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Feline Aminopeptidase N Serves as a Receptor for Feline, Canine, Porcine, and Human Coronaviruses in Serogroup I

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Two members of coronavirus serogroup I, human respiratory coronavirus HCV-229E and porcine transmissible gastroenteritis virus (TGEV), use aminopeptidase N (APN) as their cellular receptors. These viruses show marked species specificity in receptor utilization, as HCV-229E can utilize human but not porcine APN, while TGEV can utilize porcine but not human APN. To determine whether feline APN could serve as a receptor for two feline coronaviruses in serogroup I, feline infectious peritonitis virus (FIPV) and feline enteric coronavirus (FeCV), we cloned the cDNA encoding feline APN (fAPN) by PCR from cDNA isolated from a feline cell line and stably expressed it in FIPV- and FeCV-resistant mouse and hamster cells. The predicted amino acid sequence of fAPN shows 78 and 77% identity with human and porcine APN, respectively. When inoculated with either of two biologically different strains of FIPV or with FeCV, fAPN-transfected mouse and hamster cells became infected and viral antigens developed in the cytoplasm. Infectious FIPV was released from hamster cells stably transfected with fAPN. The fAPN-transfected mouse and hamster cells were challenged with other coronaviruses in serogroup I including canine coronavirus, porcine coronavirus TGEV, and human coronavirus HCV-229E. In addition to serving as a receptor for the feline coronaviruses, fAPN also served as a functional receptor for each of these serogroup I coronaviruses as shown by development of viral antigens in the cytoplasm of infected mouse or hamster cells stably transfected with fAPN. In contrast, fAPN did not serve as a functional receptor for mouse hepatitis virus (MHV-A59), which is in serogroup II and utilizes mouse biliary glycoprotein receptors unrelated to APN. Thus, fAPN serves as a receptor for a much broader range of group I coronaviruses than human and porcine APNs. The human, porcine, and canine coronaviruses in serogroup I that are able to use fAPN as a receptor have previously been shown to infect cats without causing disease. Therefore, host factors in addition to receptor specificity apparently affect the virulence and transmissibility of nonfeline serogroup I coronaviruses in the cat.

Coronaviruses are large, enveloped viruses with single-stranded, message sense RNA genomes (15). Two closely related coronaviruses that cause disease in domestic and exotic Felidae are feline infectious peritonitis virus (FIPV) and feline enteric coronavirus (FeCV) (2, 25, 28, 32). While FeCV causes inapparent or mild infection in kittens that is limited to the enteric tract (31), FIPV causes a chronic, systemic, and usually fatal disease called feline infectious peritonitis (FIP), characterized by fibrinonecrotic and pyogranulomatous peritonitis and pleuritis (2, 28, 37). FIPV can also spread to the central nervous system, where it causes granulomatous meningoencephalitis and uveitis (13, 24, 37). Isolates of FIPV from infected cats vary in virulence in vivo and cytopathogenicity in vitro. One type, exemplified by FIPV 79-1146, is highly cytopathic, causing extensive fusion and lysis of feline cells, whereas another type, exemplified by FIPV UCD-1, causes little cytopathic effect in feline cell lines. The feline coronaviruses FIPV and FeCV are members of serogroup I, which also includes two porcine coronaviruses (transmissible gastroenteritis virus [TGEV] and porcine respiratory coronavirus [PRCV]), canine coronavirus (CCV), and human coronavirus 229E (HCV-229E) (15, 40). Except for FIPV, which causes systemic disease, these viruses generally cause localized enteric and/or respiratory infections. Each serogroup I coronavirus causes disease in only a single host species (44), and these viruses generally show marked species specificity in tissue culture cell lines.

Aminopeptidase N (APN), also called CD13, is a 150-kDa glycoprotein with metalloprotease activity which is expressed on the plasma membranes of cells of the granulocyte and monocyte lineages, as well as on nonhematopoetic tissues including fibroblasts, endothelial cells, synaptic membranes in the central nervous system, epithelial cells from the renal proximal tubules and intestinal brush border, and epithelial cells of the respiratory tract (17, 21, 39). Human APN (hAPN) is a receptor for HCV-229E (47), and porcine APN (pAPN) is a receptor for TGEV and PRCV (7–9). However, HCV-229E cannot utilize pAPN as a receptor, and TGEV cannot utilize hAPN as a receptor (7, 18). Hamster cells (BHK) transfected with pAPN were not susceptible to infection with FIPV or CCV, suggesting that pAPN expressed in these cells could not function as a receptor for these viruses (9). Thus, receptor specificity appears to be an important determinant of the species specificity of HCV-229E and TGEV infection.

Since APNs serve as receptors for several group I coronaviruses, we investigated whether feline APN (fAPN) could serve as a receptor for feline coronaviruses FIPV and FeCV. Several clones of fAPN were amplified by PCR from feline cDNA, and then a full-length cDNA clone was stably transfected into FIPV-resistant mouse or hamster cell lines. The resulting fAPN-expressing mouse or hamster cell lines were chal-

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lenged with FIPV, FeCV, CCV, HCV-229E, and TGEV. All of these group I coronaviruses were able to infect fAPN-expressing mouse and hamster cells. In contrast, we found that mouse hepatitis virus (MHV), a member of serogroup II which uses mouse biliary glycoproteins as receptors (10, 11, 48), could not use fAPN as a receptor when it was expressed in hamster cells.

