Expression of CXCR4 on feline peripheral blood mononuclear cells: effect of feline immunodeficiency virus (FIV) infection

BRIAN J. WILLETT*, CELIA A. CANNON & MARGARET J. HOSIE

Retrovirus Research Laboratory, Institute of Comparative Medicine, Department of Veterinary Pathology, University of Glasgow, Glasgow G61 1QH, U.K.

Running title:CXCR4 expression in the cat

* corresponding author

Dr. B.J. Willett
Retrovirus Research Laboratory
Department of Veterinary Pathology
University of Glasgow
Bearsden Road
Glasgow G61 1QH
United Kingdom

E-mail: b.willett@vet.gla.ac.uk, Tel: +44 141 330 3274, Fax: +44 141 330 5602

SUMMARY

CXCR4 expression was analysed on feline peripheral blood mononuclear cells (PBMC). While monocytes and B lymphocytes expressed CXCR4, no CXCR4 was detected on T lymphocytes, in stark contrast to the expression pattern on T lymphocytes from human beings. In spite of the important role that CXCR4 plays in infection with feline immunodeficiency virus (FIV), expression on PBMC *in vivo* was unaffected by infection with either primary or cell culture-adapted strains of virus.

CXCR4 expression in the cat

The major pathway of infection with the primate lentiviruses involves attachment of the virus via a high affinity interaction with CD4 followed by binding to a seven transmembrane domain molecule (7TM), principally CXCR4 or CCR5. Consequently, viruses that utilize CXCR4 as a receptor target preferentially the cell types expressing CXCR4 while those using CCR5 target CCR5-expressing cells. In human beings, the expression of CCR5 on peripheral blood T cells is restricted largely to CD4+/CD45RO+ cells (memory cells), while CXCR4 is expressed on both naïve and memory T cells, albeit with higher levels on CD4+/CD45RA+ cells (naïve cells) (3, 13, 14, 16, 18). Moreover, CXCR4 expression is not restricted to T cells; significant amounts of CXCR4 are found on B cells while NK cells are effectively negative for expression (3, 13). Conversely, CCR5 is expressed on NK cells and not B cells. The differential expression of CXCR4 and CCR5 in the immune system of human beings may provide a basis for the specific targeting of memory T cells early in infection (when CCR5-dependent strains predominate) and the wider range of cell types targeted with the emergence of CXCR4-dependent strains in the later stages of infection (3, 23). Previous studies demonstrated that feline immunodeficiency virus (FIV) utilised the feline homologue of CXCR4 as a receptor (21, 22). The envelope glycoprotein from cell culture-adapted strains of FIV binds with a high affinity to CXCR4-expressing cells (11) and infection with both cell culture-adapted and primary strains of FIV is blocked by CXCR4 antagonists (9-11, 17, 19). Given the restricted expression of CXCR4 and CCR5 in the immune system of human beings, and how this affects the *in vivo* tropism of HIV, it is important to determine the expression pattern of CXCR4 in the domestic cat in order to elucidate the

in vivo cell tropism of FIV and ultimately the pathogenesis of FIV-related immunodeficiency.

CXCR4 expression *in vivo*. Using a cross-species reactive anti-human CXCR4 monoclonal antibodies (44701, 44717 and 44718, provided by M. Tsang, R&D Systems, Minneapolis) that we identified previously (11), we applied two-colour flow cytometry to examine CXCR4 expression on feline peripheral blood mononuclear cells (Fig. 1). Feline leucocytes were prepared by whole blood lysis (20) and analysed on an EPICS Elite flow cytometer (Beckman Coulter, Sheffield, UK). Lymphocyte, monocytes and granulocyte populations were identified on the basis of their forward scatter (FSC) vs. 90^{o} side scatter (SSC) characteristics (Fig. 1a). CXCR4 expression resided largely within the lymphoid gate (L) with a distinct population clustering in the region (M) consisting predominantly of monocytes (Fig. 1b). In order to confirm the identity of the monocyte population, the cells were dual-labelled with antibodies recognizing CXCR4 (44718) and CD14 (Tük 4, Dako Ltd., Ely, UK), a marker expressed at high levels on both murine and human monocytes but also to a lesser degree on a subpopulation of human B cells (12). CD14 expression resided largely within the expected monocyte gate (Fig. 1c) with a minor population of lymphocytes expressing CD14, consistent with feline CD14 having a similar expression pattern to human CD14. To further confirm the validity of the monocyte gate, a parallel sample was stained with the B cell marker, B220 (CD45R, Beckton Dickinson UK Ltd., Cowley, UK). Expression of B220 was restricted predominantly to the lymphoid gate (Fig. 1d) and did not correlate with the monocyte population identified by CD14 expression. Thus monocytes could be reliably identified on the basis of FSC versus SSC characteristics (monocyte (M) gate, Fig. 1a.) in

