Identification of the Segments of the Mouse Transferrin Receptor 1 Required for Mouse Mammary Tumor Virus Infection*§

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Most enveloped viruses enter cells through binding of virion surface envelope proteins to receptors found on the plasma membrane of the cell. The beta retrovirus mouse mammary tumor virus (MMTV) uses transferrin receptor 1 (TfR1) to enter cells in a pH-dependent mechanism, probably co-trafficking with TfR1 to an acidic compartment where virus entry occurs. We have shown here that, although mouse and rat TfR1 function as entry receptors, cat, dog, hamster, or human TfR1s do not support MMTV infection. We also demonstrated that MMTV entry is independent of transferrin, iron, and the TfR1 cofactor hereditary hemochromatosis HFE protein. Using chimeric mouse/human hybrid TfR1 constructs, we determined the site of interaction with MMTV and found that it maps to two segments physically disparate from the TfR and HFE binding sites. Thus, MMTV has apparently evolved to enter cells independently of the iron status of the host.

Much is known about the role of transferrin receptor 1 (TfR1) and its accessory proteins in the uptake of iron into cells. Iron-bound transferrin (Tf) first binds to TfR1 at neutral pH at the cell surface and traffics with the receptor to an acidic compartment where iron is released and transported to the cytoplasm via divalent metal transport protein 1 (reviewed in Ref. 1). The receptor then recycles back to the cell surface, where iron-free Tf is released and a new molecule of iron-bound Tf binds. At least one additional cell surface protein has been implicated in the process of iron uptake, the β2-globulin-associated, major histocompatibility locus-encoded hereditary hemochromatosis HFE protein, which competes with Tf for binding to TfR1 and is thought to limit iron uptake by cells in which it is co-expressed with the receptor (1). Indeed, mice with targeted deletion of the β2-globulin gene develop an iron overload disorder similar to that seen in HFE patients (2). HFE protein is found in a number of cell types, including epithelia, fibroblasts, and monocytes/macrophages (3, 4), and transcripts encoding the gene are present in many tissues, including those infected by MMTV (in vivo such as lymphoid and mammary gland (GNF Symatlas (symatlas.gnf.org/SymAtlas/) (5) and Unigene (www.ncbi.nlm.nih.gov/UniGene)).

The crystal structure of the human TfR1 ectodomain has been solved, both as a homodimer (6) and in complex with the HFE protein (7). The protein is defined by three domains, a protease-like domain (residues 122–188 and 384–606) with similarity to carboxyl- and amino-peptidases but no apparent enzymatic activity, an apical domain (residues 189–383), and a helical domain (residues 607–760) required for subunit dimerization. The solution of the structure in combination with mutagenesis studies (8–11) and cryoelectron microscopy (12) have defined overlapping and competing binding sites for Tf and HFE located in the helical region of the protein. Sequence comparison of this region from different species shows strong conservation of an RGD-like sequence that is critical for Tf and HFE binding (8, 13). However, despite this conservation, binding of Tf from one species to the receptor of another species can vary by several orders of magnitude (14, 15), indicating that other regions of the TfR1 are involved in this interaction. This is supported by mutagenesis studies implicating sequences in the protease-like domain in addition to the helical domain that affect Tf and HFE binding (8).

Even outside the Tf binding site, there is strong interspecies sequence conservation of TfR1. For example, the mouse and human TfR1 coding regions are 76% identical and 86% homologous. Despite this conservation, the murine beta retrovirus MMTV, which uses TfR1 as its entry receptor, does not infect cells expressing either human or hamster TfR1 (16). Indeed, we have shown here that only rodent TfR1s, and not cat, dog, hamster, or human molecules, support virus entry into cells. Using sequence comparison in combination with the known structure of human TfR1 as an initial starting point for design, we constructed mouse/human chimeric receptors and found that MMTV utilizes a region of TfR1 that is physically distinct from the sites of Tf and HFE binding. In support of these functional studies, we also showed that MMTV infection is not affected by the presence of Tf or iron or the absence of the HFE protein.