MATERIALS AND METHODS

Cells. *Felis catus* whole fetus (Fcwf) cells (kindly provided by Niels Pedersen, University of California at Davis, Davis), the BHK-21 line of baby hamster kidney fibroblasts, NIH 3T3 cells (from Robert Garcea, University of Colorado Health Sciences Center, Denver), ST cells (from David Brian, University of Tennessee, Knoxville), mouse 17 Cl 1 cells (from Lawrence Sturman, New York State Health Department, Albany), and A72 cells (from Leonard Binn, Walter Reed Army Institute for Research, Washington, D.C.) were grown in Dulbecco's modified Eagle's minimal essential medium supplemented with 10% heat-inactivated fetal calf serum and antibiotics (GIBCO Laboratories, Grand Island, N.Y.). L132 cells (American Type Culture Collection, Rockville, Md.) were grown in Eagle's minimal essential medium supplemented with 10% heat-inactivated fetal calf serum and antibiotics (GIBCO Laboratories).

Viruses. Two biologically distinct strains of FIPV, 79-1146 and UCD-1, and FeCV 79-1683 were propagated in Fcwf cells. CCV strain 1-71 from Leonard Binn (Walter Reed Army Institute for Research) was propagated in canine A72 cells. HCV-229E from the American Type Culture Collection was grown in human L132 cells. TGEV clone E (from David Brian, University of Tennessee) was grown in porcine ST cells. MHV-A59 was grown in 17 Cl 1 cells.

PCR amplification and cloning. Oligonucleotide primers were synthesized by GIBCO-BRL (Gaithersburg, Md.). The following primer sequences are shown in the 5′ to 3′ orientation, and their locations in the APN cDNA are shown in Fig. 1: DT003, CTGGGCCCGGCCCAGTGCCAT; DT004, CTCCGCATAGTCAG CACCCAG; DT005, CATGGCCAAGGGCTTCTACATTTCC; DT006, GGC GGAGTTGGCACAAGGGGCA; DT007, CTGGCGCTGAACAACACCCTC TTCC; DT008, AGTGGAGAATCGTCGGGTCACTGCC.

Poly(A)$^+$ RNA was prepared from 4×10^7 Fcwf cells using a FastTrack 2.0 kit (Invitrogen, San Diego, Calif.) according to the manufacturer's instructions. cDNA was synthesized by reverse transcription from 5 μg of poly(A)$^+$ RNA primed with oligo(dT), and second-strand synthesis was performed using the Copy Kit (Invitrogen). PCR products of a fAPN cDNA encoding a full-length open reading frame and of three overlapping segments of the cDNA were obtained using the Expand Long Template PCR system (Boehringer Mannheim, Indianapolis, Ind.), which contains a mixture of thermostable *Taq* and *Pwo* DNA polymerases. cDNA from Fcwf cells was amplified with 0.3 μM each primer in a Perkin-Elmer 2400 Thermocycler according to the manufacturer's instructions. The products of the reaction (2 min at 94°C, 10 s at 93°C, 30 s at 64°C, 2 min at 68°C for 10 cycles; 10 s at 94°C, 30 s at 64°C, 2 min at 68°C [with a 20-s autoextension step] for 20 cycles, followed by 7 min at 68°C) were analyzed on ethidium bromide-stained gels. The products were cut out of preparative gels, purified with a Qiaquick gel extraction column (Qiagen, Chatsworth, Calif.), and then subcloned into the pCR3 vector (Invitrogen; here called PCR_3) by T/A cloning.

DNA sequencing and analysis. The full-length fAPN clone and the three independently cloned overlapping fragments of the fAPN cDNA (Fig. 1) were sequenced by the dideoxy chain termination method (34) using Sequenase 2.0 (United States Biochemical, Cleveland, Ohio) or by automated sequencing at Macromolecular Resources (Colorado State University, Fort Collins). Each region on each of the four clones was sequenced at least twice, using primers which enabled sequencing of both strands.

The human and porcine APN sequences were obtained from the GenBank database, accession numbers M22324 and Z29522, respectively. Nucleic acid and protein sequences were analyzed using Gene Jockey (Biosoft, Cambridge, United Kingdom) and GeneWorks (IntelliGenetics, Mountain View, Calif.) software.

Transfection of hamster or mouse cells, followed by virus inoculation. PCR_3 or this plasmid containing the full-length fAPN cDNA under the control of the cytomegalovirus promoter was transfected into hamster (BHK) or mouse (NIH 3T3) cell lines using lipofectAMINE (Life Technologies, Gaithersburg, Md.) as described by the manufacturer. Pools of stably transfected cells were selected with 600 μg of G418 per ml (Life Technologies) in growth medium and then maintained in medium containing 300 μg of G418 per ml. Cells grown on coverslips were inoculated with various coronaviruses at a multiplicity of infection of 2 to 3 PFU per cell and incubated for 1 h at 37°C. At 6 or 24 h postinoculation, the cells on coverslips were fixed with cold acetone. Viral antigens in the cytoplasm of infected cells were detected by immunofluorescence with virus-specific antibodies, followed by the appropriate fluorescein-conjugated species-specific anti-immunoglobulin G as described by Dveksler et al. (11). Transfected cells were infected in two separate experiments, and multiple coverslips were analyzed for the percentage of infected cells by immunofluorescence with antiviral antibody. Production of infectious FIPV 79-1146 virions from fAPN-transfected BHK cells was demonstrated by plaque assay on Fcwf cells of supernatant medium harvested at 4 and 24 h postinfection (4).

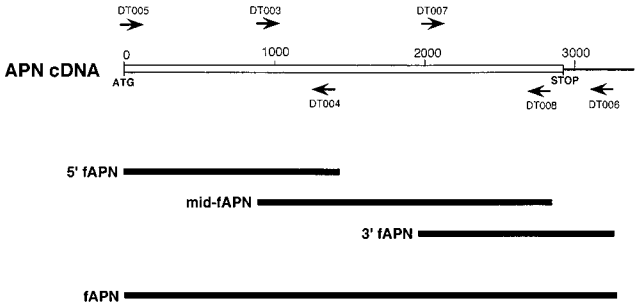

FIG. 1. PCR cloning strategy. The APN cDNA containing a full-length open reading frame and 3′ untranslated region is shown. PCR primers are shown by arrows, and the four PCR products of fAPN cDNAs which were subcloned and sequenced are indicated.