conjunction with CD14 expression. The expression of CXCR4 on CD14+ monocytes was variable, however, expression was detected consistently on monocytes from all cats tested (Fig. 1e). We examined next the expression of CXCR4 on lymphoid cells. The majority of CXCR4 expression resided in the B220+ population (B cells, Fig. 1f). While the intensity of CXCR4 expression varied amongst cats, the level of expression was consistent amongst analyses. CD5+ lymphocytes (in the domestic cat, CD5 expression is restricted to T cells (1, 4)) were ostensibly negative for CXCR4 (Fig. 1g). Similar findings were obtained using antibodies against feline CD4, CD8 and CD3 (data not shown). Anti-CXCR4 antibodies 44701 and 44717 (R&D Systems) yielded similar findings to antibody 44718 (data not shown) suggesting that the low level of reactivity with feline T cells is unlikely to result from the expression of a distinct antigenic conformation of CXCR4 on these cells. In order to compare the intensity of CXCR4 expression on feline and human lymphocytes we processed samples of feline and human blood in parallel for flow cytometry (Fig. 2). While CXCR4 expression was detected on both feline (Fig. 2b, B220+) and human (Fig. 2e, CD19+) B cells, only human T cells (Fig. 2f, CD3+) but not feline T cells (Fig. 2c, CD5+) expressed detectable levels of CXCR4. Although human lymphocytes (Fig. 2d.) were observed to be larger than feline lymphocytes (Fig. 2a.), the smaller surface area of the feline cells was insufficient to account for the failure to detect CXCR4 expression on feline T cells. Thus while expression of CXCR4 on both B cells and monocytes in the cat is similar to the expression pattern in human beings, it differs markedly in that it is undetectable on peripheral T cells. It is intriguing that the human and feline viruses induce such similar

disease conditions while the major (co)receptor distribution varies between the two species.

CXCR4 expression in FIV-infected cats. CXCR4 expression is down-modulated on some T cell subsets from HIV-infected human beings (15). Given that CXCR4 expression is down-regulated on FIV infected cells *in vitro* (11), we asked whether FIV infection affected CXCR4 expression *in vivo*. The expression of CXCR4 was examined in three groups of three age-matched animals that were either uninfected (control), or had been infected for two years with either a primary strain of FIV ($GL8_{414}$) or a cell-culture adapted strain of FIV (PET_{F14}). Mean CXCR4 expression (absolute number or fluorescence intensity) was remarkably consistent amongst the three study groups (Table 1 , Group 1), although a wide range of values was observed, in agreement with chemokine receptor expression on human PBMC (13). Further, CXCR4 expression was consistent amongst three separate analyses (data not shown). Given that it was not possible to analyse CXCR4 expression prior to infection in these animals, we monitored CXCR4 expression on T and B cells in a second group of 4 cats (vaccine study control group) during the early phase of infection with $GL8_{414}$. CXCR4 expression was analysed at 1 week prior to challenge, and at 2, 6 and 13 weeks post-infection (Table 1, Group 2). CXCR4 expression did not differ significantly on CD5+ T cells or B220+ B cells during the course of the analyses. Taken together, the data indicate that FIV infection does not induce gross alterations in the level of CXCR4 expression on feline PBMC.