EXPERIMENTAL PROCEDURES

Cells, Transfection, and Pseudovirus Infection—293T human kidney epithelial cells, XC cells, Tbh2 cells (Chinese hamster ovary cells lacking TfR1) (17), and mouse embryonic fibroblasts were grown in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum and penicillin/streptomycin (50 μg/ml). TRH3 cells (293T cells stably expressing mouse TfR1) (18) and NMU MG (normal murine mammary gland; ATCC CRL-1636) cells were grown in the same medium supplemented with genetin (100 μg/ml) or insulin (10 μg/ml), respectively. MMTV Env-pseudotyped murine leukemia virus recombinant virions containing the β-galactosidase gene under the control of the murine leukemia virus long terminal repeat (pHIT 111) (19) were prepared in 293T cells and used for infection as previously described (20). Infection was quan-

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‡ The abbreviations used are: TfR1, transferrin receptor 1; Tf, transferrin; HFE, hemochromatosis protein; MMTV, mouse mammary tumor virus; NMU MG, normal murine mammary gland; aa, amino acid.
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Itified by in situ staining of lacZ-positive colonies; the data are presented as Lac-forming units/ml of virus preparation. In some experiments, soluble β-galactosidase assays were performed with pseudovirus-infected cell extracts using a commercial kit according to the manufacturer’s instructions (Promega, Inc., Madison, WI). These data are presented as milliliters/mg/h protein. To test the wild-type, mutant, or chimeric TR1s for their ability to function as MMTV receptors, DNA was transiently transfected into 293T cells using calcium phosphate precipitation (16). Twenty-four hours after transfection, the cells were infected with MMTV pseudotypes and analyzed for infection by lacZ staining.

**Tf Competition**—Mouse apo-Tf (Sigma) was converted to holo-Tf as described (21). Mouse and human (Sigma) apo- and holo-Tf at the indicated concentrations were incubated with TRH3 cells on ice in the presence of MMTV pseudoviruses for 1 h. Cells were washed several times with medium and refed. After 48 h, infected cells were stained in situ for β-galactosidase and colonies were counted.

**Desferrioxamine and Ferric Ammonium Citrate Treatment**—NMuMG or TRH3 cells were preincubated for 1 h with 100 μM desferrioxamine mesylate (Sigma) or 100 μg/ml of ferric ammonium citrate (Sigma). MMTV pseudovirus infection was carried out in the presence of the compounds. After 2 h of incubation with virus, the cells were washed; infection was quantified 48 h later as previously described (20).

**Tf Uptake Assay**—At 24 h post-transfection, 293T cells were incubated in serum-free Dulbecco’s modified Eagle’s medium for 1 h. Fluorescein isothiocyanate- or Texas Red-conjugated Tf (12 μg/ml; Molecular Probes, Eugene, OR) was applied to cells in serum-free medium for 1 h. The cells were washed with cold phosphate-buffered saline, fixed in 3% paraformaldehyde for 10 min, and observed with a Nikon DIAPHOT 300 microscope.

**Western Blot and Fluorescence-activated Cell Sorting Analysis**—All transfected cells were harvested at 48 h post-transfection for expression analysis. For chimeras 1–6, partially purified membrane fractions were prepared (22), and 10 μg of protein/lane were electrophoresed on SDS-polyacrylamide gels (SDS-PAGE) and analyzed by Western blotting. For the other chimeras and the site-mutagenized constructs, whole cell lysates of transfected cells were prepared and separated by SDS-PAGE. Hybridoma supernatant OKT9 (a gift from Morrie Birnbaum) was used to detect proteins from whole cell lysates, and polyclonal rabbit anti-human CD71 (H-300; Santa Cruz Biotechnology, Inc., Santa Cruz, CA) to detect proteins from whole cell lysates. Rat anti-mouse TfR1 monoclonal antibody MOPC-315 (RDI, Flanders, NJ) was used to detect the mouse receptor. Different antisera/antibodies were used because the recognized epitopes were not present in all the chimeric proteins (see “Results”). For whole cell lysates and membrane preparations, the blots were stripped and probed with anti-mouse β-actin (Santa Cruz Biotechnology, Inc.) or anti-Na+/K+ ATPase (Abcam, Inc., Cambridge, MA) as loading controls. Fluorescence-activated cell sorting analysis for TfR1 was performed as previously described (23).