Antisera. FIPV and FeCV antigens were detected with polyclonal feline anti-FIPV serum (Biodesign, Kennebunk, Maine). CCV was detected with a polyclonal anti-CCV rabbit serum (Coo) prepared from a rabbit immunized with sucrose density gradient-purified, Nonidet P-40-disrupted CCV virions. Mouse monoclonal antibody 25C9.3c directed against TGEV spike protein was kindly provided by Linda Saif (Ohio State University, Wooster). Polyclonal goat anti-HCV-229E was obtained from Lawrence Sturman (New York State Health Department), and mouse monoclonal antibody directed against the N protein of MHV was kindly provided by Julian Leibowitz (Texas A & M University, College Station).

Nucleotide sequence accession number. Our fAPN sequence data have been submitted to GenBank (accession number U58920).

RESULTS

Cloning and sequence analysis of fAPN cDNA. cDNA was prepared from Fcwf cells, which are susceptible to infection with FIPV (79-1146 and UCD-1) and FeCV (79-1683), and used as a template for PCR amplification of the full-length or partial fAPN cDNAs (Fig. 1). The primers used were selected from conserved sequences of APN genes from several species, including humans, pigs, rats, and rabbits (7, 21, 42, 46).

The complete nucleotide sequence of fAPN cDNA was determined from a full-length cDNA and three independently cloned overlapping segments of the APN cDNA. Translation of a 2,901-bp open reading frame was predicted to encode a 967-amino-acid-long polypeptide (Fig. 2). The first 8 amino acids of fAPN are included in the oligonucleotide primer sequence which is identical in all four other species and constitutes most of the 9-amino-acid-long N-terminal cytoplasmic tail. Like the published sequences of pig and human APNs, the rest of the fAPN protein appears to be composed of a 23-amino-acid transmembrane domain and a 935-amino-acid ectodomain (7, 21, 26). The pentapeptide consensus sequence (His-Glu-Leu-Ala-His) of the zinc coordinating and catalytic domain of metalloproteases is found at amino acids 387 to 391 of fAPN as in hAPN and pAPN (7, 21, 26). Pairwise alignment of the amino acid sequence of fAPN with that of hAPN or pAPN revealed 78 and 77% identity, respectively (Fig. 2). The region of greatest divergence in amino acid sequence among the three sequences was observed in the stalk region immediately downstream from the membrane-spanning domain of this type II glycoprotein. Approximately half of the substitutions in this region were conservative. Additional small regions of nonconserved sequences were scattered throughout the molecule, predominantly in the carboxy-terminal portion of the ectodomain of APN. The most highly conserved region of the protein encompassed sequences flanking and including the active site of the enzyme from amino acids 335 to 390. The predicted amino acid sequence for fAPN contains eight canonical sites (Asn-X-Ser/Thr) for the potential addition of asparagine-

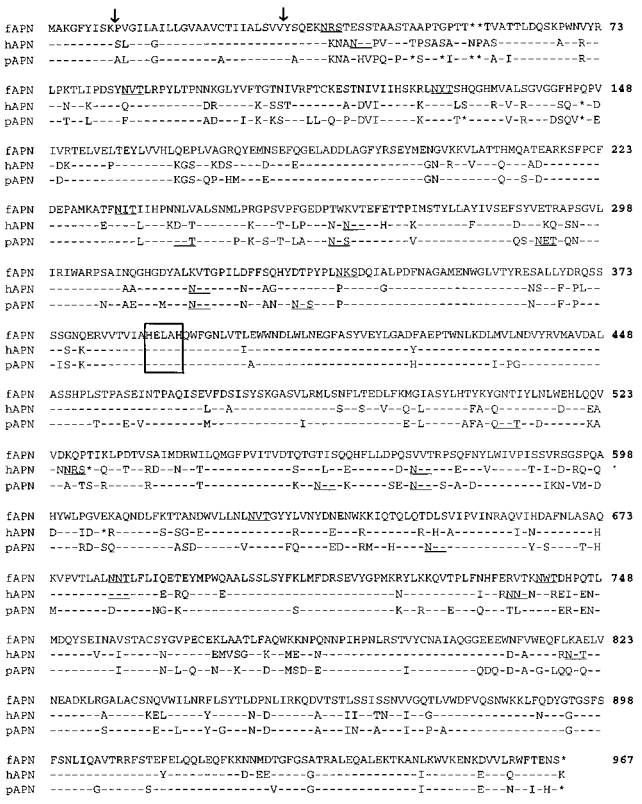

FIG. 2. Deduced amino acid sequence of fAPN cDNA and alignment with human and porcine APNs. Amino acids are numbered starting at the initial methionine of the fAPN polypeptide. The arrows indicate the probable ends of the transmembrane domain of this type II glycoprotein. Potential N-glycosylation sites are underlined, and the conserved sequence of amino acids involved in the catalytic site of APN is boxed. Gaps introduced to maximize alignment are shown by asterisks.

linked oligosaccharides, whereas hAPN has 11 and pAPN has 12. Only three potential N-glycosylation sites, located at amino acids 126, 233, and 626 of fAPN, are conserved in all three APNs. Several of the other potential N-glycosylation sites in fAPN are displaced by up to 4 amino acids compared with the sequences of the pig or human APNs (Fig. 2).