Infection with FIV is CXCR4-dependent (9, 11, 17, 22), thus the expression of CXCR4 on feline monocytes is consistent with FIV being a monocyte-tropic virus (8). Similarly, the high level of CXCR4 expression on B cells is consistent with B cells being

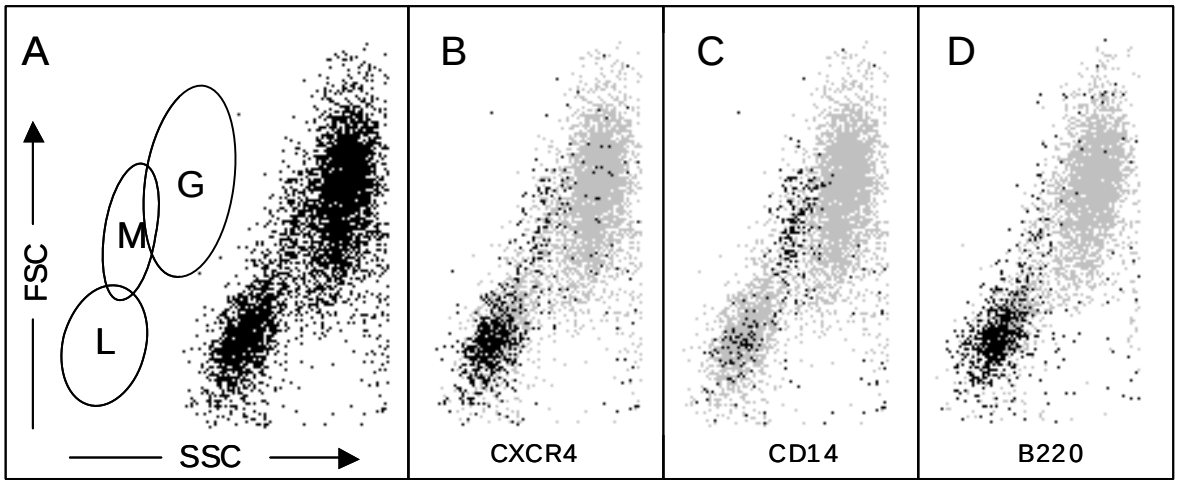
a major reservoir for proviral DNA in chronic infection (5). However, the failure to detect CXCR4 expression on the majority of T lymphocytes in peripheral blood is in contrast to the distribution of CXCR4 expression in human beings (3, 13). While it is possible that CXCR4 may be expressed on resting feline T cells in an antigenic conformation that is not recognized by the CXCR4 antibodies used in this study, previous data have demonstrated that the same anti-CXCR4 antibodies used in this study (44701, 44717 and 44718) recognize human CXCR4 on both T and B cells (2) and feline CXCR4 on a range of cell types (11). Moreover, using the same antibodies, we were able to detect CXCR4 expression on freshly isolated feline thymocytes and on a proportion of T cells within lymph nodes (data not shown), in support of a previous study showing CXCR4 expression on mitogen- activated feline T cells (17). It was shown recently that HIV-specific CD4+ T cells are infected preferentially by HIV *in vivo* (7) and given that the major reservoir for FIV in early infection is CD4+ lymphocytes, it is possible that in the early stages of infection, FIV infection is restricted to activated T cells within the lymphatic system. Thus, if the majority of CXCR4-expressing T cells within the lymph node are CD4+, then CD4+ T cells would be targeted selectively in early infection. Alternatively, early infection may require an interaction with either a non-CXCR4 receptor (6), or a primary receptor in addition to CXCR4, the expression of which is restricted to activated CD4+ lymphocytes. Further elucidation of the mechanism of FIV infection *in vivo* may reveal conservation of important host and viral factors between this animal lentivirus and its human counterpart.

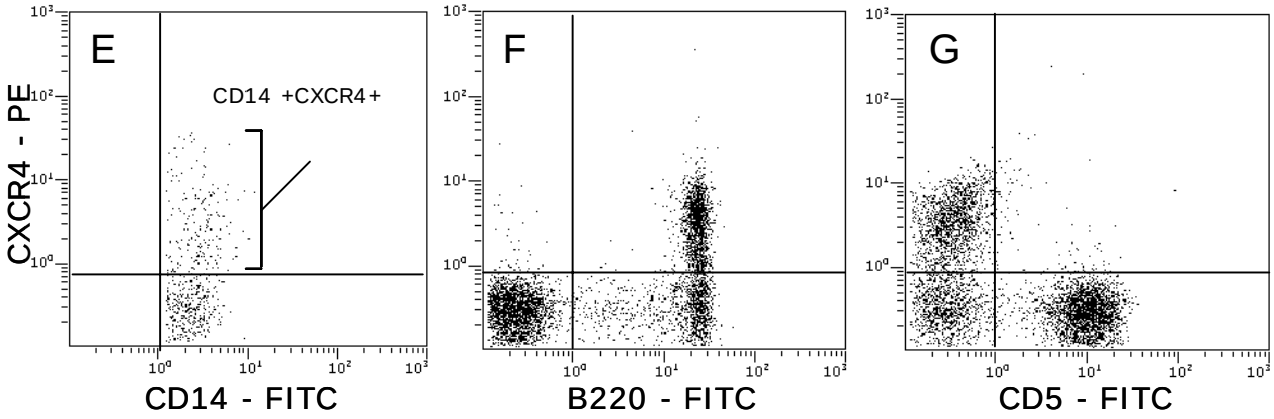
This study was funded by The Wellcome Trust and NIH grant AI049765. We thank M. Tsang for providing anti-CXCR4 antibody.