**Chimeric and Mutant Receptor Construction**—Chimeras of the human and mouse TfR1 were prepared as diagrammed in Fig. 5, using gene splicing by overlap extension (24). Details of the primers used for this method are available upon request. The cloned human and mouse receptor clone cDNAs served as the initial templates for construction of chimeras 1–3; chimeras 5–16 used chimera 3 as the backbone. All the chimeras were cloned into the expression vector pcDNA3.1(+) (Invitrogen) and sequenced to verify the alterations. Site-directed mutagenesis on chimeras 15 and 16 (Fig. 6) was performed with the QuickChange XL site-directed mutagenesis kit (Stratagene, Inc., La Jolla, CA).

**Molecular Modeling of the Mouse, Rat, and Hamster Transferrin Receptors**—The amino acid sequences of mouse TfR1 (GenBank™ accession number NM_011638), rat TfR1 (XM_340999), and hamster TfR1 (L19142) were aligned with that of human TfR1 (XM_052730) by hand. Each sequence alignment was submitted to SwissModel (25, 26) to obtain model structures based on the known crystal structure of human TfR1 (Protein Data Bank number 1CX8). Coordinate sets for the mouse, rat, and hamster TfR1 models are available upon request.

### RESULTS

**MMTV Uses Rodent, but Not Other Species, TfR1 for Virus Entry**—We previously showed that mouse, but not hamster or human, TfR1 would function as an entry receptor for MMTV (16). Additionally, work by others has shown that rat cells are susceptible to MMTV infection (27). The TfR1 coding regions from a large number of species, including mouse, rat, human, hamster, cat, and dog, have been sequenced and are highly conserved. Comparison of these sequences revealed that the mouse is more highly related to the rat than it is to the human, cat, or dog TfR1s (90% to rat versus ~76% identity to human, hamster, dog, or cat). We therefore tested whether MMTV could use the cat or dog TfR1 for infection as a means of identifying critical regions in the receptor required for entry. Expression plasmids containing the cloned TfR1 cDNAs from mouse, dog, cat, and human were transfected into 293T cells and tested for receptor expression by a Tf uptake assay (Fig. 1) or Western blot analysis (not shown) and for their ability to support infection of MMTV pseudoviruses (Table 1). Additionally, rat XC cells were tested for infection with MMTV pseudoviruses. Although all receptors supported Tf uptake, only mouse and rat, but not human, cat, or dog, TfR1 allowed infection by MMTV pseudoviruses. This allowed us to begin to define the regions of the mouse TfR1 that might be involved in interaction with the MMTV Env.

**MMTV Infection Is Not Affected by Tf or HFE**—We next tested whether Tf or HFE affected interaction of MMTV with TfR1, because both are known to change receptor conformation (7, 10, 11). In addition, the Tf binding site on TfR1 is highly conserved between species and overlaps the HFE binding site. However, there are differences in the affinity of binding of Tf from one species to the TfR1 of different species (14, 15) and recent work has indicated that amino acids outside the Tf and HFE binding site on the TfR1 affect their binding (10, 11). Thus, it was also possible that MMTV could interact with TfR1 in the same region as Tf and HFE.

We first tested whether Tf competed with MMTV for binding to TfR1, using mouse apo- and mouse and human holo-Tf. MMTV pseudovirions and increasing concentrations of the different Tf were incubated on ice with TRH3 cells (293T cells stably expressing the mouse TfR1) (18). There was no significant effect of Tf on MMTV
inhibited in cells expressing either mutant (shown for G650A in Fig. 3). When the iron chelator desferrioxamine, which reduces extra- and intracellular iron levels, was added to infected cells, the total virus-like particles (VLPs) was significantly reduced compared to untreated cells (Fig. 2B). The mutants were expressed at about the same level as wild-type receptor in transiently transfected cells (Fig. 3). However, whereas Tf uptake was inhibited in cells expressing either mutant (shown for G650A in Fig. 3B), the mutations had only a marginal effect on MMTV infection, reducing infection by at most 50% (Fig. 3C).

