MOLECULAR INTERACTIONS OF GROUP 1 CORONAVIRUSES WITH FELINE APN

Sonia M. Tusell and Kathryn V. Holmes*

1. INTRODUCTION

Most coronaviruses in phylogenetic group 1 can cause disease in only one animal species. Within group 1, porcine transmissible gastroenteritis virus (TGEV), feline infectious peritonitis virus (FIPV), human coronavirus 229E (HCoV-229E), and canine coronavirus (CCoV) use aminopeptidase N (APN) from their natural host for entry and infection of cells.[1-4] APN is a highly conserved type II transmembrane glycoprotein in mammals (70–80% at the amino acid level). Remarkably, although each of these group 1 coronaviruses uses APN of its normal host for entry, all of them can also use feline APN (fAPN) as a receptor for entry and infection in cell culture.[2] Previous studies used chimeras between APN proteins of different species to identify domains in APN that are required for coronavirus receptor activity.[4-7] Studies on receptor specificities of chimeras between human and feline APN or pig and human APN suggest that the spike glycoproteins of TGEV, FIPV, and HCoV-229E interact with two discontinous regions within APN.[6] Also, species-specific N-linked glycosylations in APN can affect receptor activity for HCoV-229E.[8] In *in vivo* studies, transgenic mice expressing human APN (hAPN) were resistant to infection with HCoV-229E, but cells harvested from the transgenic mice were susceptible to HCoV-229E.[9] hAPN transgenic mice in a Stat-1 knockout background ($hAPN^{+/+}Stat^{-/-}$) were susceptible to HCoV-229E, which was adapted for growth in cells from these double transgenic mice.[10] These studies suggest that other host factors in addition to the receptor are needed for infection *in vivo*.

In this study, we used chimeras between mouse APN (mAPN) and fAPN to identify domains of fAPN that are necessary for entry by group 1 coronaviruses. Baby hamster kidney (BHK-21) cells, which are resistant to infection by TGEV, FIPV, CCoV, and HCoV-229E, were transfected with cDNAs encoding wild-type mAPN, fAPN, or mouse-feline APN (MF) chimeras. The transfected cells were inoculated with TGEV clone E, FIPV 79-1146, HCoV-229E, or CCoV 1-71 virus strains. Virus entry and infection was demonstrated by immunofluorescence with antiviral antibodies.

* University of Colorado Health Sciences Center, Aurora, Colorado 80045.

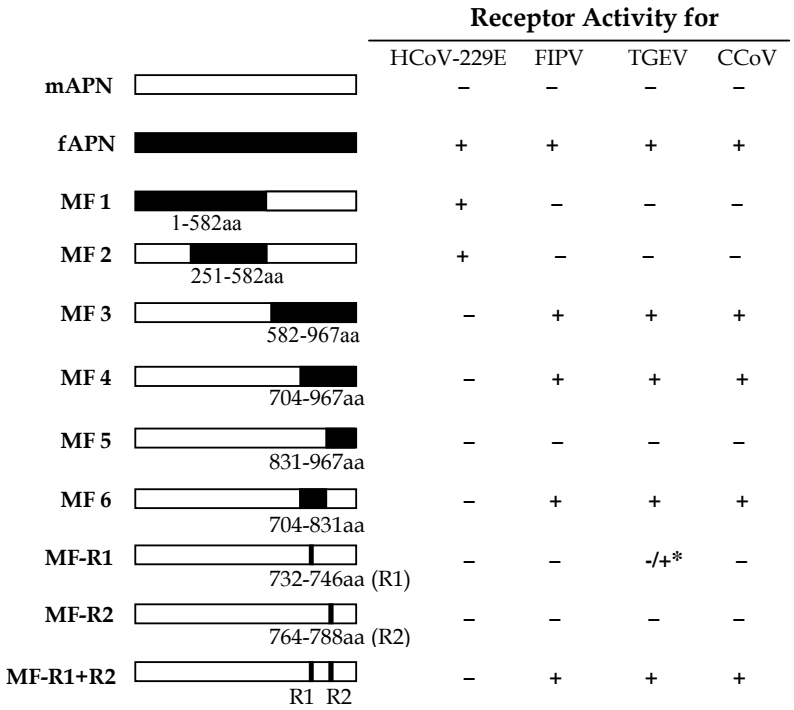

Figure 1. Coronavirus receptor activities of mouse APN (mAPN), feline APN (fAPN), and mouse-feline APN (MF) glycoproteins. All constructs were cloned into pCDNA3.1 TOPO/D V5 his and transiently expressed in BHK-21 cells. Forty-eight hours after transfection, cells were inoculated with each of the coronaviruses, and 10 or 24 hours after virus inoculation, cells were fixed and immunolabeled with antibodies against viral antigens. *Very few cells stained positive for TGEV antigens in BHK-21 cells transfected with MF-R1.