Challenge of murine and hamster cells expressing recombinant fAPN with FIPV. To determine whether the fAPN glycoprotein is a functional receptor for FIPV, the full-length cDNA encoding fAPN was stably expressed in hamster (BHK) or mouse (NIH 3T3) cell lines, which are normally resistant to FIPV infection. Genomic DNA from mouse or hamster cells transfected with vector alone or fAPN cDNA was analyzed by PCR using fAPN-specific oligonucleotide primers which do not amplify murine or hamster APN. This experiment demonstrated that fAPN cDNA was present in the population of fAPN-transfected cells (data not shown). The stably transfected cells were inoculated with either of two strains of FIPV (79-1146, a cytopathic strain [23, 38], or UCD-1, a noncytopathic strain [27, 38]) or mock infected with medium. Antigens of FIPV 79-1146 were detected in the cytoplasm of 5% of the fAPN-transfected mouse cells and 30% of fAPN-transfected hamster cells by 6 h postinoculation, but no viral antigens were detected in hamster or mouse cells stably transfected with the vector alone and then inoculated with FIPV (Fig. 3). The UCD-1 strain of FIPV also infected fAPN-transfected hamster and mouse cells (Fig. 4 and 5) but with a decreased efficiency

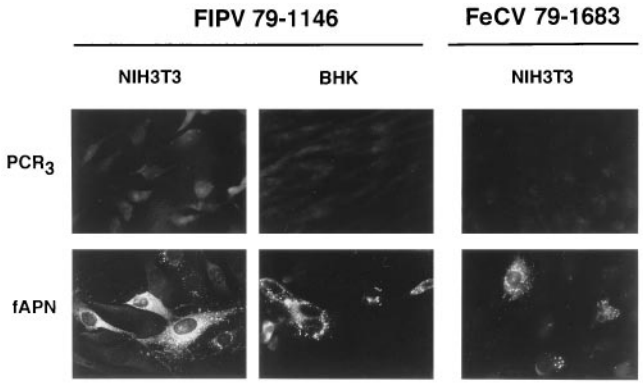

FIG. 3. FIPV infection of fAPN-transfected mouse and hamster cells and FeCV infection of fAPN-transfected mouse cells. Mouse NIH 3T3 or hamster BHK cells stably transfected with vector alone (PCR_3) or with fAPN cDNA in PCR_3 (fAPN) were inoculated with FIPV 79-1146 or FeCV 79-1683 and fixed after 6 h. The presence of viral antigens in the cytoplasm of infected cells was detected with cat anti-FIPV serum and fluorescein isothiocyanate-conjugated goat anti-cat immunoglobulin G. Magnification, ca. ×356.

(2%). This virus also did not infect cells transfected with vector alone (data not shown). Hamster and mouse cells transfected with fAPN or vector alone were also inoculated with FeCV, an enterotropic feline coronavirus closely related to FIPV. FeCV viral antigens were detected in the cytoplasm of 10% of fAPN-transfected NIH 3T3 cells (Fig. 3) and 15% of BHK cells (data not shown), but not in cells transfected with vector alone. Thus, fAPN serves as a receptor for two types of feline coronaviruses, including two biologically different strains of FIPV and one of FeCV. Expression of recombinant fAPN in mouse or hamster cells is sufficient to allow virus binding and entry and the synthesis of viral antigens.

To determine whether fAPN-transfected hamster cells could produce infectious FIPV, these cells were inoculated with FIPV 79-1146 and the infectious virus released into the super-

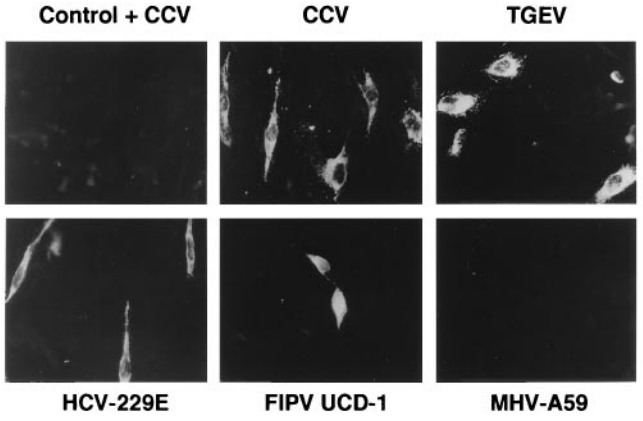

FIG. 4. Infection of fAPN-transfected hamster cells with feline, canine, porcine, and human coronaviruses. BHK cells stably transfected with vector alone or with fAPN cDNA in PCR_3 were inoculated with CCV, porcine coronavirus (TGEV), human coronavirus (HCV-229E), FIPV strain UCD-1, or murine coronavirus (MHV-A59). Viral antigens were detected by immunofluorescence 6 h after inoculation of fAPN-transfected BHK cells with CCV or TGEV and 24 h after inoculation with HCV-229E and FIPV UCD-1. "Control + CCV" shows BHK cells transfected with vector alone and then inoculated with CCV. Similarly, no viral antigens developed in BHK cells transfected with vector alone after inoculation with each of the other viruses (data not shown) or in fAPN-transfected BHK cells inoculated with murine coronavirus MHV-A59. Magnification, ca. ×500.