Finally, we tested whether the absence of the HFE molecule, which binds to TfR1 at sites both overlapping and distinct from Tf, affects MMTV infection. Embryonic fibroblasts were prepared from C3H/HeN and β2-microglobulin knock-out (C3H/HeN background) mice and tested for infection with MMTV pseudotypes. Cells from the two mouse strains were infected at similar efficiencies (Fig. 4). Additionally, we tested in vivo infection of β2-microglobulin mice and saw no difference in infection compared to wild-type mice (not shown), as has been previously reported (29). These data indicated that MMTV might interact with a different region of TfR1 than Tf or HFE.

Identification of the Site of Interaction of MMTV with TfR1—The data described in the preceding section indicated that MMTV/TfR1 contact was not affected by either Tf or HFE and suggested that the viral envelope protein might interact at a distinct site. Because of the high level of amino acid identity/homology, we made the assumption that the mouse, rat, hamster, cat, and dog TfR1 molecules all folded into similar structures and performed sequence comparison of the TfR1s from these species. This analysis showed three potential regions of highest dissimilarity between the different species that, when placed on the crystal structure, indicated they were on the surface of the TfR1 molecule (aa 196–237, 247–370, and 560–643). Using the human TfR1 molecule as a backbone, we constructed a series of chimeric molecules between the mouse and human TfR1s to determine whether any of the three regions were required for MMTV infection (Fig. 5A). To ensure that the chimeric receptors were expressed at similar levels, we used Tf uptake assays (not shown) and Western blot analysis of both total cell extracts (Figs. 5B and 5D) and partially purified membranes (Fig. 5B). All the chimeric receptors that demonstrated Tf uptake showed similar levels of expression by Western blot analysis. We used rabbit anti-human TfR1 directed against aa 461–760 of the human receptor or monoclonal antibody OKT9, which detects human but not mouse TfR1. The rabbit polyclonal antisera only weakly interacted with region aa 461–760 of the mouse TfR1 (for example, see mouse and chimera 3 and 4 in Fig. 5B). Moreover, OKT9 only interacted with chimeras that contained human sequence aa 305–366 (i.e. chimera 6, 9, 11, 12, and 13, but not 5, 7, and 8; Fig. 5B). The chimeras (Fig. 5A, 1–16) that were expressed and supported Tf uptake were then tested for their ability to serve as an MMTV entry receptor.

Chimeras 3 and 4, but not 1 and 2, supported MMTV infection, thereby eliminating the first candidate region (aa 196–237) from further consideration and defining the minimum region of mouse TfR1 required to support MMTV between amino acids 238–643 (Fig. 5A). This left two potential areas of interaction, based on their divergent sequence between rodents and other species and their location on the surface of the receptor, amino acids 247–370 and 560–643. Accordingly, we constructed chimera 5 and showed that it functioned as a virus receptor, allowing infection at levels similar to the mouse TfR1. Additional constructs allowed us to map the minimal region required from the mouse TfR1 from amino acids 285–296 (Fig. 5B, segment 1) and 569–589 (segment 2) (see chimeras 6–11 and 16 in Fig. 5C). Both segments 1 and 2 were required, because chimeras 14 and 15, containing only mouse segment 2 (aa 569–589) or segment 1 (aa 285–296), respectively, did not
function as MMTV entry receptors (Fig. 5C). Chimeras 14, 15, and 16 were all expressed at similar levels (Fig. 6B and data not shown).

