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Chemokine receptors and co-stimulatory molecules: unravelling feline immunodeficiency virus infection

Brian J. Willett* and Margaret J. Hosie

Retrovirus Research Laboratory, Institute of Comparative Medicine, Faculty of Veterinary Medicine, University of Glasgow, Bearsen Road, Glasgow G61 1QH. United Kingdom.

Abstract

Feline immunodeficiency virus (FIV) infection of the domestic cat induces an immunodeficiency characterised by a gradual depletion of CD4+ T-helper lymphocytes. The virus targets T-helper cells by way of an interaction between its envelope glycoprotein (Env) and the cell surface molecule CD134 (OX40), a member of the nerve growth factor receptor/tumour necrosis factor receptor superfamily. The Env-CD134 interaction is a necessary prerequisite for the subsequent interaction with CXCR4, the only chemokine receptor identified to date to act as a co-receptor for FIV. As T-helper cell expression of CD134 and CXCR4 is restricted to activated cells, FIV targets selectively antigen-specific T-helper cells. With disease progression the cell tropism of the virus expands; this may be the result of changes in the way in which Env interacts with CD134, a less stringent Env-CD134 interaction enabling the Env to interact more readily with CXCR4 and thus broadening the cell tropism of virus. In contrast, viruses that are present in early infection may have a narrower cell tropism, reflecting a more stringent interaction with CD134. Accordingly, “early” viruses may target CD134-expressing cells more efficiently and be more resistant to neutralising antibody. It is these early viruses that may be transmitted and should be considered as candidates for the development of vaccine regimes and novel therapeutic agents.

Keywords

FIV; receptor; CD134; OX40; CXCR4

Introduction

Feline immunodeficiency virus (FIV) is a widespread pathogen of the domestic cat; in the United Kingdom alone, an estimated 500,000 cats are infected with FIV. Infection leads to the development of an immune dysfunction characterised by a progressive decline in CD4+ helper T lymphocytes (T_h cells), leading ultimately to an AIDS-like syndrome marked by clinical signs of wasting, gingivitis/stomatitis and neuropathies. Thus FIV has been studied widely as both an important veterinary pathogen and an animal model for HIV/AIDS. FIV and the primate

*corresponding author, b.willett@vet.gla.ac.uk, tel: +44 141 330 3274, fax: +44 141 330 2271, Retrovirus Research Laboratory, Institute of Comparative Medicine, Faculty of Veterinary Medicine, University of Glasgow, Bearsen Road, Glasgow G61 1QH. United Kingdom.

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lentiviruses (HIV and SIV) are unique amongst the lentiviruses in targeting CD4+ helper T cells selectively and inducing immunodeficiency; the caprine, ovine and equine lentiviruses induce a disease state that is more typical of a chronic inflammatory condition while infection the bovine lentivirus appears to be largely inapparent. Why then do FIV, HIV and SIV target CD4+ lymphocytes? Do they share common receptors or do they follow distinct entry pathways to achieve the same insidious outcome?

HIV receptors

The specificity of the virus-receptor interaction is a primary determinant of cell tropism and a critical factor in the pathogenesis of viral disease. HIV targets T_H cells and cells of the monocyte macrophage lineage by way of a high affinity interaction between the viral envelope glycoprotein and CD4^{18,36,39}; the viral cell tropism correlating with the cellular distribution of CD4. Further complexity is added to the cell tropism of HIV in that for infection to proceed, the primary CD4 interaction must be followed by a secondary interaction between the viral envelope glycoprotein and a molecule from the seven transmembrane domain (7TM) superfamily, typically the chemokine receptors CXCR4^{7,13,29,46} and CCR5^{4,23,26,63}. Although a range of other 7TM molecules support infection with a limited number of strains of HIV^{10,12,13,25,43,52}, some 7TM molecules (for example CCR3) appear to be used efficiently as viral co-receptors by biologically relevant primary strains of virus^{1,48}. Moreover, restricted usage of the promiscuous chemokine receptor D6 by primary isolates of HIV-1 and HIV-2 may contribute to astrocyte tropism and CNS dissemination⁴⁴. In humans T cells, CXCR4 is expressed widely on naïve CD45RO+ cells while CCR5 expression is restricted to memory CD45RA+ cells⁸. Accordingly, CXCR4-dependent strains of HIV such as LAI⁵ and 3B⁵¹ have a broader T cell tropism while CCR5-dependent strains such as JRFL³⁷ and YU2³⁸ target selectively memory/antigen-stimulated T cells. As CXCR4 is expressed widely in the thymus, these cells are susceptible to selective targeting and destruction by X4 strains of HIV¹⁷.