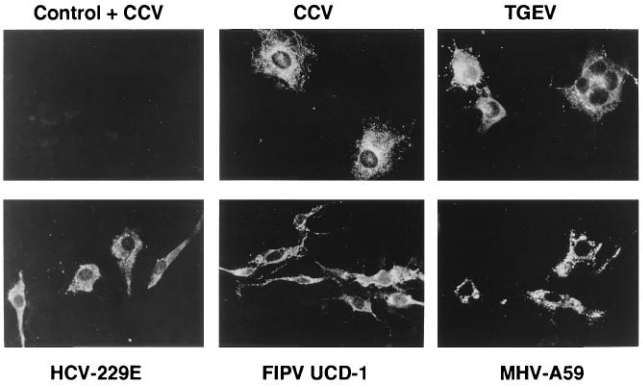

FIG. 5. Infection of fAPN-transfected mouse NIH 3T3 cells with feline, canine, porcine, human, and murine coronaviruses. NIH 3T3 cells stably transfected with vector alone or with fAPN cDNA in PCR_3 were inoculated with CCV, porcine coronavirus (TGEV), human coronavirus (HCV-229E), FIPV strain UCD-1, or murine coronavirus (MHV-A59). Viral antigens were detected 6 h after inoculation of fAPN-transfected NIH 3T3 with CCV, TGEV, and MHV-A59 and after 24 h with HCV-229E and FIPV UCD-1. "Control + CCV" shows NIH 3T3 cells transfected with vector alone 6 h after inoculation with CCV. Similarly, no viral antigens developed in NIH 3T3 cells transfected with vector alone and then challenged with TGEV, HCV-229E, or FIPV UCD-1 (data not shown). However, mouse NIH 3T3 cells transfected with vector alone were as susceptible to infection with murine coronavirus MHV-A59 as were the fAPN-transfected NIH 3T3 cells, because the biliary glycoprotein-related receptors for MHV are expressed on these cells (data not shown). Magnification, ca. ×390.

natant medium was titered by plaquing on Fcwf cells. Table 1 shows that infectious virions were produced and released into the medium from fAPN-transfected BHK cells but not from BHK cells transfected with vector alone.

Infection of fAPN-expressing murine or hamster cell lines with other coronaviruses. Because Fcwf cells are susceptible to infection by other coronaviruses in group I including CCV, TGEV, and HCV-229E in addition to FIPV and FeCV (18), we tested these viruses for infectivity on fAPN-transfected BHK and NIH 3T3 cells. The fAPN-transfected BHK and NIH 3T3 cells or control cells transfected with vector alone were inoculated with CCV, HCV-229E, TGEV, or MHV-A59, a coronavirus in serogroup II. The fAPN-transfected hamster and mouse cells were susceptible to infection with each of the coronaviruses in serogroup I as shown by viral antigen production in the cytoplasm of some of the transfected cells (Fig. 4 and 5). CCV infected approximately 20% of fAPN-transfected BHK cells and 5% of fAPN-transfected NIH 3T3 cells, while TGEV infected 30% of fAPN-transfected BHK cells and 20% of fAPN-transfected NIH 3T3 cells, and HCV-229E infected 10% of fAPN-transfected BHK cells and 5% of fAPN-transfected NIH 3T3 cells. Neither mouse nor hamster cells transfected with vector alone were infected by CCV (Fig. 4 and 5), HCV-229E, or TGEV (data not shown). Thus, fAPN also served as a receptor for CCV, HCV-229E, and TGEV.

The fAPN-transfected BHK cells were not susceptible to infection with murine coronavirus MHV-A59 (Fig. 4). As expected, both NIH 3T3 cells transfected with vector alone (data not shown) and fAPN-transfected NIH 3T3 cells were susceptible to MHV infection (Fig. 5) because mouse cells express the biliary glycoproteins that serve as receptors for MHV (10, 11, 48).

DISCUSSION

The *Coronaviridae* family includes many viruses which infect humans and animals and cause disease in a species-restricted manner (15, 44). To date, the receptors for several coronaviruses have been identified and include different classes of molecules. MHV utilizes mouse biliary glycoproteins as receptors (10, 11, 48). Bovine coronavirus employs a carbohydrate moiety, *N*-acetyl-9-*O*-acetylneuraminic acid, as a receptor (35, 36). Human HCV-229E and porcine coronaviruses TGEV and PRCV utilize APN glycoproteins of their normal host species as receptors (7, 9, 47).

In these studies, we showed that feline coronaviruses FIPV and FeCV, which are antigenically related to HCV-229E and TGEV, also utilize APN as a receptor. Coronaviruses that cause feline diseases include both cytopathic and noncytopathic strains of FIPV as well as FeCV (23, 27, 38). FIPV and FeCV are antigenically related, and it is not yet clear whether FeCV is a distinct coronavirus from FIPV or whether the two are strains of a feline coronavirus which differ in tissue tropism and virulence. These feline coronaviruses possess a broad spectrum of virulence that ranges from acute, self-limiting, asymptomatic infection to production of the severe systemic disease known as FIP. FeCV isolates do not cause FIP and are usually avirulent, but some isolates induce symptomatic enteritis (23). In cats, macrophages are infected with FIPV (29, 30), whereas FeCV replicates only in the intestine and not in macrophages (41). Perhaps FeCV cannot spread to other tissues because it cannot infect macrophages. Because the common receptor for FIPV and FeCV is fAPN, which is found both on macrophages and on the intestinal brush border membrane (BBM), the different tissue tropisms of these two very closely related feline coronaviruses are apparently not determined by the availability of the receptor, but probably also depend upon cell or tissue factors that affect steps in virus replication that occur after receptor binding.

Comparison of the predicted amino acid sequence of fAPN with those of hAPN and pAPN reveals a high degree of homology and 78 and 77% amino acid identity, respectively. The carboxy-terminal portion of this type II glycoprotein contains the most divergent sequences, as expected since this is the extracellular domain. The sequences encompassing and flanking the catalytic site of the enzyme and its zinc-binding domain are very highly conserved among all three APNs (Fig. 2). Only 3 of 8 to 12 potential N-glycosylation sites are conserved among the three APN glycoproteins, and it is not yet known whether glycosylation of APN affects virus-receptor interactions. The sites of interaction between hAPN and HCV-229E or between TGEV and pAPN have not yet been defined. Anti-hAPN monoclonal antibody RBS, which blocks HCV-229E infection, also inhibits the enzymatic activity of hAPN (47). A mutant hAPN lacking the catalytic site of the enzyme did not bind HCV-229E or anti-hAPN antibodies which inhibited infection (47). These observations could be explained either by a conformational change of the mutant protein or by a role in virus binding for some residues at or near the catalytic site.

