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Sequential CD134-CXCR4 Interactions in Feline Immunodeficiency Virus (FIV): Soluble CD134 Activates FIV Env for CXCR4-Dependent Entry and Reveals a Cryptic Neutralization Epitope

Aymeric de Parseval,[1]* Chris K. Grant,[2] K. Jagannadha Sastry,[3] and John H. Elder[1]*

The Scripps Research Institute, Department of Molecular Biology, La Jolla, California 92037[1]; Custom Monoclonals, Inc., West Sacramento, California[2]; and The University of Texas M. D. Anderson Cancer Center, Department of Immunology, Houston, Texas 77030[3]

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Recombinant soluble CD134 (sCD134) facilitated feline immunodeficiency virus (FIV) entry into CXCR4-positive, cell surface CD134-negative target cells. sCD134-activated entry was dose dependent and CXCR4 dependent. We used the sCD134 activation system to explore the neutralization by four anti-V3 monoclonal antibodies (MAbs). V3 MAbs weakly neutralized FIV infection using target cells expressing both CD134 and CXCR4 but potently inhibited sCD134-activated entry into target cells expressing CXCR4 alone. These findings provide direct evidence for a sequential interaction of FIV Env with CD134 and CXCR4 and reveal the presence of a cryptic epitope in V3 that is masked in the mature envelope oligomers.

As with human immunodeficiency virus type 1 (HIV-1), feline immunodeficiency virus (FIV) targets primarily $CD4^+$ T cells but uses CD134 and not CD4 as a primary receptor (4, 24). CD134 is a T-cell activation antigen in the tumor necrosis factor receptor superfamily (12, 15). Human CD134 fails to support FIV infection (3, 4), and exchange of domains between feline and human CD134 has shown that the first domain binds FIV (3). Aspartate residues at positions 60 and 62 are critical for CD134-FIV interaction (3) and, by homology with the known structure of the tumor necrosis factor alpha receptor, map to the upper edge of domain 1 (D1) (3). Other local amino acids, including Ser45, Gly59, and Lys64, are also involved (3).

HIV entry into target cells requires the sequential interaction of gp120 with CD4 and a coreceptor, typically either CXCR4 or CCR5 (2, 10). Gp120 binds to CD4, which exposes a highly conserved binding site for the coreceptor, which in turn induces additional conformational changes and leads to fusion with the target cell membrane. Evidence suggests a similar model for entry of FIV, in which FIV gp95 interacts sequentially with CD134 and CXCR4 to facilitate entry into the host cell. CXCR4 alone is insufficient for virus entry (4, 5, 24), and infection of CXCR4-positive cells by field strain FIVs occurs only when cells also express CD134 (4, 5, 24). Most importantly, soluble CD134 (sCD134) can induce a CXCR4-dependent infection of cells lacking CD134 (3).

FIV Env is the primary target for antibody-mediated neutralization, and epitope mapping using sera from infected cats identified a major immunodominant domain in the V3 region of gp95 (1, 7, 16, 20). However, anti-V3 monospecific sera and monoclonal antibody (MAb) elicited against V3 peptides only weakly neutralize virus infection (7, 13, 14, 16–19, 22, 23, 25), and V3 monospecific sera or monoclonal antibodies poorly recognize V3 on the mature Env oligomer (19, 22). Thus, V3 may be inaccessible due to masking by carbohydrates and/or tertiary or quaternary interactions within the Env oligomeric complex. Given our results, we hypothesized that binding of FIV Env to CD134 may expose the CXCR4 binding site on gp95 as well as antibody-sensitive epitopes located in the coreceptor binding site.

sCD134 activation of FIV Env-mediated viral entry. In order to address our predictions, we tested whether sCD134 could induce Env-mediated virus entry into target cells expressing CXCR4 but lacking CD134. CrFK cells, a CD134-negative, CXCR4-positive, feline, epithelial kidney cell line, are refractory to infection by primary FIV isolates. CrFK cells were challenged with β-galactosidase (β-Gal)-expressing FIV pseudotyped with FIV Env after preincubation in the absence or presence of 100 nM of sCD134-Fc, a recombinant soluble CD134-Fc adhesin (3). Virus entry was assessed 2 days later by a β-galactosidase assay. No entry was detected in the absence of sCD134, consistent with previous studies demonstrating that cells expressing CXCR4 alone do not support infection by primary FIV isolates (Fig. 1a) (5, 26). AMD3100 blocked sCD134-activated infection, demonstrating dependence on CXCR4 for FIV entry. Two soluble forms of CD134 were compared, each capable of binding to FIV gp95: D1 sCD134, containing only domain 1 of CD134, and wild-type sCD134, containing the entire extracellular region of CD134 (Fig. 1b). Wild-type sCD134-Fc adhesin activated viral entry of CD134-negative CrFK target cells (Fig. 1c) in a dose-dependent manner, up to 350 nM.

