The role of the chemokine receptor CXCR4 in infection with feline immunodeficiency virus

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Summary

Infection with feline immunodeficiency virus (FIV) leads to the development of a disease state similar to AIDS in man. Recent studies have identified the chemokine receptor CXCR4 as the major receptor for cell culture-adapted strains of FIV, suggesting that FIV and human immunodeficiency virus (HIV) share a common mechanism of infection involving an interaction between the virus and a member of the seven transmembrane domain superfamily of molecules. In this article, we review the evidence for the involvement of chemokine receptors in FIV infection and contrast these findings with similar studies on the primate lentiviruses HIV and SIV (simian immunodeficiency virus).

Introduction

Feline immunodeficiency virus (FIV) is a widespread pathogen of the domestic cat, infection resulting in an immune dysfunction characterised by a progressive depletion of CD4+ lymphocytes from the peripheral blood (Ackley et al. 1990; Novotney et al. 1990). Lentiviruses have also been identified in non-domestic felids such as the Pallas cat (Barr et al. 1997), pumas (Langley et al. 1994) and lions

(Brown et al. 1994). Phylogenetic analyses have demonstrated that the lion and puma lentiviruses (LLV and PLV respectively) are more closely related to FIV than to either the primate or the ungulate lentiviruses. However, whether PLV or LLV induce an immunodeficiency in their natural host is unclear; in many cases infection may be inapparent as has been observed with SIV_{SM} infection in the sootey mangabey. Indeed, amino acid sequence comparisons between the envelope glycoproteins of FIV and PLV-14 revealed only 8% similarity (37% nucleic acid similarity) (Langley et al. 1994) suggesting an ancient divergence between FIV and PLV.

FIV infects a range of CD4-negative cell types including monocyte/macrophages (Brunner and Pedersen, 1989), B cells (Dean et al. 1996; English et al. 1993) and cells of neuronal lineage (Dow et al. 1990), and CD4 expression *in vitro* does not correlate with susceptibility to infection with FIV (Willett et al. 1991). Indeed, CD4 expression in the cat is restricted to T-helper lymphocytes and their thymic precursors and unlike in human beings it is not expressed on monocytes and macrophages. While the principal *in vivo* target of FIV during early infection appears to be the $CD4^+$ T cell, with increased time post-infection the major reservoir for proviral DNA appears to be $CD21^+$ lymphocytes (B-cells) and $CD8^+$ T-cells (Dean et al. 1996; English et al. 1993). Whether B-cells are productively infected with FIV, or whether viral uptake occurs via a complement-dependent mechanism as described for HIV (Boyer et al. 1991; Robinson et al. 1990) has yet to be ascertained, however, these studies provide compelling evidence for the existence of a non-CD4 receptor for FIV. Indeed, ectopic expression of feline CD4 on feline cell lines of non-lymphoid origin does not render the cells permissive for infection with lymphotropic isolates of FIV (Norimine et al. 1993).

FIV can be isolated readily from cultures of mitogen-stimulated peripheral blood mononuclear cells (PBMC) from the infected domestic cat, virus production being accompanied by a cytopathic effect characterised by syncytium formation, ballooning-degeneration and cell lysis. The majority of these primary isolates of FIV can be propagated only in cultures of fresh mitogen-stimulated PBMCs, thymocytes or interleukin-2 (IL-2)-dependent T cell lines. However, some isolates of FIV adapt readily to growth in established feline cell lines such as the Crandell feline kidney cell line (CrFK). Indeed, two molecular clones of the Petaluma isolate of FIV, namely F14 (Olmsted et al. 1989) and 34TF10 (Talbott et al. 1989) were derived from infected CrFK cells. These "CrFK-adapted" strains of FIV have an expanded cell tropism which permits infection of a wide range of feline cell lines. Furthermore, several non-feline cell lines, such as the canine cell line MDCK and the human cell line HeLa (Egberink et al. 1991), are susceptible to infection with CrFK-adapted strains of FIV. Originally, it was thought that the mechanism underlying this expanded cell tropism of FIV was pseudotypic mixing between FIV and the feline endogenous retrovirus RD114 (Egberink et al. 1991), a retrovirus produced in large quantities by many feline cell lines including CrFK. While pseudotypic mixing between FIV and RD114 has not been excluded, subsequent studies have demonstrated that transfection of human cells with plasmid vectors expressing the FIV envelope glycoprotein gene (*env*) from a CrFK-adapted strain of FIV resulted in syncytium formation (Pancino et al. 1995), suggesting that the viral envelope glycoprotein (Env) determines the expanded cell tropism of these types of virus. In agreement with these findings, the critical determinant of CrFK-tropism was localised to the third variable region (V3 loop) of the Env protein. Two independent studies demonstrated that an increase in the net charge of the V3 loop was sufficient to render

some primary isolates of FIV "CrFK-tropic" (Siebelink et al. 1995; Verschoor et al. 1995). The increase in charge resulted from the mutation of either of two glutamate residues (407 and 409) in the V3 loop to lysine residues (Figure1). These studies mirrored observations made with HIV regarding the switch from an NSI (non-syncytium inducing) to an SI (syncytium inducing) phenotype (de Jong et al. 1992) and suggested that CrFK-adapted strains of FIV and SI strains of HIV shared a common mechanism of infection.