Segments 1 and 2 map to opposing parts of a groove found at the outside edges of the TfR1, a region completely distinct from the Tf and HFE binding site (Fig. 7A). To further define the amino acids important for MMTV interaction in segment 1, we carried out site-specific mutagenesis, using chimera 16 as a backbone. Two amino acids in segment 1 of the mouse TfR1, Lys-287 and Asp-296, which are located on either side of conserved loop extending into the pocket (supplemental Fig. S1), were important for MMTV infection, because changing either the former alone to its human counterpart (K287Q) or both simultaneously (K287Q and D296E) reduced infection levels 60 and 87%, respectively (Fig. 6C).

To further define the MMTV interaction site in segment 2, we created two additional chimeras, 12 and 13 (Fig. 5A), containing aa 569–577 and 578–589, respectively, as well as segment 1, from the mouse. Chimera 12 functioned weakly as a receptor, supporting pseudovirus infection about 2 orders of magnitude lower than the wild-type mouse or functional chimeric receptors. In contrast, chimera 13 did not support infection. The ability to fully support infection was restored to chimera 12 when amino acids 578 (Glu to Gln), 582 (Glu to Gln), 585 (Lys to Gln), and 589 (Ala to Thr) were all changed from human to mouse (chimera 52 in Fig. 6, A and D).

**DISCUSSION**

The region of TfR1 containing the RGD sequences that constitute the Tf and HFE binding sites are highly conserved among mouse, rat, human, hamster, cat, and dog, but MMTV was unable to infect cells of any but the first two species. We therefore used the inability of MMTV to infect cells expressing human TfR1 to map the domains required for infection. By comparing the sequences of the mouse, rat, human, cat, and dog TfR1s and placing the regions of greatest dissimilarity on the human crystal structure, we found three potential regions of interaction and constructed mouse/human chimeras to test their role in infection. We were able to determine that two segments were critical determinants of infection, aa 285–296 located within the apical domain and 569–589 found in the membrane-distal region of the protease domain. Although the two segments are distinct on the linear map, in the folded molecule they form opposing sides of a groove or depression located on the surface of the outer edge of the TfR1 dimer and distant from the Tf and HFE binding sites (Fig. 7).

To ensure that the chimeric receptors were expressed at similar levels, we used Tf uptake assays and Western blot analysis of both total cell extracts and partially purified membranes. All the chimeric receptors that demonstrated Tf uptake showed similar levels of expression by Western blot analysis. Although it is possible that some of the chimeras were not expressed on the cell surface at the same levels, virus infection can occur when only small amounts of receptor are present (16).

Indeed, because we used transient transfection to achieve expression of the chimeric TfR1s, all were highly overexpressed relative to the endogenous hTfR1 expressed by 293T cells (compare the no DNA lanes in Figs. 5B and 6B to the lanes containing extracts from transfected cells). Thus, it is likely that the inability of certain chimeras to function as MMTV entry receptors is a reflection of sequence and not level of expression.

Mouse and man differ at six of the twelve residues in the first segment

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3 A. Sankin and S. Ross, unpublished observations.
residues 285–296 of human TfR1), yet most of the differences in this segment do not correlate with receptor function. For example, the asparagine at residue 292 in human TfR1 (Asn-292) differs from the glutamate (Glu-294) in the mouse receptor, but non-susceptible hamster cells also have a glutamate in this position. Further, the third and last residues in this segment differ between the mouse and rat even though the virus infects both species. To understand this discordance we modeled the mouse, rat, and hamster TfR1s based on the published crystal structure of human TfR1 (supplemental Fig. S1). In the human TfR1 and the models, the side chains of all but two of the divergent residues are not at the surface but instead fold within the protein, providing a plausible explanation for their lack of influence on infection. Of the two that are surface exposed, Gln-285 of human TfR1 correlates best with lack of receptor function. Indeed, the only single change that decreased the receptor function of mouse TfR1 by more than half was a change from lysine to glutamine in the corresponding residue (K287Q).