Prior to the elucidation of 7TM molecule function in viral entry, HIV strains were often classified as T cell tropic or macrophage-tropic^{14,30}. While co-receptor usage and macrophage tropism are linked in that the majority of macrophage-tropic strains of HIV use CCR5, other determinants within the viral envelope glycoprotein (Env) protein contribute to macrophage tropism. Indeed, several primary strains of HIV have been identified which are macrophage-tropic and which use CXCR4 as a co-receptor⁵⁸ while conversely, some R5 strains of HIV infect macrophages very poorly⁴⁹. The major determinant of macrophage tropism would appear to be the ability to use low levels of CD4 and co-receptor efficiently^{32,48,49,62}, this being particularly marked with viruses isolated from the CNS or following adaptation for growth in microglia^{31,42,49}.

The restricted expression pattern of the co-receptors CXCR4 and CCR5 may lead to “filtering” of viruses during transmission, the so-called “gatekeeper” hypothesis, whereby CXCR4-dependent strains are readily inactivated by mucins on mucosal surfaces while CCR5-dependent strains may selectively traverse the cells of the sub-mucosa by transcytosis due to local expression of CCR5 and not CXCR4 (reviewed in Margolis and Shattock, 2006⁴¹).

FIV receptors

CXCR4

Infection with HIV requires a sequential interaction between the HIV Env and CD4 followed by a second interaction with a “co-receptor”, typically the chemokine receptors CXCR4 and CCR5. Prior to CD4 binding, the chemokine receptor binding site on Env is hidden; attachment to CD4 triggers a conformational change in Env that reveals the chemokine receptor binding

site enabling attachment to the co-receptor. The Env/co-receptor interaction in turn triggers a rearrangement of the transmembrane protein gp41/TM that ultimately leads to exposure of the fusion peptide and fusion of the viral and cellular membranes. FIV infection, like HIV infection, requires an interaction with a co-receptor^{50,71,74}. The identity of the co-receptor for FIV was revealed in advance of the discovery of the primary viral receptor, taking advantage of the fusogenic properties of the Envs of cell-culture (CrFK)-adapted strains of FIV. During the process of adaptation for growth in CrFK cells, the net charge of the third variable (V3) loop of FIV Env increases and the Env acquires a syncytium-inducing phenotype^{57,66}. This process of CrFK-adaptation shared many similarities with CD4-independent infection with HIV, thus, the usage of chemokine receptors by CrFK-tropic strains of FIV was investigated and it was revealed that CXCR4 expression alone was sufficient to mediate syncytium formation^{71,74} and viral entry^{33,74}. Further, FIV infection could be abrogated by the natural ligand for CXCR4 (SDF-1) and Scatchard analysis predicted a CXCR4-binding affinity for FIV Env of $\sim 1\text{nM}$ ³³. Subsequent studies revealed that all primary strains of FIV tested to date use CXCR4 as the co-receptor for infection, infection being inhibited by the potent and selective CXCR4 antagonist AMD3100^{28,53}. The interaction between FIV Env and CXCR4 would appear to be very similar to that between the Env of X4 strains of HIV and CXCR4, with the second extracellular loop being critical to its function as a viral co-receptor^{9,67}.