Identification of CXCR4 as a receptor for FIV

The identification of the orphan seven transmembrane domain receptor LESTR (lymphocyte expressed seven transmembrane domain receptor, subsequently re-named CXCR4 - the receptor for the α-chemokine SDF-1) as the principal cofactor for CD4-dependent infection with SI strains of HIV-1 (Feng et al. 1996) provided a molecular basis for the adaptation of HIV to grow in T cell lines; primary isolates of HIV were not able to use CXCR4 as a cofactor whereas those selected for growth in established cell lines interacted with CXCR4. CXCR4 was therefore implicated in infection with CrFK-adapted strains of FIV. As the envelope glycoprotein of CrFK-adapted strains of FIV is able to induce cell-cell fusion in human cell lines (Pancino et al. 1995), the role of CXCR4 in FIV infection could be addressed directly by examining the ability of the anti-CXCR4 antibody 12G5 to inhibit fusion between FIV-infected CrFK cells and the human cell line HeLa. Incubation of the cells in the presence of the antibody 12G5 resulted in a dose-dependent inhibition of syncytium formation (Willett et al. 1997). In contrast, cell fusion was not inhibited by incubation with either antibody recognising feline CD9 or a polyclonal goat serum raised against the envelope glycoprotein of the feline endogenous retrovirus RD114. However, cell

fusion was inhibited efficiently by a monoclonal antibody recognising the FIV envelope glycoprotein as well as serum from an FIV-infected cat (Figure 2). These data suggested that CXCR4 functioned as a cofactor for fusion mediated by CrFK-adapted strains of FIV. In order to address the role of CXCR4 in FIV infection directly, cDNAs encoding the feline homologue of CXCR4 were amplified by the polymerase chain reaction (PCR) utilising primers designed from regions of nucleotide sequence homology between the human, bovine and ovine CXCR4 sequences. Using mRNA derived from either the feline T cell line Mya-1 or the CrFK cell line, cDNA clones were amplified by PCR, cloned and the nucleic acid sequence determined (Willett et al. 1997). The predicted amino acid sequence of feline CXCR4 displayed 94.9% amino acid identity to human CXCR4 compared with 90.5% identity to murine CXCR4. The high degree of homology between human and feline CXCR4 provided support for the proposal that fusion between the human cell line HeLa and FIV-infected CrFK cells was mediated by CXCR4. Subsequent studies demonstrated that transfection of the human cell line U87 with either human or feline CXCR4 rendered these cells permissive for fusion with FIV-infected CrFK cells (Willett et al. 1997).

In order to demonstrate that FIV interacts directly with CXCR4, binding studies were performed using ^{125}I-labelled SDF-1α and a derivative of the human cell line U87 transfected stably with feCXCR4 (Hosie et al. 1998). SDF-1 was found to interact with feline CXCR4 with a high affinity (SDF-1α with a K_D of 12.0 $\pm$ 5.3 nM, SDF-1β with a K_D of 10.4 $\pm$ 1.5nM). Immunoaffinty purified Env protein from the CrFK-adapted isolate of FIV (FIV_{PET}) competed with SDF-1 for binding to feline CXCR4, K_D = 0.48 $\pm$ 0.16nM. Previous studies using HIV-1 IIIB gp120 (Hesselgesser et al. 1997) had demonstrated displacement of SDF-1 binding, K_D=70 $\pm$

26nM, indicating that the binding affinity of FIV Env and CXCR4 was very high. However, the HIV-1 IIIb gp120 binding studies were performed with CD4-negative human neurones and infection with HIV-1 IIIB is CD4-dependent; this may account for the lower affinity interaction between the IIIB gp120 and CXCR4. As yet, binding of envelope glycoprotein from CD4-independent strains of HIV to CXCR4 has not been examined but studies on the binding of envelope glycoproteins to the β-chemokine receptor CCR5 in the presence of CD4 have demonstrated half-maximal inhibition of ^{125}I-labelled MIP-1α (a CCR5 ligand) at concentrations from approximately 1 to 5nM (Hill et al. 1997; Wu et al. 1996). Also, SIVmac239 gp120 binds rhesus macaque CCR5 in the absence of CD4 with a K_D of 14.36$\pm$3.90nM (Martin et al. 1997). Therefore it appears that there is indeed a high affinity interaction between FIV gp120 and CXCR4, implicating CXCR4 itself as the primary receptor for CrFK-adapted strains of FIV. Our recent studies have demonstrated that transfection of murine or hamster cell lines with CXCR4 renders the cells permissive for fusion with FIV-infected CrFK cells (Willett et al. 1998) suggesting that either a highly conserved component of the feline, murine and hamster cell surface acts as the primary viral receptor or infection is mediated by CXCR4 alone, analogous to infection with CD4-independent strains of HIV-2. In agreement with these findings, recent studies by Poeschla and Looney (Poeschla and Looney, 1998) have demonstrated that replication-defective FIV pseudotypes based on the 34TF10 molecular clone of FIV will infect human cells in the presence, but not in the absence, of human CXCR4. Furthermore, the post-entry block to FIV infection that exists in human cells (Ikeda et al. 1996) would appear to be due to poor promoter activity of the FIV LTR U3 element (Poeschla and Looney, 1998) as when a heterologous promoter is substituted, efficient production of infectious FIV occurs.

