Mapping the Domains of CD134 as a Functional Receptor for Feline Immunodeficiency Virus

Brian J. Willett,1* Elizabeth L. McMonagle,1 Francesca Bonci,2 Mauro Pistello,2 and Margaret J. Hosie1

Retrovirus Research Laboratory, Institute of Comparative Medicine, Faculty of Veterinary Medicine, University of Glasgow, Bearsden Road, Glasgow G61 1QH, United Kingdom, and Department of Experimental Pathology, Retrovirus Center and Virology Section, University of Pisa, Via San Zeno 35, I-56127 Pisa, Italy2

Received 10 April 2006/Accepted 4 May 2006

The feline homologue of CD134 is the primary binding receptor for feline immunodeficiency virus (FIV), targeting the virus preferentially to activated CD4+ helper T cells. However, strains of FIV differ in utilization of CD134; the prototypic strain PPR requires a minimal determinant in the first cysteine-rich domain (CRD1) of feline CD134 to confer near-optimal receptor function, while strains such as GL8 require additional determinants in the CRD1 CRD2. We map this determinant to a loop in CRD2 governing the interaction between the receptor and its ligand; the amino acid substitutions S78N-S79Y-K80E restored full viral receptor activity to the CRD2 of human CD134 in the context of feline CD134, with tyrosine-79 appearing to be the critical residue for restoration of receptor function.

The initial event in the process of virus entry into a target cell is the interaction between the virus and its cellular receptor; the specificity of this interaction determines both the cell tropism and the pathogenicity of the virus. The primary receptor for feline immunodeficiency virus (FIV) is CD134 (OX40) (13), a member of the tumor necrosis factor receptor (TNFR)/nerve growth factor receptor family of molecules. CD134 expression is greatest on activated CD4+ T-helper cells (7); thus, infection results in a progressive depletion of T-helper cells and AIDS-like illness. Primary isolates of FIV use CD134 as the binding receptor in conjunction with the chemokine receptor CXCR4 as a cofactor for infection (11, 13, 15). FIV binds specifically to CD134-expressing cells (13); FIV Env interacts directly with CD134 (4), and pretreatment of virus with soluble CD134 facilitates infection of CD134+/CXCR4+ cells (3).

The binding site for the PPR strain of FIV has been mapped to the first cysteine-rich domain (CRD1) (3); human CD134 is not a functional receptor for FIV (13), and substitution of CRD1 of human CD134 with that of feline CD134 renders the molecule functional as a receptor for the PPR strain of FIV. However, expression of feline CD134 CRD1 in the context of human CD134 is insufficient to confer receptor function on many primary strains of FIV; additional determinants in CRD2 of feline CD134 are required to restore function (16), indicating differential utilization of CD134 by diverse strains of FIV (16). In order to map the determinants in CRD2, we compared the amino acid sequences of the CRD2s of feline and human CD134 (previously we identified the fragment spanning amino acids 65 to 82 as containing the critical determinant[s] (16)) and identified amino acid sequence differences (Fig. 1B). Next, using the human CRD2-containing chimera FFHH as our template, we proceeded to mutate the remaining amino acids in CRD2 from the human sequence to the feline sequence by PCR amplification from the FFHH template by using a mutagenic internal oligonucleotide (3′) carrying a BsrGI restriction site and a nonmutagenic external flanking (5′) primer carrying a BamHI restriction site. The following mutations were introduced: R66L-P67Q (RP→LQ), G69A-P70S (GP→AS), D75E-V76A (DV→EA), S78N-K80E (SK→NE), and S78N-S79Y-K80E (SSK→NYE). They were then cloned into the BamHI/BsrGI-digested FFHH construct. The nucleic acid sequence of each mutant was confirmed; the constructs were then cloned into the retroviral pDONAI and packaged into MLV pseudotypes, as previously described (16), and used to transduce HeLa cells. Stable transductants were selected in G418, and surface expression of the chimeric receptors was confirmed by flow cytometry using the BerACT35 monoclonal antibody. Flow cytometric analysis confirmed that, following G418 selection, the constructs were expressed at comparable levels at the cell surface (not shown).

The stable cell lines were then seeded into 96-well plates and infected with human immunodeficiency virus (HIV) (FIV) pseudotypes bearing FIV Env identified previously as displaying a marked preference for the chimera FFHH (amino acids 65 to 82, feline in origin) over the chimera FFHH (amino acids 65 to 82, human in origin)—Envs GL8, CPG41, and 0827—displaying no or little preference between FFHH and FFHH—Envs 1419, B2542, and PPR (16). Each of the virus pseudotypes infected parent feline CD134 (FF)-expressing cells and the FFHH chimera-expressing cells with similar efficiencies, while human CD134 (HHH)-expressing cells did not differ significantly from control HeLa cells transduced with vector only. Introduction of the R66L-P67Q, G69A-P70S, and D75E-V76A substitutions into the FFHH chimera did not render the molecule a functional receptor for the GL8, CPG41, and 0827 Env-bearing pseudotypes. S78N-K80E restored partial function for the CPG41 and 0827 Envs, while S78N-S79Y-K80E restored full viral entry receptor function to FFHH for...
infection with GL8, CPG41, and 0827 Env-bearing pseudotypes. The data suggest that the NYE (Asn78-Tyr79-Glu80) motif in feline CD134 CRD2 is a critical determinant of receptor function for viruses such as GL8, CPG41, and 0827; of these residues, Y79 would appear to be the most important. Although strains 1419 and B2542 showed only weak selectivity for usage of FFHH over FFFH (<10-fold), the S78N-S79Y-K80E mutation restored full functionality. In contrast, strain PPR was at the other extreme from strains GL8, CPG41, and 0827, utilizing all chimeras with similar efficiency.