CD134

FIV and HIV share a similar cell tropism, targeting preferentially CD4+ T-helper cells and cells of the monocyte/macrophage lineage. As human CD4 is expressed on T-helpers and monocyte/macrophages there is a good concordance between expression of the primary viral receptor and cell tropism. However, CD4 expression in the cat is restricted solely to T-helper cells and is not expressed on monocyte/macrophages², indicating that despite a shared cell tropism with HIV, FIV may not share usage of CD4 as a primary viral receptor^{34,45,70}. Does FIV require a primary receptor for infection? CXCR4 expression alone is sufficient to render cells permissive for infection with CrFK-adapted strains of FIV^{33,67,74}, however, although high level expression of CXCR4 permits a variable degree of viral entry with primary strains of FIV^{21,68}, the existence of a high affinity primary receptor for FIV was indicated by the failure of CXCR4 expression alone to render cells permissive for production infection with primary strains of FIV such as GL8⁷⁴ despite infection being CXCR4-dependent⁵³. Further, FIV Env “immunoadhesins” (fusion proteins between the SU component of Env and the Fc domain of IgG) appeared to bind specifically to a 40kDa protein on feline cells known to be susceptible to infection with primary strains of FIV²⁴. In order to identify the primary receptor for FIV, a cDNA expression library from the IL2-dependent feline T cell line (MYA-1) was prepared and screened by “panning” using FIV-coated plates⁵⁶. A single cDNA was identified that conferred reproducible FIV binding upon murine myeloma cells following ectopic expression. The cDNA encoded the feline homologue of OX40 (CD134), a member of the tumour necrosis factor receptor/nerve growth factor receptor (TNFR/NGFR) superfamily⁵⁵. Ectopic expression of feline CD134 rendered cells permissive for productive infection with primary strains of FIV; viral entry following challenge with HIV(FIV) pseudotypes; and syncytium formation following transfection with expression vectors encoding *env* genes from primary strains of FIV⁵⁵. The species specificity of FIV infection was revealed by the failure of FIV to use human CD134 as a functional receptor⁵⁵. Further, CD134-dependent infection required co-expression of CXCR4; infection being abrogated by the CXCR4 antagonist AMD3100⁵⁵. Ectopic expression of CD134 renders feline cells permissive for infection with all strains of FIV tested to date irrespective of geographical origin and phylogenetic subtype⁷².

CD134 was first described as a marker for activated rat CD4+ T cells^{40,47} and studies have revealed that CD134 is expressed on IL2-dependent feline T cells *in vitro*⁵⁵, and on lymph

node-derived CD4⁺ T cells *in vivo*³⁵. Further, immunoblotting studies have confirmed high level expression of feline CD134 in mitogen-stimulated CD4⁺ T cells²⁰ (and not CD8⁺ T cells). Together, these data are consistent with the selective targeting of activated CD4⁺ T_{helper} cells by the virus and the gradual depletion of this lymphocyte subset with the progression towards AIDS in infected animals. In humans and mice, CD134 is also expressed at low levels on activated CD8⁺ T cells^{3,6}, macrophages and activated B cells²⁷. Given that in chronic infection with FIV there appears to be a shift in cell tropism from CD4⁺ T-helper cells towards CD8⁺ T cells and B cells, the tropism of FIV would appear to be consistent with the predicted expression pattern of CD134.

Mapping the FIV Env-CD134 interaction

FIV PPR SU binds to a determinant in the first cysteine-rich domain (CRD1) of CD134 and chimaeric CD134 molecules expressing this sequence in the context of human CD134 (human CD134 does not act as a functional receptor for FIV) generates a function receptor for this strain of virus.¹⁹ However, such chimaeras either support infection very inefficiently or fail to support infection with a range of other strains of FIV suggesting that additional determinants in CD134 are required for infection with these viruses⁷². Systematic mutational analysis of CRD2 of CD134 has revealed that determinants in the A1 loop of CRD2 are required for efficient infection with strains of FIV such as GL8 and CPG41⁷³. Functional studies have indicated that critical residues 78N, 79Y, 80E, form the binding site for OX40L (CD134L) and these studies are in agreement with the recently solved crystal structures of the human CD134:CD134L complex (Figure 1). Given that this region is remote from the predicted FIV Env binding site, the data suggest that the conformation of CD134 is critical to infection with GL8-like viruses.