Inhibition of FIV infection by CXCR4 antagonists

The natural ligand for CXCR4 has been identified as the chemokine SDF-1 (stromal cell derived factor) (Bleul et al. 1996; Oberlin et al. 1996). As we had demonstrated both a direct interaction between FIV Env and CXCR4 as well as competition between SDF-1 and FIV Env for binding to CXCR4, the antiviral activity of SDF-1 against FIV infection was evaluated. Incubation of CrFK cells with either recombinant or synthetic SDF-1 resulted in a dose-dependent inhibition of infection with FIV_{PET} (a subtype A isolate) (Hosie et al. 1998). Subsequent experiments suggested that the inhibition of infection of CrFK cells was mediated by steric hindrance and not by down-modulation of CXCR4 expression as has been implicated as the antiviral principle for HIV-1 (Amara et al. 1997) since prolonged incubation of CrFK cells with SDF-1 led to enhanced expression of CXCR4 and increased susceptibility to FIV infection (Hosie et al. 1998). Recent studies have suggested that SDF-1 has broad anti-viral activity against FIV and that the inhibition of infection extends not only to other antigenically distinct subtype A isolates, but also to isolates from subtype B and D (Endo et al. 1998). Importantly, SDF-1 did not inhibit infection of the T-lymphoblastoid cell line Mya-1 with FIV_{PET} (Hosie et al. 1998), suggesting the existence of a CXCR4-independent pathway of infection with FIV. However, Endo *et al* have observed inhibition of FIV infection by SDF-1β in the lymphoid cell line Kumi-1 (Endo et al. 1998) and Egberink *et al* demonstrated that infection of thymocytes with FIV_{UT113} is inhibited by the CXCR4 antagonist AMD3100 (Egberink et al. 1998). The studies by Endo et al and Egberink et al. are significant in that they suggest a role for CXCR4 in infection with primary strains of FIV. It is conceivable that the restricted tropism of primary isolates may the reflect a

requirement for expression of a high affinity primary receptor in conjunction with CXCR4 for infection to occur, analogous to the use of CD4 as a primary receptor by HIV, and that cell culture adaptation allows the virus to infect using CXCR4 alone. A precedent for this model has already been set with the discovery that CD4-independent strains of HIV-2 use CXCR4 as the sole receptor for infection (Endres et al., 1996). Future studies should therefore address the mechanism of FIV infection of Mya-1 cells in the presence of CXCR4 antagonists to clarify whether infection of these cells is indeed CXCR4-independent.

The development of CXCR4 antagonists provides a potential source of therapeutic agents for the treatment of FIV-infected cats and HIV-infected human beings. We have compared a number of CXCR4-antagonists for the ability to inhibit FIV infection of CrFK cells and many of these agents have potent anti-viral activity. Figure 3 illustrates a comparison of the ability of three CXCR4 antagonists, namely AMD3100 (Donzella et al. 1998), ALX404C (Doranz et al. 1997) and Met-SDF, to inhibit infection of CrFK cells with FIV_{PET}. Each of the antagonists inhibited FIV infection with a similar efficiency; 100% inhibition being observed at concentrations of between 10 and 100nM. The concentrations of AMD3100 and ALX404C that inhibited FIV infection were similar to those inhibiting HIV-1 infection (Donzella et al. 1998; Doranz et al. 1997), again suggesting that FIV and HIV interact in a similar way with CXCR4 to promote infection.

Mapping of domains on CXCR4 involved in FIV infection

The discovery that FIV interacts specifically with the chemokine receptor CXCR4 suggests that despite the evolutionary divergence of FIV and HIV, the envelope glycoproteins of FIV and HIV share conserved structural features enabling a

high affinity interaction. Several studies have demonstrated that the alterations in the V3 loop that generate an SI phenotype select for usage of CXCR4 as a coreceptor (Choe et al. 1996; Speck et al. 1997), suggesting that the V3 loop contributes to the CXCR4 binding site. Given that mutations in the V3 loop of FIV select for CrFK adaptation and usage of CXCR4 as a receptor, the data suggest that the CXCR4 binding sites of both viruses are structurally similar and that the V3 loop contributes to the structure. The interaction between HIV and CXCR4 has been investigated using a series of chimaeric CXCR4 molecules generated between CXCR4 and the related α-chemokine receptor CXCR2. These studies demonstrated that the first and second extracellular loops of CXCR4 were found to be important determinants of the interaction with CD4-dependent T-tropic or dual-tropic strains of HIV(Lu et al. 1997). In contrast, M-tropic strains could only use CXCR4 when the amino terminus was substituted with that of CCR5(Lu et al. 1997). Similarly, when chimaeras were generated between human CXCR4 and the rat homologue of CXCR4, the second extracellular loop was found to be the principal determinant of CXCR4-usage by the NDK strain of HIV-1 (Brelot et al. 1997). In agreement with these findings, chimaeras constructed between murine and human CXCR4 have demonstrated that the second loop of human CXCR4 expressed in the context of murine CXCR4 renders canine thymocytes susceptible to infection with HIV-1 strains that are unable to use murine CXCR4 as a receptor (Parolin et al. 1998).