FIGURE LEGENDS

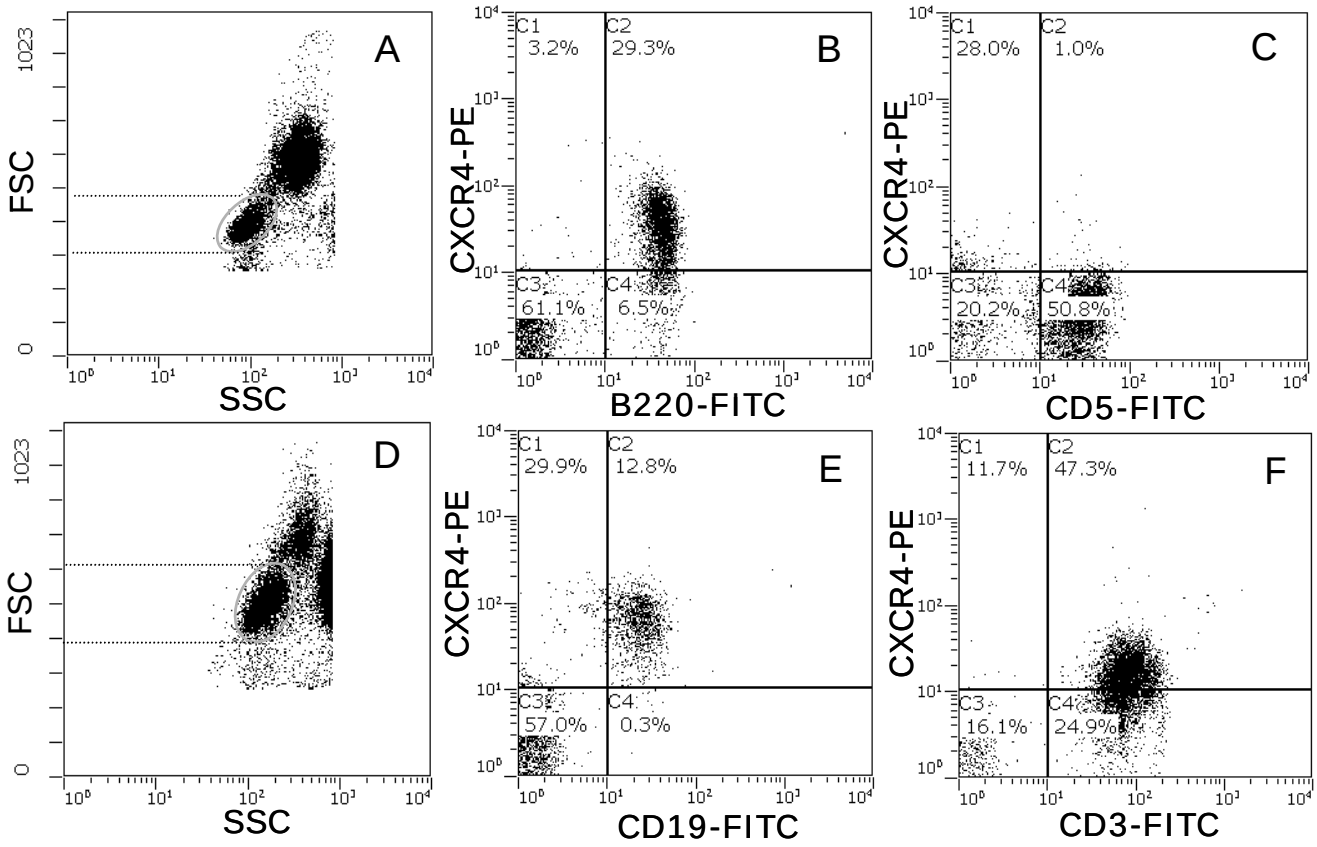
Figure 1. CXCR4 expression on feline peripheral blood mononuclear cells. A. Discrimination of lymphocytes (L), monocytes (M) and granulocytes (G) by forward scatter (FSC) vs. 90^{o}C side scatter (SSC). CXCR4+ (B), CD14+ (C) and B220+ (D) cells back-gated on a plot of FSC vs. SSC. Two colour analysis of CXCR4 expression (phycoerythrin, PE) vs. CD14 (E), B220 (F) or CD5 (G) coupled to fluorescein isothiocyanate (FITC). Each figure represents 10,000 events, data analysis used Expo ADC software (Applied Cytometry Systems, Sheffield, U.K.).