Previous studies looking at the interaction between herpes simplex virus glycoprotein D (HSV gD) and its cellular receptor HveA (herpes simplex virus entry mediator A) have demonstrated that binding of HSV gD to HveA can be inhibited by the binding of the natural ligands for HveA (LIGHT and LT- α). However, the inhibition of HSV gD binding is not by direct competition for binding to a shared point of interaction, rather the inhibitory effect is thought to be allosteric such that LT- α and LIGHT engagement with HveA alters the conformation of HveA such that HSV gD can no longer bind. It is likely then that GL8-like viruses recognise a conformation of CD134 that requires the presence of an intact CRD2. Given that this site forms the CD134 ligand binding site, infection with such viruses may be susceptible to modulation by CD134L, either enhancing or inhibiting infection.

Receptor usage in non-domestic cats

To date, 27 of 35 felid species surveyed have shown seroreactivity against feline lentiviral antigens and infection has been confirmed in 11 of these species following PCR amplification of viral genomic material and nucleic acid sequencing (reviewed in Vande Woude and Apetrei⁶⁴). Little is known about the receptor usage of the non-domestic cat lentiviruses however it is known that both the lion lentivirus (LLV (or FIVple)) and puma lentivirus (PLV (or FIVpco)) will grow in domestic cat PBMC and cell lines⁶⁵. When LLV and PLV are grown in feline IL2-dependent T cells, neither CD134 nor CXCR4 are down-regulated⁷³. In contrast, both CD134 and CXCR4 are down-regulated following infection with FIV of diverse origins⁷³, consistent with their roles as viral receptor and co-receptor respectively⁶⁰. Further, some LLVs and PLVs appear to infect the CD134-negative cell line 3201⁵⁹ readily, with the caveat that isolation of replication competent LLV and PLV often requires a prolonged co-culture of lion or puma PBMCs with feline T cells in order to recover virus. This may indicate that a degree of cell culture adaptation is occurring during viral growth and that this may, ultimately, alter receptor usage. Infection with LLV and PLV is not blocked by either the

CXCR4-specific antagonist AMD3100 or SDF-1 α , the CXCR4 ligand (ref 59 and Figure 2). Thus the data to date would indicate that the non-domestic cat lentiviruses may use receptors distinct from CXCR4 and CD134, the receptors used by the domestic cat viruses.

Significance of receptor usage to pathogenesis

In humans, CXCR4 is expressed widely on T cells while CCR5 expression is restricted to memory CD45RA⁺ cells⁸; thus R5 strains of HIV, the strains that predominate in early infection, selectively target this T cell subpopulation. In contrast, expression of feline CXCR4 on domestic cat T cells is restricted to activated cells^{35,53,69} (mitogen-stimulation of T cells *in vitro* results in upregulation of CXCR4) suggesting that the virus should be able to target both CD4⁺ and CD8⁺ T cells providing they express the primary receptor. Given that the majority of CD134 expression is found on CD4⁺ T cells, receptor usage would predict that the primary target for FIV would be activated CD4⁺ T cells, for example those residing in the lymphoid tissues. FIV, like HIV, appears to be trapped by DC-SIGN²² on dendritic cells in lymphoid tissues, facilitating presentation of the virus to its target population during the process of antigen presentation and the formation of an immunological synapse. This would lead to the selective destruction of antigen-specific T cells during an active immune response.