In recent studies, we have investigated the interaction between FIV and CXCR4 using the panels of chimaeric CXCR4 molecules described above. Using rat CXCR4/human CXCR4 chimaeras (Brelot et al. 1997) the principal determinant of CXCR4 usage by FIV was localised to the second extracellular loop; a chimaeric molecule derived almost entirely from rat CXCR4, except for the second extracellular

loop which was derived from human CXCR4, supported both fusion and infection with similar efficiency to the parent human CXCR4 molecule (Willett et al. 1998). When similar studies were performed using human CXCR2/human CXCR4 chimaeras, substitution of the first or third extracellular domains of CXCR4 with the corresponding domains of CXCR2 rendered the molecule non-functional as an FIV receptor. The data suggest that either the first and third extracellular loops of CXCR4 contribute to the FIV Env binding site or that these domains are required to present the second extracellular loop in the appropriate conformation for the interaction with the FIV Env. Previous studies have suggested that the N-terminal extracellular domain of CXCR4 is required for CXCR4 to function as a coreceptor for some strains of HIV (Lu et al. 1997). However, mutant CXCR4 molecules in which the N-terminus was either replaced with the corresponding domain of CXCR2, or was removed completely to form a truncated molecule, supported both fusion and infection with CrFK-adapted FIV (Willett et al. 1998), suggesting that this portion of the molecule is not required for the interaction with FIV Env.

Conclusions

Adaptation of FIV to replicate in the CrFK cell line is accompanied by the selection of viruses that are capable of interacting with the chemokine receptor CXCR4. These CXCR4-dependent strains of FIV may be analogous to CD4-independent strains of HIV-2 that appear to interact directly with CXCR4 (Endres et al. 1996). Indeed, it has been demonstrated recently that the second extracellular loop of CXCR4 is the principal determinant of CXCR4 usage by HIV-2 ROD/B (Reeves et al. 1998). There are a number of striking similarities between CrFK-adapted strains of FIV and HIV-2 ROD/B. Adaptation of FIV to a CrFK-tropic phenotype is

accompanied by a glutamate to lysine switch in the V3 loop of the virus, increasing the net charge of the V3 loop. Also, a similar mutation in the V3 loop of HIV-2 ROD-B (mutation of glutamine 310 to lysine) enhances the CD4-independent phenotype(Reeves and Schultz, 1997) of the virus. Furthermore, while a methionine 751 to threonine mutation in the TM protein of FIV is associated with the CrFK-tropic phenotype(Vahlenkamp et al. 1997), a similar alanine 526 to threonine mutation in the TM protein of HIV-2 ROD is observed upon adaptation to a CD4-independent phenotype(Reeves and Schultz, 1997), in each case the mutations are located adjacent to the region of TM thought to be involved in the formation of a coiled-coil domain involved in oligomerization, viral entry and neutralization (Cao et al. 1993; Dubay et al. 1992; Ho et al. 1987; Muster et al. 1993; Wild et al. 1993). Although mutations in other regions of the *env* gene are thought to be necessary to confer a CrFK-tropic phenotype on FIV (Siebelink et al. 1995; Vahlenkamp et al. 1997; Verschoor et al. 1995), and a CD4-independent phenotype on HIV-2 (Reeves and Schultz, 1997), both viruses adapt in a similar way to the use of pathways for infection that result ultimately in the use of CXCR4 alone as a viral receptor. These studies suggest that common structural motifs remain despite the divergence in amino acid sequence between FIV and HIV. By comparing the mechanism of infection with the feline and human lentiviruses, it may be possible to define the regions of the Env protein that are essential for the chemokine receptor interaction and ultimately to design novel therapeutic agents that can be used in the treatment of lentiviral diseases.

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References

Ackley, C.D., Yamamoto, J.K., Levy, N., Pedersen, N.C. and Cooper, M.D. (1990) Immunologic abnormalities in pathogen-free cats experimentally infected with feline immunodeficiency virus. Journal of Virology 64, 5652-5655.

Amara, A., LeGall, S., Schwartz, O., Salamero, J., Montes, M., Loetscher, P., Baggiolini, M., Virelizier, J.L. and ArenzanaSeisdedos, F. (1997) HIV coreceptor downregulation as antiviral principle: SDF-1 alpha- dependent internalization of the chemokine receptor CXCR4 contributes to inhibition of HIV replication. Journal of Experimental Medicine 186, 139-146.

Barr, M.C., Zou, L.L., Long, F., Hoose, W.A. and Avery, R.J. (1997) Proviral organization and sequence analysis of feline immunodeficiency virus isolated from a pallas' cat. Virology 228, 84-91.

Bleul, C.C., Farzan, M., Choe, H., Parolin, C., Clark-Lewis, I., Sodroski, J. and Springer, T.A. (1996) The lymphocyte chemoattractant SDF-1 is a ligand for LESTR/Fusin and blocks HIV-1 entry. Nature 282, 829-833.

Boyer, V., DesGranges, C., Trabaud, M.-A., Fischer, E. and Kazatchkine, M.D. (1991) Complement mediates human immunodeficiency virus type 1 infection of a human T cell line in a CD4- and antibody-independent fashion. Journal of Experimental Medicine 173, 1151.

Brelot, A., Heveker, N., Pleskoff, O., Sol, N. and Alizon, M. (1997) Role of the first and third extracellular domains of CXCR-4 in human immunodeficiency virus coreceptor activity. Journal of Virology 71, 4744-4751.

Brown, E.W., Yuhki, N., Packer, C. and OBrien, S.J. (1994) A lion lentivirus related to feline immunodeficiency virus -epidemiologic and phylogenetic aspects. Journal of Virology 68, 5953-5968.