The second segment of the bipartite groove differs at twelve of the twenty-one residues between mouse and man but only six are non-conservative, and these show more consistent correlation with receptor function among the species. No single change in the highly divergent

FIGURE 5. Mouse/human TfR1 chimeras. A, diagram of the chimeras. Filled box, mouse sequences; open box, human sequences. Segment 1 in the human TfR1 (aa 285–296) aligns with aa 287–298 in the mouse TfR1. B, Western blot of selected chimeras. Equal amounts of partially purified membrane proteins or cell extracts, as indicated, were loaded in each lane, and the blots were probed with the indicated antibodies. The blots were stripped and probed with antisera to the ubiquitously expressed Na+/K+ ATPase membrane protein or to mouse β-actin to ensure equal loading of proteins. C, pseudovirus infection levels supported by the different chimeras.

FIGURE 6. Site-directed mutants. A, diagram of chimeras 15 and 16 and the amino acids subjected to point mutations. B, Western blot analysis of selected mutants using OKT-9 antibody for detection. The blots were stripped and probed with antisera to mouse β-actin to ensure equal loading of proteins. C, infection levels of point mutants in segment 1. D, infection levels of segment 2 point mutants.
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FIGURE 7. The site required for MMTV infection is formed by adjacent segments on the surface of human TfR1 but distant from the Tf and HFE binding sites. A, a homodimer of human TfR1 ectodomain is depicted in space-filled form drawn from the crystal structure coordinates (1CX8) of human TfR1 using the molecular visualization tool RasMol (4 – 8). The residues in segment 1 (S1, aa 285 – 296) are shown in yellow. Segment 2 (S2, aa 569 – 589) is shown in cyan. The locations of Tf and HFE when bound to TfR1 are shown in light green and light blue, respectively; their overlap appears dark blue-green. The positions of the two ligands were located on TfR1 by superimposing a space-filled form of the human TfR1-Tf complex drawn from the crystal structure coordinates (1SUV) and then reducing the opacity of the superimposed complex until the space-filled residues of TfR1 (1CX8) could be seen through the light green image of Tf. The location of bound HFE protein was drawn on TfR1 by a similar superimposition using the crystal coordinates of a human TfR1-HFE complex (1DE4), the reduced opacity resulting in a light blue image of bound HFE. B, a closer view of the groove formed by S1 and S2 on human TfR1.

residues conferred receptor activity to the hTfR1. More importantly, there is a consistent correlation between the abundance and distribution of charged residues in this segment and receptor function. The most striking differences occur at four surface-exposed residues (574, 575, 578, and 585) that lie near the center of the groove. These are all charged amino acids in human TfR1 (lysine, glutamate, glutamate, and lysine, respectively), but only the first is charged in mouse and rat and that one is of the opposite charge (glutamate, supplemental Fig. S1). Other non-susceptible species also display charges in at least three of these four positions (supplemental Fig. S1).

We previously identified the putative receptor binding site on the MMTV envelope protein consisting of the sequence PDEHGFRNMS-GNVHFEG (single letter amino acid code) in its amino-terminal domain (18). The aromatic third residue of this segment may directly interact with mouse TfR1 because changing it to serine or to alanine abrogated infection and binding whereas a change to an aromatic tyrosine did not alter infection. The lower net charge in the lower portion of the groove of mouse and rat receptors could provide a highly favorable surface for this critical interaction.

Enveloped viruses use molecules found on the cell membrane to gain access to cells. In many cases, the presence or absence of these receptors determines the cell type- or species-specific tropism of viruses. Thus, it is somewhat surprising that MMTV predominantly infects lymphoid and mammary epithelial cells in vivo, because most cells require iron as a cofactor for growth and have the potential to express TfR1. However, closer examination of TfR1 expression reveals that this receptor is only produced at significant levels on cells that are highly metabolically active, a time when iron uptake is critical. Cells that are actively dividing express the highest levels of TfR1; in vivo, TfR1 is a known activation marker on lymphocytes (30, 31), and pregnant and lactating mammary glands dramatically up-regulate expression (32). Retroviruses like MMTV do not infect non-dividing cells, because they require nuclear membrane breakdown or factors at the nuclear pore present only on activated cells to accomplish trafficking of the viral double-stranded DNA pre-integration intermediate into the nucleus (33–35). Thus, the induction of TfR1 expression on activated lymphocytes and dividing mammary epithelial cells explains why these cells in particular are the primary targets for MMTV.