* Corresponding author. Mailing address: Department of Molecular Biology, MB-14, The Scripps Research Institute, 10550 N. Torrey Pines Rd., La Jolla, CA 92037. Phone for Aymeric de Parseval: (858) 784-2932. Fax: (858) 784-2750. E-mail: parseval@scripps.edu. Phone for John H. Elder: (858) 784-8270. Fax: (858) 784-2750. E-mail: jelder@scripps.edu.

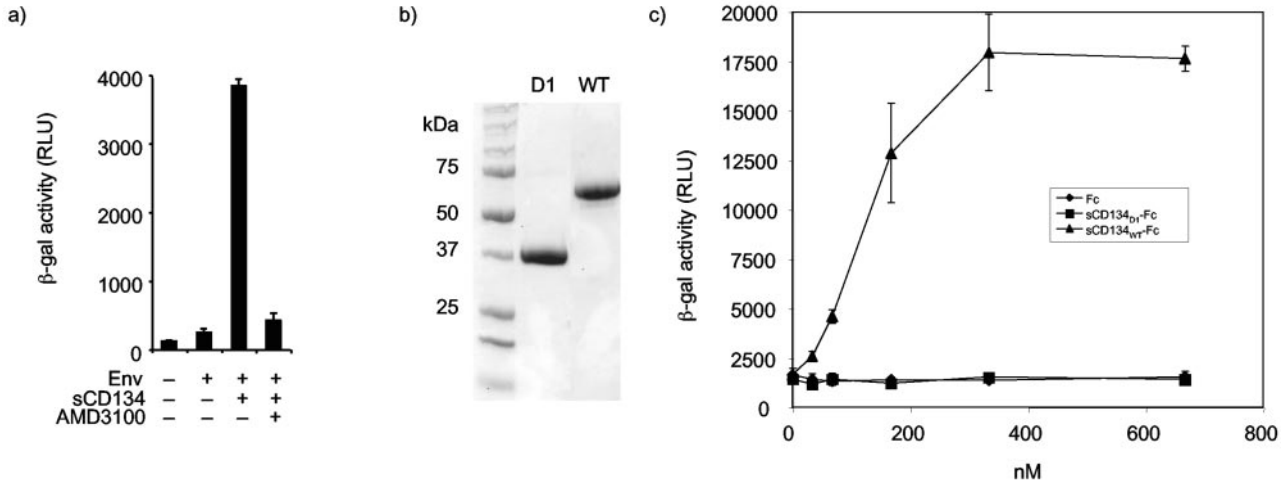

FIG. 1. sCD134 activation of FIV Env. (a) sCD134 activation of FIV Env-mediated, CXCR4-dependent viral entry. Target CrFK cells were infected with β-Gal FIV particles pseudotyped with the Env of FIV-PPR, a primary FIV isolate (21). Particles were preincubated in the absence or presence of sCD134-Fc (100 nM). Viral entry was detected 48 h later by a β-Gal assay. Infection in the presence of AMD3100 (1 μg/ml), a CXCR4 antagonist, was used to show the strict dependence on CXCR4 for viral entry. (b) One-domain (D1) and wild-type (WT) CD134-Fc fusion proteins were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and revealed by Coomassie blue staining. (c) Dose-response curve of sCD134-Fc for activation of viral entry. Target CrFK cells were infected with β-Gal FIV particles pseudotyped with the Env of FIV-PPR, a primary FIV isolate. Particles were preincubated in the presence of Fc alone, one-domain sCD134 ($sCD134_{D1}$-Fc), wild-type soluble CD134 ($sCD134_{WT}$-Fc) at the indicated concentrations. Viral entry was assessed 48 h later by a β-Gal assay. RLU, relative light units.