Brunner, D. and Pedersen, N.C. (1989) Infection of peritoneal macrophages in vitro and in vivo with with feline immunodeficiency virus. Journal of Virology 63, 5483-5488.

Cao, J., Bergeron, L., Helseth, E., Thali, M., Pepke, H. and Sodroski, J. (1993) Effects of amino acid changes in the extracellular domain of the human immunodeficiency virus type 1 gp41 envelope glycoprotein. Journal of Virology 67, 2747-2755.

Choe, H., Farzan, M., Sun, Y., Sullivan, N., Rollins, B., Ponath, P.D., Wu, L., Mackay, C.R., LaRosa, G., Newman, W., Gerard, N., Gerard, C. and Sodroski, J. (1996) The β-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. Cell 85, 1135-1148.

de Jong, J.J., de Ronde, A., Keulen, W., Tersmette, M. and Goudsmit, J. (1992) Minimal requirements for the human immunodeficiency virus type 1 V3 domain to support the syncytium-inducing phenotype: analysis by single amino acid substitution. Journal of Virology 66, 6777-6780.

Dean, G.A., Reubel, G.H., Moore, P.F. and Pedersen, N.C. (1996) Proviral burden and infection kinetics of feline immunodeficiency virus in lymphocyte subsets of blood and lymph node. Journal of Virology 70, 5165-5169.

Donzella, G.A., Schols, D., Lin, S.W., Este, J.A., Nagashima, K.A., Maddon, P.J., Allaway, G.P., Sakmar, T.P., Henson, G., DeClercq, E. and Moore, J.P. (1998) AMD3100, a small molecule inhibitor of HIV-1 entry via the CXCR4 co- receptor. Nature Medicine 4, 72-77.

Doranz, B.J., GrovitFerbas, K., Sharron, M.P., Mao, S.H., Goetz, M.B., Daar, E.S., Doms, R.W. and Obrien, W.A. (1997) A small-molecule inhibitor directed against the chemokine receptor CXCR4 prevents its use as an HIV-1 coreceptor. Journal of Experimental Medicine 186, 1395-1400.

Dow, S.W., Poss, M.L. and Hoover, E.A. (1990) Feline immunodeficiency virus: a neurotropic lentivirus. J. AIDS 3, 658-668.

Dubay, J.W., Roberts, S.J., Brody, B. and Hunter, E. (1992) Mutations in the leucine zipper of the human immunodeficiency virus type 1 transmembrane glycoprotein affect fusion and infectivity. Journal of Virology 66, 4748-4756.

Egberink, H.F., Calafat, J., Ederveen, J., Koolen, M.J.M. and Horzinek, M.C. (1991) Non-feline cells are infectable by FIV harvested from CrFK cultures. (Unpublished observations).

Egberink, H.F., De Clercq, E., Henson, G., Balzarini, J., Horzinek, M.C. and Schols, D. (1998) The bicyclam AMD3100, a selective antagonist of the human chemokine receptor CXCR4, inhibits feline immunodeficiency virus (FIV) replication in vitro. Fourth International Feline Retrovirus Research Symposium A3.3, 26.

Endo, Y., Mizuno, T., Nishimura, Y., Goto, Y., Okayama, T., Watari, T., Tsujimoto, H. and Hasegawa, A. (1998) Inhibitory effect of SDF-1β on feline immunodeficiency virus replication. Fourth International Feline Retrovirus Research Symposium A3.4, 26.

Endres, M.J., Clapham, P.R., Marsh, M., Ahuja, M., Turner, J.D., McKnight, A., Thomas, J.F., Stoebenau-Haggarty, B., Choe, S., Vance, P.J., Wells, T.N.C., Power, C.A., Sutterwala, S.S., Doms, R.W., Landau, N.R. and Hoxie, J.A. (1996) CD4-independent infection by HIV-2 is mediated by fusin. Cell 87, 745-756.

English, R.V., Johnson, C.M., Gebhard, D.H. and Tompkins, M.B. (1993) In vivo lymphocyte tropism of feline immunodeficiency virus. Journal of Virology 67, 5175-5186.

Feng, Y., Broder, C.C., Kennedy, P.E. and Berger, E.A. (1996) HIV-1 entry co-factor:functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. Science 272, 872-877.

Hesselgesser, J., HalksMiller, M., DelVecchio, V., Peiper, S.C., Hoxie, J., Kolson, D.L., Taub, D. and Horuk, R. (1997) CD4-independent association between HIV-1 gp120 and CXCR4: Functional chemokine receptors are expressed in human neurons. Current Biology 7, 112-121.

Hill, C.M., Deng, H.K., Unutmaz, D., KewalRamani, V.N., Bastiani, L., Gorny, M.K., Zolla-Pazner, S. and Littman, D.R. (1997) Envelope glycoproteins from human immunodeficiency virus types 1 and 2 and simian immunodeficiency virus can use human CCR5 as a coreceptor for viral entry and make direct CD4-dependent interactions with this chemokine receptor. Journal of Virology 71, 6296-6304.

Ho, D.D., Sarngadharan, M.G., Hirsch, M.S., Schooley, R.T., Rota, T.R., Kennedy, R.C., Chanh, T.C. and Sato, V.L. (1987) Human immunodeficiency virus neutralizing antibodies recognize several conserved domains on the envelope glycoprotein. Journal of Virology 61, 2024-2028.