TABLE 1. Yield of infectious FIPV 79-1146 from hamster cells transfected with fAPN cDNA in PCR_3 or with control vector PCR_3

Cells	Titer (10^4/ml)[a]	
	4 h p.i.	24 h p.i.
BHK-PCR_3	3.7 ± 0.2	1.1 ± 0.2
BHK-fAPN	7.3 ± 0.1	760 ± 17

[a] Titers of infectious FIPV in supernatant medium at 4 or 24 h postinoculation (p.i.) were measured by plaque assay in Fcwf cells. Numbers indicate the mean titer and standard deviation.

Using chimeras between pAPN and hAPN, Delmas et al. identified a region on pAPN between amino acids 717 and 813, located 330 amino acids downstream from the active site, that was required to be of porcine origin in order to permit TGEV infection (7). This region varies markedly in amino acid sequence between the different species of APN. Unexpectedly, using these same chimeras, the corresponding region of hAPN was not responsible for the species specificity of HCV-229E infection (20). Whether this region of pAPN affects TGEV binding or subsequent steps in virus entry remains to be determined. Further studies are required to define the APN residues that interact with coronavirus spike proteins and other moieties that may be required for membrane fusion.

In addition to serving as a receptor for FIPV and FeCV, recombinant fAPN also functioned as a receptor for other group I coronaviruses, including CCV, HCV-229E, and porcine TGEV. In marked contrast, the human and porcine APNs function only as receptors for HCV-229E and porcine TGEV, respectively (7, 19). Since pools of mouse or hamster cells stably transfected with fAPN were tested for susceptibility to coronavirus infection in these studies, we observed varying percentages of cells infected by the different coronaviruses. Further studies will be performed on stably transfected subcloned cell lines expressing known amounts of fAPN in order to compare the receptor activities of fAPN for each of these coronaviruses.

Our laboratory showed that, unlike MHV, which binds only to intestinal BBMs from MHV-susceptible mice and not to BBMs from other species, coronaviruses from serogroup I (FIPV, CCV, HCV-229E, and TGEV) bind to BBMs from all four species naturally susceptible to these viruses, including humans, pigs, dogs, and cats, but not to mouse BBMs (6, 14). The present data are compatible with the suggestion that on cat BBMs these viruses bound to fAPN. If so, then the species specificity of virus infection observed with coronaviruses in serogroup I may in some cases be restricted at a step in the virus replication cycle that follows virus binding to a receptor. For example, the species specificity of HCV-229E infection can be determined at multiple steps in its replication cycle. The first possible site of restriction is determined by the virus-binding specificity of the APN, as hamster and mouse cells are resistant to HCV-229E infection because they do not bind virions on their membranes. The second possible site of coronavirus host range restriction in tissue culture appears to occur after binding but prior to viral protein synthesis, since HCV-229E binds to membranes of dog and pig cell lines, but does not infect them, whereas these cells can be infected by transfection of HCV-229E genomic RNA (18).

In general, the coronaviruses from serogroup I cause disease only in their normal host species. However, it has been reported that some strains of CCV and TGEV can infect other species such as cats without causing disease (3, 33, 38), and likewise FIPV can infect piglets (45). When cats were experimentally inoculated with TGEV, CCV, or HCV-229E, they seroconverted and in some cases shed virus, although no signs of clinical disease were observed (1, 3, 33, 38). In addition, infection with these serologically related viruses did not provide protection from FIPV infection. The ability of these human, canine, and porcine coronaviruses to use fAPN as a receptor explains how they can infect cats. Host factors that affect stages in the virus life cycle after receptor binding apparently determine the yield and spread of virus and the ability to cause disease in the infected host.

The ability of nonfeline group I coronaviruses to utilize fAPN as a receptor has important implications for the evolution of this group of viruses. RNA viruses can undergo rapid evolution because of high error rates and lack of proofreading by RNA polymerases (12) and also by reassortment of viral segments as seen in influenza virus (43) or by recombination as in coronaviruses (16, 22). Possibly recombination between different coronaviruses could occur in a cat that is simultaneously infected with FeCV or FIPV and another coronavirus in group I. The nonfeline virus could enter cat cells via the fAPN receptor and would not necessarily have to undergo a productive infection in order to recombine with the feline virus. Thus, cats may serve as "mixing vessels" for coronaviruses in group I, as pigs do for influenza A viruses (5). Simultaneous infection of a single feline cell with two different coronaviruses would probably be an extremely rare event. Nevertheless, recombinants between different coronaviruses could have properties different from either parent and might show altered host range, tissue tropism, antigenicity, and/or virulence, possibly resulting in emergence of a new disease.

Because APN glycoproteins are used as a receptor by human, porcine, and feline coronaviruses in group I, it appears likely that the S glycoproteins of these viruses recognize conserved amino acids in their APN receptors. Most of the monoclonal anti-hAPN antibodies that we have tested do not react with APNs of other species (19). The virus-binding moieties of APNs may lie not on the exposed surface of the glycoprotein, but in a depression inaccessible to antibodies. Possibly, these residues are more readily accessible to viral spike glycoproteins in fAPN than in hAPN or pAPN. Detailed analysis of the binding of the S proteins of group I coronaviruses to the APNs of different species will elucidate the determinants of receptor specificity and provide insight into the role of receptor specificity in determining the host ranges of these viruses.