D1 sCD134 failed to activate viral entry, although it efficiently inhibited the binding of gp95 to CD134 (3; data not shown). Thus, additional regions of CD134 may be important for postbinding events.

sCD134 activation unmasks a cryptic neutralization site. As mentioned earlier, the V3 region of FIV gp95 is one of the major immunodominant domains of FIV, but V3 monoclonal antibodies and/or feline V3-specific sera only weakly neutralize FIV infection. We generated 20 monoclonal antibodies by immunizing mice with a soluble form of gp95 fused to the Fc domain of immunoglobulin G1, an immunoadhesin previously developed to analyze Env-receptor interactions (5). Four of these antibodies recognized a linear epitope within the V3 region of gp95 (data not shown). We assessed these V3 MAbs for the ability to block gp95-Fc binding to CD134 by flow cytometry (Fig. 2). None of the antibodies inhibited gp95-Fc binding to CD134, whereas sCD134 specifically inhibited the binding of gp95 to 104-C1 cells (Fig. 2a). No inhibition of gp95-Fc binding was detected with AMD3100, consistent with previous studies demonstrating that the detected binding of gp95 to 104-C1 T cells is solely a measure of CD134 interaction (4–6). In contrast, assessment of influence on CXCR4 binding using CD134-negative, CXCR4-positive 3201 cells revealed that all four V3 MAbs inhibited gp95-Fc binding to CXCR4 (Fig. 2b).

We next assessed the FIV neutralization potential of the V3 MAbs in both a standard infection system and an sCD134-dependent system. The V3 MAbs and a control MAb were preincubated for 1 h in the absence (standard assay) or presence (activation assay) of sCD134 with β-Gal FIV particles pseudotyped with the Env of FIV-PPR, a primary FIV isolate (21). The mixture was incubated for 2 h at 37°C with CXCR4-positive CrFK cells engineered to express cell surface CD134 (CrFK-CD134) (4) for the standard assay or parental CD134-

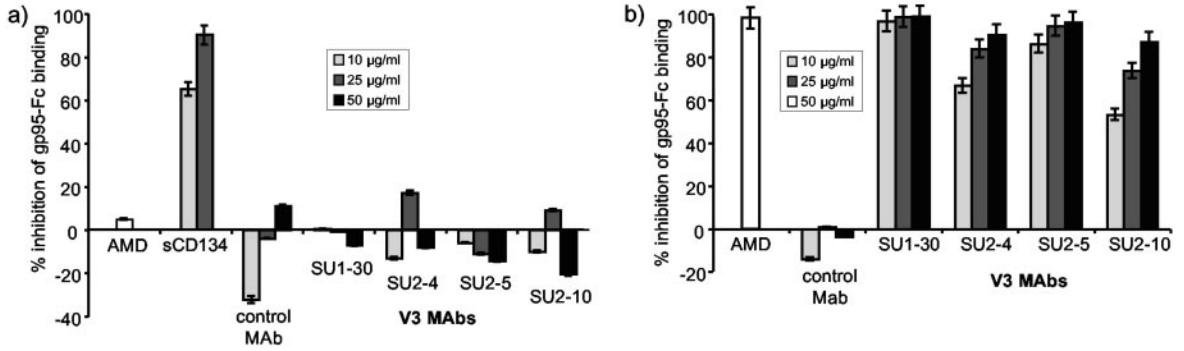

FIG. 2. V3 MAbs block gp95-CXCR4 interaction. V3 MAbs failed to inhibit gp95 binding to CD134 (a), whereas they strongly inhibited gp95 binding to CXCR4 (b). AMD3100 (AMD) was used at 1 μg/ml. Data are expressed as percentages of inhibition of gp95-Fc binding activity in the absence of inhibitor.

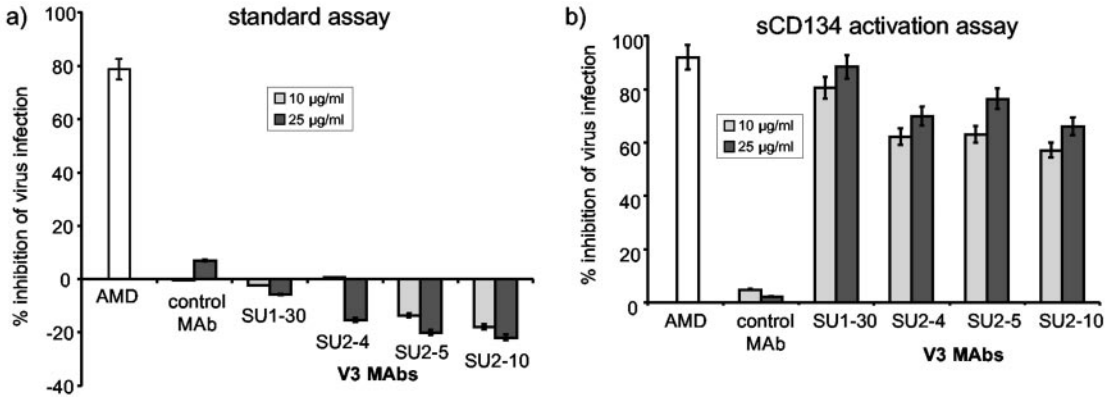

FIG. 3. V3 MAbs are CD134-induced antibodies. The neutralizing activity of the V3 MAbs was assessed in standard (a) and sCD134 activation (b) assays. Neutralization of FIV entry was observed only in the sCD134 activation assay, indicating that the V3 MAbs are CD134-induced antibodies. AMD3100 (AMD) was used at 1 μg/ml. Data are expressed as percentages of inhibition of β-galactosidase activity obtained in the absence of MAb.