Hosie, M.J., Broere, N., Hesselgesser, J., Turner, J.D., Hoxie, J.A., Neil, J.C. and Willett, B.J. (1998) Modulation of feline immunodeficiency virus infection by stromal cell-derived factor (SDF-1). Journal of Virology 72, 2097-2104.

Ikeda, Y., Tomonaga, K., Kawaguchi, Y., Kohmoto, M., Inoshima, Y., Tohya, Y., Miyazawa, T., Kai, C. and Mikami, T. (1996) Feline immunodeficiency virus can

infect a human cell-line (molt- 4) but establishes a state of latency in the cells. Journal of General Virology 77, 1623-1630.

Langley, R.J., Hirsch, V.M., OBrien, S.J., Adgerjohnson, D., Goeken, R.M. and Olmsted, R.A. (1994) Nucleotide-sequence analysis of puma lentivirus (PLV-14) - genomic organization and relationship to other lentiviruses. Virology 202, 853-864.

Lu, Z.H., Berson, J.F., Chen, Y.H., Turner, J.D., Zhang, T.Y., Sharron, M., Jenks, M.H., Wang, Z.X., Kim, J., Rucker, J., Hoxie, J.A., Peiper, S.C. and Doms, R.W. (1997) Evolution of HIV-1 coreceptor usage through interactions with distinct CCR5 and CXCR4 domains. Proceedings of the National Academy of Sciences USA 94, 6426-6431.

Martin, K.A., Wyatt, R., Farzan, M., Choe, H., Marcon, L., Desjardins, E., Robinson, J., Sodroski, J., Gerard, C. and Gerard, N.P. (1997) CD4-independent binding of SIV gp120 to rhesus CCR5. Science 278, 1470.

Muster, T., Steindl, F., Purtscher, M., Trkola, A., Klima, A., Himmler, G., Ruker, F. and Katinger, H. (1993) A conserved neutralizing epitope on gp41 of human immunodeficiency virus type 1. Journal of Virology 67, 6642-6647.

Norimine, J., Miyazawa, T., Kawaguchi, Y., Tomonaga, K., Shin, Y.-S., Toyosaki, T., Kohmoto, M., Niikura, M., Tohya, Y. and Mikami, T. (1993) Feline CD4 molecules expressed on feline non-lymphoid cell lines are not enough for productive infection of highly lymphotropic feline immunodeficiency virus isolates. Archives of Virology 130, 171-178.

Novotney, C., English, R.V., Housman, J., Davidson, M.G., Nasisse, M.P., Jeng, C.R., Davis, W.C. and Tompkins, M.B. (1990) Lymphocyte population changes in cats naturally infected with feline immunodeficiency virus. AIDS 4, 1213-1218.

Oberlin, E., Amara, A., Bachelerie, F., Bessia, C., Virelizier, J.-L., Arenzana-Seisdedos, F., Schwartz, O., Heard, J.-M., Clark-Lewis, I., Legler, D.F., Loetscher, M., Baggiolini, M. and Moser, B. (1996) The CXC chemokine SDF-1 is the ligand for LESTR/fusin and prevents infection by T-cell-line-adapted HIV-1. Nature 382, 833-835.

Olmsted, R.A., Hirsch, V.M., Purcell, R.H. and Johnson, P.R. (1989) Nucleotide-sequence analysis of feline immunodeficiency virus -genome organization and relationship to other lentiviruses. Proceedings Of The National Academy Of Sciences Of The United States Of America 86, 8088-8092.

Pancino, G., Castelot, S. and Sonigo, P. (1995) Differences in feline immunodeficiency virus host-cell range correlate with envelope glycoprotein fusogenic properties. Virology 206, 796-806.

Parolin, C., Borsetti, A., Choe, H., Farzan, M., Kolchinsky, P., Heesen, M., Ma, Q., Gerard, C., Palu, G., Dorf, M.E., Springer, T. and Sodroski, J. (1998) Use of murine CXCR-4 as a second receptor by some T-cell-tropic human immunodeficiency virus. Journal of Virology 72, 1652-1656.

Poeschla, E.M. and Looney, D.J. (1998) CXCR4 requirement for a nonprimate lentivirus:heterologous expression of FIV in human, rodent and feline cells. Journal of Virology 72, 6858-6866.

Reeves, J.D., Heveker, N., Brelot, A., Alizon, M., Clapham, P.R. and Picard, L. (1998) The second extracellular loop of CXCR4 is involved in CD4-independent entry of human immunodeficiency virus type 2. Journal of General Virology 79, 1793-1799.

Reeves, J.D. and Schultz, T.F. (1997) The CD4-independent tropism of human immunodeficiency virus type 2 involves several regions of the envelope protein and

correlates with a reduced activation threshold for envelope-mediated fusion. Journal of Virology 71, 1453-1465.

Robinson, W.E., Montefiori, D.C. and Mitchell, W.M. (1990) Complement-mediated antibody-dependent enhancement of HIV-1 infection requires CD4 and complement receptors. Virology 175, 600-604.

Siebelink, K.H.J., Karlas, J.A., Rimmelzwaan, G.F., Osterhaus, A.D.M.E. and Bosch, M.L. (1995) A determinant of feline immunodeficiency virus involved in CrFK tropism. Veterinary Immunology and Immunopathology 46, 61-69.