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REFERENCES

1. **Barlough, J. E., C. M. Johnson-Lussenburg, C. A. Stoddart, R. H. Jacobson, and F. W. Scott.** 1985. Experimental inoculation of cats with human coronavirus 229E and subsequent challenge with feline infectious peritonitis virus. Can. J. Comp. Med. **49:**303–307.
2. **Barlough, J. E., and C. A. Stoddart.** 1990. Feline coronaviral infections, p. 300–312. *In* C. E. Greene (ed.), Infectious diseases of the dog and cat. W. B. Saunders Co., Philadelphia.
3. **Barlough, J. E., C. A. Stoddart, G. P. Sorresso, R. H. Jacobson, and F. W. Scott.** 1984. Experimental inoculation of cats with canine coronavirus and subsequent challenge with feline infectious peritonitis virus. Lab. Anim. Sci. **34:**592–597.
4. **Boyle, J. F., N. C. Pedersen, J. F. Evermann, A. J. McKeirnan, R. L. Ott, and J. W. Black.** 1984. Plaque assay, polypeptide composition and immunohistochemistry of feline infectious peritonitis virus and feline enteric coronavirus isolates. Adv. Exp. Med. Biol. **173:**133–147.
5. **Castrucci, M. R., I. Donatelli, L. Sidoli, G. Barigazzi, Y. Kawaoka, and R. G. Webster.** 1993. Genetic reassortment between avian and human influenza A viruses in Italian pigs. Virology **193:**503–506.
6. **Compton, S. R., C. B. Stephensen, S. W. Snyder, D. G. Weismiller, and K. V. Holmes.** 1992. Coronavirus species specificity: murine coronavirus binds to a mouse-specific epitope on its carcinoembryonic antigen-related receptor glycoprotein. J. Virol. **66:**7420–7428.
7. **Delmas, B., J. Gelfi, E. Kut, H. Sjöström, O. Norén, and H. Laude.** 1994. Determinants essential for the transmissible gastroenteritis virus-receptor interaction reside within a domain of aminopeptidase N that is distinct from the enzymatic site. J. Virol. **68:**5216–5224.
8. **Delmas, B., J. Gelfi, R. L'Haridon, L. K. Vogel, H. Sjöström, O. Norén, and H. Laude.** 1992. Aminopeptidase N is a major receptor for the enteropathogenic coronavirus TGEV. Nature (London) **357:**417–419.
9. **Delmas, B., J. Gelfi, H. Sjöström, O. Norén, and H. Laude.** 1994. Further characterization of aminopeptidase N as a receptor for coronaviruses. Adv. Exp. Med. Biol. **342:**293–298.