negative CrFK cells for the sCD134 activation assay. None of the V3 MAbs neutralized FIV infection in the standard assay (Fig. 3a), whereas a dose-dependent neutralization of FIV infection was observed in the sCD134 activation assay (Fig. 3b). In both assays, AMD3100 inhibited β-Gal activity, consistent with CXCR4-dependent viral entry.

V3 MAbs neutralize genetically diverse FIV isolates. The above analyses used FIV-PPR, a clade A primary isolate (21). We also tested the blocking activities of SU1-30 MAb against the Envs of two divergent primary viruses, 36C, a clade C isolate (8), and B2452, a clade B isolate (11), in standard (Fig. 4a) and sCD134-activated (Fig. 4b) infectivity assays. No blocking activity against any of the primary FIVs was observed in the standard assay (Fig. 4a), whereas MAb SU1-30 inhibited sCD134-activated entry of both clade B and C primary isolates (Fig. 4b), consistent with the conserved amino acid sequence in the C-terminal region of V3 (Fig. 4c).

The findings indicate a common mechanism of Env activation for HIV and FIV, in spite of the use of distinct primary binding receptors. Exposure of cryptic neutralization sites after primary-binding-receptor interaction offers an interesting target to disrupt virus infection in both virus systems. CD4-based molecules, such as sCD4-17b, that enhance the exposure of CD4-induced epitopes have been described and have shown promising results (9). Cats may offer an excellent venue for in vivo testing of this novel intervention strategy.

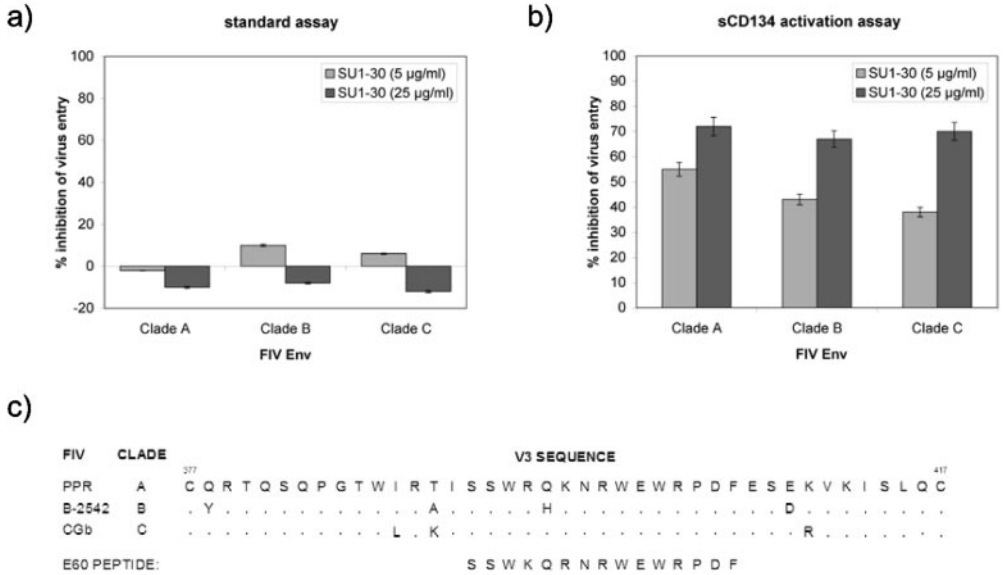

FIG. 4. MAb SU1-30 neutralizes genetically divergent FIV strains. The neutralizing activity of the MAb SU1-30 was assessed in standard (a) and sCD134 activation (b) assays using viruses pseudotyped with the Envs of FIV-PPR (clade A), FIV-B2542 (clade B), and FIV-36C (clade C). (c) V3 sequence of three genetically divergent FIV strains. The V3 sequences of FIV-PPR (clade A), FIV-B2542 (clade B), and FIV-36C (clade C) are shown. Also shown below the alignment is the sequence of the peptide (E60) that is specifically recognized by MAbs SU1-30, SU2-4, SU2-5, and SU2-10. Note that the sequence of the E60 peptide is highly conserved between the three isolates.

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