Speck, R.F., Wehrly, K., Platt, E.J., Atchison, R.E., Charo, I.F., Kabat, D., Chesebro, B. and Goldsmith, M.A. (1997) Selective employment of chemokine receptors as human immunodeficiency virus type 1 coreceptors determined by individual amino acids within the V3 loop. Journal of Virology 71, 7136-7139.

Talbott, R.L., Sparger, E.E., Lovelace, K.M., Fitch, W.M., Pedersen, N.C., Luciw, P.A. and Elder, J.H. (1989) Nucleotide-sequence and genomic organization of feline immunodeficiency virus. Proceedings Of The National Academy Of Sciences Of The United States Of America 86, 5743-5747.

Vahlenkamp, T.W., Verschoor, E.J., Schuurman, N.N.M.P., van Vliet, A.L.W., Horzinek, M.C., Egberink, H.F. and de Ronde, A. (1997) A single amino acid substitution in the transmembrane envelope glycoprotein of feline immunodeficiency virus alters cellular tropism. Journal of Virology 71, 7132-7135.

Verschoor, E.J., Boven, L.A., Blaak, H., van Vliet, A.R.W., Horzinek, M.C. and de Ronde, A. (1995) A single mutation within the V3 envelope neutralization domain of feline immunodeficiency virus determines its tropism for CRFK cells. Journal of Virology 69, 4752-4757.

Wild, C., Greenwell, T. and Matthew, T. (1993) A synthetic peptide from HIV-1 is a potent inhibitor of virus-mediated cell-cell fusion. AIDS Research and Human Retroviruses 9, 1051-1053.

Willett, B.J., Adema, K., Heveker, N., Brelot, A., Picard, L., Alizon, M., Turner, J.D., Hoxie, J.A., Peiper, S., Neil, J.C. and Hosie, M.J. (1998) The second extracellular loop of CXCR4 determines its function as a receptor for feline immunodeficiency virus. Journal of Virology 72, 6475-6481.

Willett, B.J., Hosie, M.J., Dunsford, T.H., Neil, J.C. and Jarrett, O. (1991) Productive infection of T-helper lymphocytes with feline immunodeficiency virus is accompanied by reduced expression of CD4. AIDS 5, 1469-1475.

Willett, B.J., Hosie, M.J., Neil, J.C., Turner, J.D. and Hoxie, J.A. (1997) Common mechanism of infection by lentiviruses. Nature 385, 587.

Willett, B.J., Picard, L., Hosie, M.J., Turner, J.D., Adema, K. and Clapham, P.R. (1997) Shared usage of the chemokine receptor CXCR4 by the feline and human immunodeficiency viruses. Journal of Virology 71, 6407-6415.

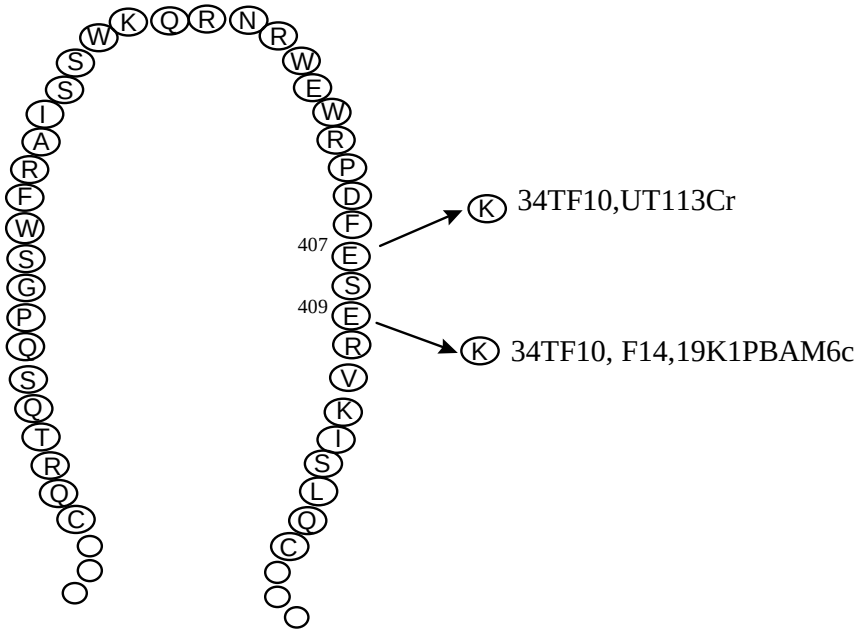
Wu, L., Gerard, N.P., Wyatt, R., Choe, H., Parolin, C., Ruffing, N., Borsetti, A., Cardosso, A.A., Desjardin, E., Newman, W., Gerard, C. and Sodroski, J. (1996) CD4-induced interaction of primary HIV-1 gp120 glycoproteins with the chemokine receptor CCR5. Nature 384, 179-183.

Figure Legends

Figure 1. Amino acid sequence mutations in the V3 loop of FIV that select for growth in the CrFK cell line. Adaptation involves the mutation of either of two glutamate residues, residues 407 and 409 (numbering according to the FIV_{PET}-F14 sequence, Genbank accession number M25381) to lysine, increasing the net charge of the V3 loop. E to K mutations are present in the 34TF10 (Talbott et al. 1989), F14 (Olmsted et al. 1989), UT113Cr (Verschoor et al. 1995) and 19K1PBAM6 (Siebelink et al. 1995) strains of FIV, all of which have been selected for growth in CrFK cells.

