLV-C (48, 49, 66). Multiple membrane transport receptors are also used by other retroviruses, including beta- and deltaretroviruses. For example, certain human endogenous retroviruses and baboon endogenous virus use as receptors the neutral amino acid transporters ASCT1 and ASCT2 (50, 65). Human T-cell leukemia virus type 1 also appears to require a transport protein for infection, the glucose transporter Glut1 (36). Thus, the finding that the thiamine transport protein THTR1 is a receptor for FeLV-A is in keeping with the general preference for multiple membrane transport molecules as receptors for retroviruses.

The human THTR1 protein rendered MDTF cells permissive to FeLV-A, but MDTF/huTHTR1 cells were less readily infected than MDTF/feTHTR1 cells. These findings are consistent with previous findings showing that human cells were less permissive to FeLV-A than feline cells (9, 41, 42). Indeed, we found that FeLV-A infection was ~50- to 100-fold lower in human cells (HEK293T) and ~10- to 30-fold lower in MDTF-huTHTR1 cells compared to feline cells (AH927) and MDTF-feTHTR1 cells. This difference in susceptibility between human 293T cells and MDTF-huTHTR1 cells could be due to lower endogenous expression of the huTHTR1 in 293 cells. In addition, binding by FeLV-A was less readily detected with the human receptor versus the feline receptor. This may suggest that human

THTR has a lower binding affinity for FeLV-A, which may explain the lower infectivity observed with this receptor orthologue.

The THTR1 transporter belongs to the reduced folate family of transporters, SLC19, of which there are three members. There are two thiamine transporters, THTR1 and THTR2 and a reduced folate carrier, RFC1. The THTR1 and thiamine transporter 2 (THTR-2), which share ~65% sequence homology, both transport thiamine but not folate (14–16); the third member, RFC1, transports folate but not thiamine (40, 47, 70). Human THTR1 is expressed in absorptive tissue such as the small intestine, liver, and kidney, and is also expressed in skeletal muscle and peripheral blood leukocytes (12, 14). This pattern of THTR1 expression appears similar in feline tissue, where high levels of feTHTR1 are found in small intestine, liver, and kidney. FeTHTR1 was also expressed at relatively high levels in cells of the lymphoid system; the expression pattern of huTHTR1 has not been examined for the lymphoid system in humans.

This broad pattern of feTHTR1 expression is consistent with the observation that FeLV-A infects many tissues in the infected cat (21, 55). It will be of interest to examine the expression of different feTHTR1 cell populations in the oral mucosa, as this is a presumed site of FeLV-A shedding as well as a possible portal of entry for the virus (19, 27). The observed expression of feTHTR1 in lymphoid cells is consistent with reports that in the early phase of FeLV-A infection, the virus can be found replicating in mononuclear leukocytes in the local lymph nodes of the head and neck (55).

It is perhaps noteworthy that the autosomal recessive disorder Rogers syndrome, or thiamine responsive megaloblastic anemia, has been linked to mutations in the THTR1 gene (12, 32, 51). Rogers syndrome is characterized by the occurrence of megaloblastic anemia, diabetes mellitus, sensorineural deafness, and abnormalities of the retina and heart anomalies. The decrease in intracellular thiamine in these patients leads to decreased activity of enzymatic reactions that are dependent on thiamine pyrophosphate (46). These include enzymes in the pentose phosphate shunt pathway, namely the enzyme transketolase, which is involved in the production of ribose synthesis required for nucleic acids that are important for replication and cell function (46). Because megaloblastic anemia has been described in some cats with FeLV-infection (11, 20, 35), it is tempting to speculate that FeLV-A infection may disrupt thiamine transport function.

Down-regulation of this particular transporter in persistent retroviral infection could contribute in some manner to FeLV-induced disease. FeLV-A defines a unique receptor interference group among retroviruses; no other retroviruses are thought to share the same receptor (45). This receptor was one of the few remaining elusive retroviral receptors to be defined and may be of particular importance given the role of the A subgroup in FeLV transmission. Moreover, recent studies have indicated a role for the FeLV-C receptor, which functions in heme export, in the specific pathology associated with this FeLV subgroup (1, 49). Thus, the identification of feTHTR1 as the receptor for FeLV-A will allow further investigations into the mechanism of FeLV transmission and pathogenesis, including whether the function of the receptor in thiamine transport has any specific role in the disease process.

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