These findings may indicate that the PPR/CD134 interaction is more relaxed than the interactions with strains GL8, CPG41, and 0827. This may be the result of a higher tolerance of the PPR Env for amino acid changes within CRD2, or, in contrast to GL8, CPG41, and 0827, PPR Env binding to CD134 may not involve CRD2. Consistent with this hypothesis, the binding site for FIV PPR Env has been mapped to CRD1 of feline CD134 in the region encompassing residues D60 and D62 (3). If the structural predictions for feline CD134 prove to be accurate, then the determinant identified in this study lies in CRD2 on the face of the molecule opposite that of the Env binding site (Fig. 2A). Tyr79 sits at the crown of a loop defined previously as the “A1 module” of an A1B2 cysteine-rich domain (CRD2) (2). The crystal structures of the TNF-TNFR (1) and Apo2L-DR5 (6) complexes place this loop at the ligand-receptor interface (Fig. 2B). Comparison of the structural predictions for feline and human CD134 illustrates that feline CD134 would present a bulky tyrosine residue (Tyr79) at the ligand binding face of the receptor and that this would be flanked by a negatively charged glutamate residue (E80). In contrast, human CD134 would have a smaller serine residue (Ser79) flanked by a positively charged lysine (Lys80). We have constructed the following models to account for the effect of the substitution of residues 78NYE80 with 78SSK80 in feline CD134 on the function of feline CD134 as a viral receptor.

(i) Allosteric effect. Mutation of residues 78NYE80 in feline CD134 may have an allosteric effect on the FIV Env binding domain in CRD1, disrupting recognition of the domain by the GL8, CPG41, and 0827 Envs, or on CD134 structure per se, disrupting a postbinding event in the viral entry process. Previous studies of the interaction between herpes simplex virus type 1 (HSV-1) gD and its receptor HveA (for “herpes simplex
viruses (light) as HSV-1 gD and LT-HveA inhibits the interaction between HveA and function as a receptor for HSV-1. Conversely, binding of ligand LT-H9251 virus entry mediator A") revealed that binding of the HveA ligand LT-α (lymphotoxin A) or LIGHT to HveA perturbs its function as a receptor for HSV-1. Conversely, binding of HSV-1 gD to HveA inhibits the interaction between HveA and LIGHT (9). As HSV-1 gD and LT-α/LIGHT bind to distinct sites on HveA (12), the data indicated that ligand binding to HveA altered the conformation of the receptor, perturbing its function. By analogy with HveA, the 78NYE80 region in feline CD134 may be required for maintenance of the Env binding domain (in CRD1); the NYE→SSK substitution may alter the conformation of the virus binding domain sufficiently to ablate recognition by the GL8, CPG41, and 0827 Envs. Further dissection of the role of the receptor-ligand interaction in viral receptor function will require the cloning and expression of functional feline CD134L. However, it is notable that cell lines known to be highly susceptible to FIV express not only CXCR4 and CD134 but also CD134L (Table 1).

Given that the interaction between the GL8, CPG41, and 0827 Envs would appear to be complex and to involve regions of the CRD2 of CD134 predicted to lie at the receptor-ligand interface, our studies raise the possibility that infection by such viruses may well be modulated (inhibited or enhanced) by the natural ligand for feline CD134 (CD134L or OX40 ligand).

The biological significance of differences in the affinity of virus-receptor interaction has been revealed by studies of the adaptation of HIV for growth in cell culture, with primary strains displaying differential requirements for levels of CD4 and CXCR4 (14). These findings are mirrored by analyses of the molecular determinants of viral growth in microglia, where microglia-tropic strains utilize CD4 more efficiently by way of

### Table 1. Real-time reverse transcription-PCR measurement of CXCR4, CD134, and CD134L

<table>
<thead>
<tr>
<th>Sample cell line</th>
<th>Relative gene expressiona</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GAPDH (CT)</td>
</tr>
<tr>
<td></td>
<td>C_T</td>
</tr>
<tr>
<td>MBM</td>
<td>20.14</td>
</tr>
<tr>
<td>MYA-1</td>
<td>18.44</td>
</tr>
</tbody>
</table>

*Total RNA was extracted from the T-lymphoid cell lines MBM and MYA, reverse transcribed into cDNA by using random hexamers, and amplified for feline CD134, CD134L, and CXCR4; the housekeeping protein glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control (M. Pistello et al., submitted for publication). Primers and probes are available by e-mail on request.

Relative gene expression is given as the difference between the threshold cycle (C_T) of the target and the C_T of the internal control (ΔC_T).
a higher-affinity Env-CD4 interaction and increased exposure of the chemokine receptor binding site (8). Further, a high-affinity interaction between the prCBL23 Env and CD4 may mediate Lv2 restriction (10). Revealing the biological significance of the differential utilization of CD134 by FIVs may inform the design of novel strategies for vaccination and therapy and further strengthen the comparative value of FIV infection of the domestic cat as a nonprimate model for HIV and AIDS.

This work was supported by Public Health Service grant AI049765 to B.J.W. and M.J.H. from the National Institute of Allergy and Infectious Diseases.

REFERENCES