Figure 2. Inhibition of syncytium formation between FIV-infected CrFK cells and Hela cells by anti-CXCR4 antibody. Hela cells were incubated for 1 hour with 25μg/ml of either the anti-CXCR4 antibody 12G5, the anti-CD9 antibody vpg15 or the anti-FIV envelope antibodies vpg67 and 71.2; a 1:20 dilution of a serum from an FIV infected cat (Q71) or a 1:20 dilution of a polyclonal goat serum raised against the feline endogenous retrovirus RD114. FIV_{PET}-infected CrFK cells were then added and incubated for 18 to 24 hours at which time the cells were fixed, stained and the number of syncytia per field enumerated.

Figure 3. Inhibition of FIV infection by CXCR4 antagonists. CrFK cells were incubated with a dilution series of the CXCR4 antagonists AMD3100, ALX404C and met-SDF for 1 hour. Virus was added to the cells and incubated for 1 hour at 37^{o}C. The cells were then washed twice with culture medium and then re-fed with medium containing antagonist. Supernatants were collected 5 days post-infection and assayed for viral core protein p24 by ELISA.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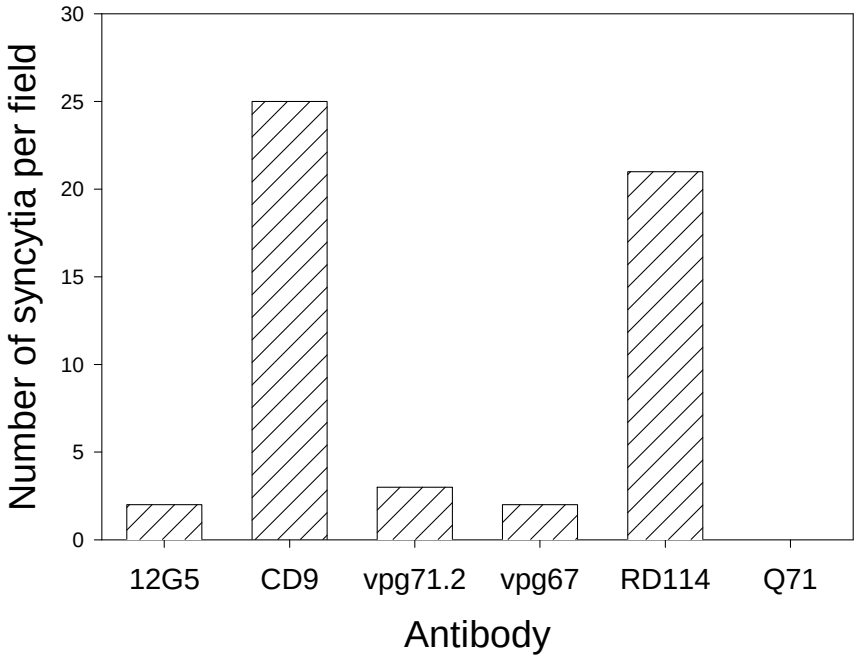
Number of syncytia per field
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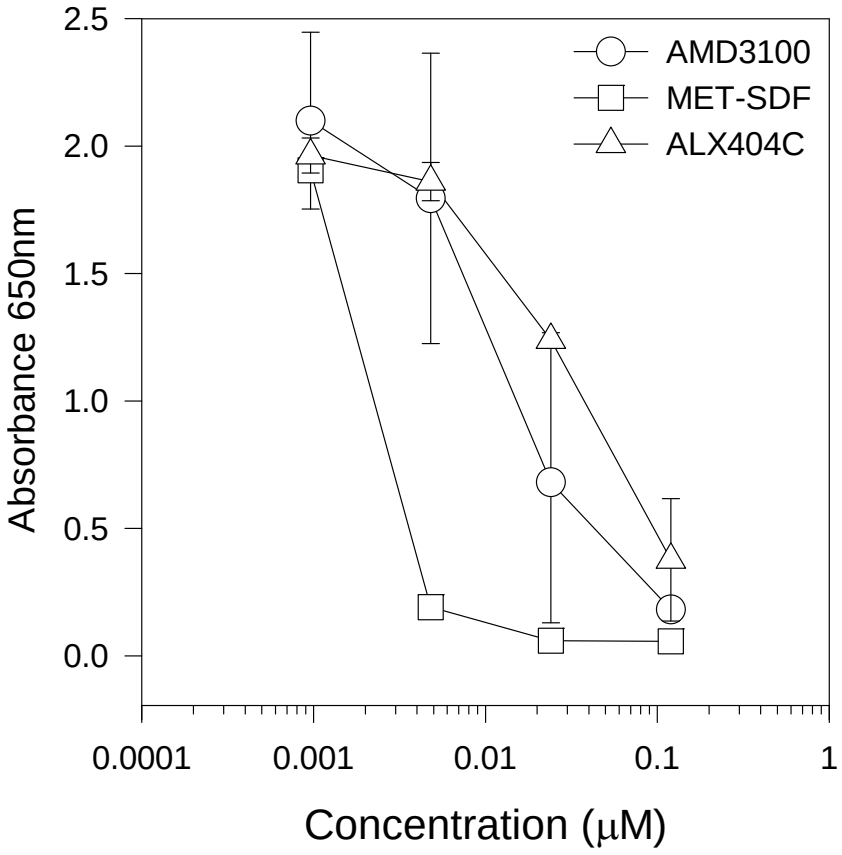
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Concentration (μM)