2. RESULTS AND DISCUSSION

BHK-21 cells transfected with mAPN cDNA remained resistant to infection with FIPV, TGEV, CCoV, and HCoV-229E. Chimera MF1, which consists of amino acids 1 to 582 of fAPN in a mAPN backbone, promoted entry of HCoV-229E only, while MF3 promoted entry of FIPV, TGEV, and CCoV, but not HCoV-229E. Construct MF2 consisting of a smaller region of fAPN (amino acids 251–582) in a mAPN backbone functioned as a receptor only for HCoV-229E. The receptor activities of MF1 and MF3 indicated that amino acids 582 to 967 of fAPN were necessary and sufficient for entry of the cat, pig, and dog coronaviruses, but not for HCoV-229E. In contrast, amino acids closer to the N terminus of the protein (251–582aa) of fAPN were required for entry of HCoV-229E. Additional chimeras, MF 4, 5, and 6 were constructed to identify a smaller region in fAPN that would be sufficient for FIPV, TGEV, and CCoV entry. Importantly, MF6 identified a sequence of 127 amino acids in fAPN (aa 704–831) that when substituted into mAPN was sufficient for FIPV, TGEV, and CCoV receptor activity. These data agree with previously published conclusions based on other chimeric APN proteins.[4, 6, 7] Further mutational analysis identified several amino acid residues in aa

704–831 of fAPN that when introduced into mAPN were sufficient to promote entry of the pig, dog, and cat viruses. These residues were located in two discontinuous segments corresponding to amino acids 732–746 (R1) and amino acids 764–788 (R2). Interestingly, TGEV was able to enter BHK-21 cells transfected with the MF-R1 construct, although virus entry was very inefficient relative to TGEV receptor activity of fAPN. However, MF-R1 had no receptor activity for FIPV and CCoV. MF-R2 had no receptor activity for any of these viruses. Efficient receptor activity for these three group 1 coronaviruses was only detected when R1 and R2 from fAPN were substituted together into mAPN.

In summary, amino acid residues in fAPN that are important for entry of FIPV, TGEV, and CCoV were localized to two discrete regions within the C terminal region of fAPN, whereas HCoV-229E entry required an N-terminal domain of fAPN. Without a crystal structure for APN, it is unclear whether these functionally important regions are adjacent in the three-dimensional structure of the receptor glycoprotein. These observed differences in fAPN receptor utilization correlate well with differences in the spike glycoproteins of these viruses, as the cat, pig, and dog virus spike glycoproteins are more closely related to each other at the amino acid level than to the HCoV-229E spike glycoprotein. Characterization of the molecular interactions between the spikes of these group 1 coronaviruses and their APN receptors will identify residues that affect the host ranges of these viruses and provide insight into the evolution of group 1 coronaviruses.

3. ACKNOWLEDGMENTS

This research was supported by NIH grant R01-AI-26075. Sonia Tusell was supported by NIH grant AI 52066.

4. REFERENCES

1. B. Delmas, J. Gelfi, R. L'Haridon, et al., Aminopeptidase N is a major receptor for the entero-pathogenic coronavirus TGEV, *Nature* **357**, 417-420 (1992).
2. D. B. Tresnan, R. Levis, and K. V. Holmes, Feline aminopeptidase N serves as a receptor for feline, canine, porcine, and human coronaviruses in serogroup I, *J. Virol.* **70**, 8669-8674 (1996).
3. C. L. Yeager, R. A. Ashmun, R. K. Williams, et al., Human aminopeptidase N is a receptor for human coronavirus 229E, *Nature* **357**, 420-422 (1992).
4. L. Benbacer, E. Kut, L. Besnardeau, et al., Interspecies aminopeptidase-N chimeras reveal species-specific receptor recognition by canine coronavirus, feline infectious peritonitis virus, and transmissible gastroenteritis virus, *J. Virol.* **71**, 734-737 (1997).
5. A. F. Kolb, A. Hegyi, J. Maile, et al., Molecular analysis of the coronavirus-receptor function of aminopeptidase N, *Adv. Exp. Med. Biol.* **440**, 61-67 (1998).
6. A. Hegyi and A. F. Kolb, Characterization of determinants involved in the feline infectious peritonitis virus receptor function of feline aminopeptidase N, *J. Gen. Virol.* **79**, 1387-1391 (1998).
7. A. F. Kolb, A. Hegyi, and S. G. Siddell, Identification of residues critical for the human coronavirus 229E receptor function of human aminopeptidase N, *J. Gen. Virol.* **78**, 2795-2802 (1997).
8. D. E. Wentworth and K. V. Holmes, Molecular determinants of species specificity in coronavirus receptor aminopeptidase N (CD13): Influence of N-linked glycosylation, *J. Virol*. **75**, 9741-9752 (2001).
9. D. E. Wentworth, D. B. Tresnan, B. C. Turner, et al., Cells of human aminopeptidase N (CD13) transgenic mice are infected by human coronavirus-229E in vitro, but not in vivo, *Virology* **335**, 185-197 (2005).
10. C. Lassnig, C. M. Sanchez, M. Egerbacher, et al., Development of a transgenic mouse model susceptible to human coronavirus 229E, *Proc. Natl. Acad. Sci. USA* **102**, 8275-8280 (2005).