Progression from an acute to a chronic infection with FIV is accompanied by an expansion of cell tropism from CD4⁺ T cells to CD4⁺ and CD8⁺ T cells, and B cells. In HIV infection, there is often a switch in the biological phenotype of the virus from R5 to X4R5 or X4 with disease progression¹⁶, resulting in an expanded cell tropism of the virus. All strains of FIV tested to date interact with CXCR4. In the absence of an alternative co-receptor, an alternative mechanism underlying the shift in cell tropism with disease progression must be sought. Feline CXCR4 is expressed on B cells and monocyte/macrophages⁶⁹, thus an expanded tropism would be consistent with continued usage of CXCR4 however which feature of the virus in chronic (late) infection is it that governs the expanded tropism? One possible mechanism may be a relaxation of the requirement for an interaction with CD134, such that the virus evolves towards CD134-independence. Such strains of virus may be more readily neutralised by the humoral immune response and thus only arise with the development of a functional immunodeficiency. Accordingly, strains of FIV such as GL8 and CPG41 (early?) appear to recognise a complex determinant on CD134⁷³ while isolates such as PPR and B2542 (late?) require only a minimal determinant on CD134 for infection^{19,73}. Thus, while infection will all primary strains of FIV would require co-expression of both CD134 and CXCR4, levels of CXCR4 expression would play a major role in determining susceptibility to infection with “late” viruses. Consistent with this hypothesis, over-expression of human CXCR4 is sufficient to render cells permissive to infection with FIV PPR²¹ while the *in vivo* distribution of FIV NCSU³⁵ indicates that levels of CXCR4 expression are a critical determinant of cell tropism for this virus.

The complex interaction between Env and CD134 observed with GL8 and CPG41 may be advantageous to the virus in that the chemokine receptor (CXCR4) binding site may be well-hidden from the humoral immune response, only being exposed upon full engagement between Env and CD134. “Late” viruses would have an increased propensity for CD134-independent infection resulting from a more exposed CXCR4-binding site (or a reduced threshold for exposure of the CXCR4-binding site). As a consequence of this, the CXCR4 binding site in may be more readily accessible to neutralising antibodies. This model is consistent with observations with HIV-2 where both X4 and R5 strains appear more readily neutralizable when utilizing a CD4-independent route of infection⁶¹ and with HIV-1 where recently-emerged X4 variants have an enhanced sensitivity to neutralization compared to co-existing R5 variants¹¹.

Conclusions

Infection of the domestic cat with FIV induces a disease state similar to AIDS. By using CD134 as a primary receptor, a molecule that is preferentially expressed on activated feline CD4⁺ T cells, the virus targets a similar population of T cells to the CD45RA⁺ population targeted by R5 strains of HIV. The restricted expression of feline CXCR4 to activated T cells facilitates further the specificity of the viral cell tropism in early infection; however, with disease progression, alterations in the way in which the virus interacts with CD134 may allow the virus to interact with CXCR4 more readily and disseminate to a broader range of CXCR4-expressing cells. The significance of this hypothesis would be that viruses in early infection would have a biological phenotype distinct from those in late infection, with implications for the selection of biologically relevant challenge strains and viral strains for vaccine production and immunogen design. Moreover, the biological properties of an early virus may affect sensitivity to receptor antagonists and novel therapeutic agents. Further dissection of the contribution of the virus-receptor interaction to the pathogenesis of disease will be critical to the development of novel vaccine approaches and therapeutic interventions.

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Figure 1.

Model of the feline CD134:CD134L complex. The 3D-structures of feline CD134L (white) and CD134 (grey) were modelled on the solved crystal structure of the human CD134:CD134L complex¹⁵ using the SWISS-MODEL automated protein homology server⁵⁴. Residues involved in binding of FIV PPR immunoadhesin¹⁹ are shown in pink while residues 78N, 79Y and 80E involved in infection with GL8 and CPG41⁷³ are shown in blue.

Figure 2.

Infection of AH927 cells by PLV is independent of CXCR4. AH927 cells stably expressing feline CXCR4 were exposed to FIV-GL8, FIV-PET, and the puma viruses CoLV and PLV-14. Infections were performed in the presence (+) or absence (-) of AMD3100 (1 μ g/ml). Supernatants were assayed for reverse transcriptase at 9 days post-infection.