When the virion surface protein of enveloped viruses such as MMTV binds to a cellular receptor, there is a conformational change in the envelope protein. As a result, the fusion peptide on the transmembrane domain that mediates virus/cell membrane fusion is uncovered and the virus cores are released into the cell (36). This mechanism does not perturb membrane architecture, and virus entry per se is not pathogenic. However, some viruses that use critical cell surface receptors have been shown to interfere with their function, leading to pathogenic effects. For example, it has recently been proposed that the pathogenic effects of human T cell leukemia virus I, which uses the glucose transporter 1 protein as its entry receptor, are due in part to its interference with glucose uptake in infected cells expressing the envelope protein, a process termed receptor interference (37). Unlike HTLV-I, MMTV infection does not result in obvious cellular pathologies until it induces breast cancer, a relatively late event in the virus infection pathway. Previous work by our laboratory has demonstrated that endogenous MMTV envelope expression does not result in receptor interference (38), thus making it unlikely that virus infection would interfere with iron uptake. Additionally, we have shown here that the virus utilizes a completely different interaction site on TfR1 than Tf or HFE. This arrangement has the potential to promote MMTV attachment selectively to metabolically active target cells able to sustain all steps of its replication pathway without disrupting iron uptake. Although addition of high levels of MMTV virions can cause some down-regulation of TfR1 from the cell surface in cultured cells (16), it is unlikely that during in vivo infection there is sufficient virus to achieve significant loss of functional receptor on the cell surface, as evidenced by the lack of receptor interference by envelope expression.

Several viruses that infect cells from multiple species seem to bind to conserved regions of their entry receptor required for function. For example, gamma retroviruses such as gibbon-ape leukemia virus, feline leukemia type C, and amphotropic murine leukemia viruses use the phosphate transporter molecules Pit1 and Pit2 of several species for entry (39). It has been suggested that this ability to interact with a functionally conserved region of the receptor is advantageous for the virus, because such regions are less likely to undergo changes (39, 40). Although utilization of a site on TLR1 that does not interfere with iron uptake probably benefits the ability of MMTV to infect target cells, it has the potential to limit virus species tropism. Recent work by several laboratories indicates that MMTV-like envelope sequences are found integrated in human breast cancer biopsies, but not normal tissue from the same patient (41–43), and in tissue from patients with primary biliary cirrhosis (44). Additionally, there is a recent report that MMTV can infect human tissue culture cell lines (45). Interestingly, the envelope protein sequences of these human viruses are virtually indistinguishable from the mouse virus and thus should show the same species tropism (18). One possible mechanism by which infection of humans by an MMTV-like virus could occur would be that these individuals have TfR1 polymorphisms that allow entry. However, although a number of nucleotide polymorphisms have been found in the human TfR1 gene, most do not cause amino acid changes; the one non-conservative change, G142S (46), is found in a region of the protein that we showed is not involved in MMTV interaction. We also previously showed that pseudotyped viruses bearing the envelope protein from an MMTV thought to be adapted to infect human tissue culture cells did not have altered species tropism but instead still infected only mouse cells (18). However, it is possible that low level zoonotic transmission of MMTV...
could occur by a non-TfR1-mediated entry mechanism, especially in the infection of tissue culture cells.

MMTV infection is one of the few retroviruses whose infection is pH dependent (16, 47). It is well established that the TfR1 dimer in complex with holo-Tf traffics to the acidic recycling endosome. Future studies will address whether MMTV interaction at a site distinct from Tf also traffics to this compartment or directs TfR1 to some other acidic compartment.

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TfR1 plasmids, Tanya Golovkina for the Acknowledgments—We thank Colin Parrish for the Tvb.2 cells and dog and cat compartment.

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