10. **Dveksler, G. S., C. W. Dieffenbach, C. B. Cardellichio, K. McCuaig, M. N. Pensiero, G.-S. Jiang, N. Beauchemin, and K. V. Holmes.** 1993. Several members of the mouse carcinoembryonic antigen-related glycoprotein family are functional receptors for the coronavirus mouse hepatitis virus-A59. J. Virol. **67:**1–8.
11. **Dveksler, G. S., M. N. Pensiero, C. B. Cardellichio, R. K. Williams, G.-S. Jiang, K. V. Holmes, and C. W. Dieffenbach.** 1991. Cloning of the mouse hepatitis virus (MHV) receptor: expression in human and hamster cell lines confers susceptibility to MHV. J. Virol. **65:**6881–6891.
12. **Holland, J., K. Spindler, F. Horodyski, E. Grabau, S. Nichol, and S. Vandepol.** 1982. Rapid evolution of RNA genomes. Science **215:**1577–1585.
13. **Holmberg, C. A., and D. H. Gribble.** 1973. Feline infectious peritonitis diagnostic gross and microscopic lesions. Feline Pract. **3:**11–14.
14. **Holmes, K. V., and S. R. Compton.** 1995. Coronavirus receptors, p. 55–71. *In* S. G. Siddell (ed.), The *Coronaviridae*. Plenum Press, New York.
15. **Holmes, K. V., and M. M. C. Lai.** 1996. Coronaviridae: the viruses and their replication, p. 1075–1093. *In* B. N. Fields, D. M. Knipe, and P. M. Howley (ed.), Virology (Fields), 3rd ed. Lippincott-Raven Publishers, Philadelphia.
16. **Keck, J. G., S. Makino, L. H. Soe, J. O. Fleming, S. A. Stohlman, and M. M. C. Lai.** 1987. RNA recombination of coronavirus. Adv. Exp. Med. Biol. **218:**99–107.
17. **Kenny, A. J., and S. Maroux.** 1982. Topology of microvillar membrane hydrolases of kidney and intestine. Physiol. Rev. **62:**91–128.
18. **Levis, R., C. B. Cardellichio, C. A. Scanga, S. R. Compton, and K. V. Holmes.** 1995. Multiple receptor-dependent steps determine the species specificity of HCV-229E infection. Adv. Exp. Med. Biol. **380:**337–343.
19. **Levis, R., and K. V. Holmes.** Unpublished data.
20. **Levis, R., K. V. Holmes, B. Delmas, and H. Laude.** Unpublished data.
21. **Look, A. T., R. A. Ashmun, L. H. Shapiro, and S. C. Peiper.** 1989. Human myeloid plasma membrane glycoprotein CD13 (gp150) is identical to aminopeptidase N. J. Clin. Invest. **83:**1299–1307.
22. **Makino, S., J. G. Keck, S. A. Stohlman, and M. M. C. Lai.** 1986. High frequency RNA recombination of murine coronaviruses. J. Virol. **56:**729–737.
23. **McKeirnan, A. J., J. F. Evermann, A. Hargis, L. M. Miller, and R. L. Ott.** 1981. Isolation of feline coronaviruses from two cats with diverse disease manifestations. Feline Pract. **11(3):**16–20.
24. **Montali, R. J., and J. D. Strandberg.** 1972. Extraperitoneal lesions in feline infectious peritonitis. Vet. Pathol. **9:**109–121.
25. **O'Brien, S. J., M. E. Roelke, L. Marker, A. Newman, C. A. Winkler, D. Meltzer, L. Colly, J. F. Evermann, M. Bush, and D. E. Wildt.** 1985. Genetic basis for species vulnerability in the cheetah. Science **227:**1428–1434.
26. **Olsen, J., G. M. Cowell, E. Kønigshøfer, E. M. Danielsen, J. Møller, L. Laustsen, O. C. Hansen, K. G. Welinder, J. Engberg, W. Hunziker, M. Spiess, H. Sjöström and O. Norén.** 1988. Complete amino acid sequence of human intestinal aminopeptidase N as deduced from cloned cDNA. FEBS Lett. **238:**307–314.
27. **Pedersen, N. C.** 1976. Morphologic and physical characteristics of feline infectious peritonitis virus and its growth in autochthonous peritoneal cell cultures. Am. J. Vet. Res. **37:**567–572.
28. **Pedersen, N. C.** 1983. Feline infectious peritonitis and feline enteric coronavirus infections. Part 2. Feline infectious peritonitis. Feline Pract. **13:**5–20.
29. **Pedersen, N. C.** 1987. Virologic and immunologic aspects of feline infectious peritonitis virus infection. Adv. Exp. Med. Biol. **218:**529–550.
30. **Pedersen, N. C., and J. F. Boyle.** 1980. Immunologic phenomena in the effusive form of feline infectious peritonitis. Am. J. Vet. Res. **41:**868–876.
31. **Pedersen, N. C., J. F. Boyle, K. Floyd, A. Fudge, and J. Barker.** 1981. An enteric coronavirus infection of cats and its relationship to infectious peritonitis. Am. J. Vet. Res. **42:**368–377.
32. **Pfeifer, P. L., J. F. Evermann, M. E. Roelke, A. M. Gallina, R. L. Ott, and A. J. McKeirnan.** 1983. Feline infectious peritonitis in a captive cheetah. J. Am. Vet. Med. Assoc. **183:**1317–1319.
33. **Reynolds, D. J., and D. J. Garwes.** 1979. Virus isolation and serum antibody responses after infection of cats with transmissible gastroenteritis virus. Arch. Virol. **60:**161–166.
34. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
35. **Schultze, B., H.-J. Gross, R. Brossmer, and G. Herrler.** 1991. The S protein of bovine coronavirus is a hemagglutinin recognizing 9-*O*-acetylated sialic acid as a receptor determinant. J. Virol. **65:**6232–6237.
36. **Schultze, B., and G. Herrler.** 1992. Bovine coronavirus uses *N*-acetyl-*O*-acetylneuraminic acid as a receptor determinant to initiate infection of cultured cells. J. Gen. Virol. **73:**901–906.
37. **Scott, F. W.** 1986. Feline infectious peritonitis and other feline coronaviruses, p. 1059–1062. *In* R. W. Kirk (ed.), Current veterinary therapy IX. W. B. Saunders Co., Philadelphia.
38. **Scott, F. W.** 1986. Immunization against feline coronaviruses. Adv. Exp. Med. Biol. **218:**569–576.
39. **Semenza, G.** 1986. Anchoring and biosynthesis of stalked brush border membrane proteins: glycosidases and peptidases of enterocytes and renal tubuli. Annu. Rev. Cell Biol. **2:**255–313.
40. **Siddell, S., H. Wege, and V. ter Meulen.** 1983. The biology of coronaviruses. J. Gen. Virol. **64:**761–776.
41. **Stoddart, C. A., and F. W. Scott.** 1989. Intrinsic resistance of feline peritoneal macrophages to coronavirus infection correlates with in vivo virulence. J. Virol. **63:**436–440.
42. **Watt, V. M., and C. C. Yip.** 1989. Amino acid sequence deduced from a rat kidney cDNA suggests it encodes the Zn-peptidase aminopeptidase N. J. Biol. Chem. **264:**5480–5487.
43. **Webster, R. G., W. G. Laver, G. M. Air, and G. C. Schild.** 1982. Molecular mechanisms of variation in influenza viruses. Nature (London) **296:**115–121.
44. **Wege, H., S. Siddell, and V. Ter Meulen.** 1982. The Biology and pathogenesis of coronaviruses. Curr. Top. Microbiol. Immunol. **99:**165–200.
45. **Woods, R. D., N. F. Cheville, and J. E. Gallagher.** 1981. Lesions in the small intestine of newborn pigs inoculated with porcine, feline and canine coronaviruses. Am. J. Vet. Res. **42:**1163–1169.
46. **Yang, X. F., P. E. Milhiet, F. Gaudoux, P. Crine, and G. Boileau.** 1993. Complete sequence of rabbit kidney aminopeptidase N and mRNA localization in rabbit kidney using in situ hybridization. Biochem. Cell Biol. **71:** 278–287.
47. **Yeager, C. L., R. A. Ashmun, R. K. Williams, C. B. Cardellichio, L. H. Shapiro, A. T. Look and K. V. Holmes.** 1992. Human aminopeptidase N is a receptor for human coronavirus 229E. Nature (London) **357:**420–422.
48. **Yokomori, K., and M. M. C. Lai.** 1992. Mouse hepatitis virus utilizes two carcinoembryonic antigens as alternative receptors. J. Virol. **66:**6194–6199.