Figure 2. Comparison of CXCR4 expression on feline and human T cells. Feline PBMC and human PBMC were processed for flow cytometry by whole blood lysis. Lymphocytes were identified on the basis of forward scatter (FSC) vs. 90^{o}C side scatter (SSC) characteristics (dotted lines illustrate the respective sizes of feline (A) and human (D) lymphocytes, ellipse delineates the analysis gate). Two colour analysis of CXCR4 expression (PE) vs. B220 (B) and CD5 (C) on feline lymphocytes and CD19 (E) and CD3 (F) on human lymphocytes. Each figure represents 10,000 events, data are presented without baseline offset.


A
B
C
D
G
M
L
FSC
SSC
CXCR4
CD14
B220


E
F
G
CD14 +CXCR4+
CXCR4 - PE
CD14 - FITC
B220 - FITC
CD5 - FITC

CXCR4 expression in the cat



REFERENCES

1. **Ackley, C. D. and M. D. Cooper**. 1992. Characterization of a feline T-cell-specific monoclonal antibody reactive with a CD5-like molecule. Am. J. Vet. Res. **53**:466-471.

2. **Baribaud, F., T. G. Edwards, M. Sharron, A. Brelot, N. Heveker, K. Price, F. Mortari, M. Alizon, M. Tsang, and R. W. Doms** . 2001. Antigenically distinct conformations of CXCR4. J. Virol. **75**:8957-8967.

3. **Bleul, C. C., L. J. Wu, J. A. Hoxie, T. A. Springer, and C. R. Mackay**. 1997. The HIV coreceptors CXCR4 and CCR5 are differentially expressed and regulated on human T lymphocytes. Proc. Natl. Acad. Sci. USA **94**:1925-1930.

4. **Brown, A. L., T. H. Dunsford, O. Jarrett, B. J. Willett, and M. J. Hosie**. 2002. Demonstration of biological activity of CD40 ligand (CD154) in the domestic cat. Cytokine **17**:140-148.

5. **Dean, G. A., G. H. Reubel, P. F. Moore, and N. C. Pedersen**. 1996. Proviral burden and infection kinetics of feline immunodeficiency virus in lymphocyte subsets of blood and lymph node. J. Virol. **70**:5165-5169.

6. **deParseval, A. and J. H. Elder**. 2001. Binding of recombinant feline immunodeficiency virus surface glycoprotein to feline cells: role of CXCR4, cell-surface heparans, and an unidentified non-CXCR4 receptor. J. Virol. **75**:4528-4539.

7. **Douek, D. C., J. M. Brenchley, M. R. Betts, D. R. Ambrozak, B. J. Hill, Y. Okamoto, J. P. Casazza, J. Kuruppu, K. Kunstman, S. Wolinsky, Z. Grossman, M. Dybul, A. Oxenius, D. A. Price, M. Connors, and R. A. Koup**. 2002. HIV preferentially infects HIV-specific CD4+ T cells. Nature **417**:95-98.

8. **Dow, S. W., C. K. Mathiason, and E. A. Hoover**. 1999. In vivo monocyte tropism of pathogenic feline immunodeficiency viruses. J. Virol. **73**:6852-6861.

9. **Egberink, H. F., E. De Clerq, A. L. Van Vliet, J. Balzarini, G. J. Bridger, G. Henson, M. C. Horzinek, and D. Schols**. 1999. Bicyclams, selective antagonists of the human chemokine receptor CXCR4, potently inhibit feline immunodeficiency virus replication. J. Virol. **73**:6346-6352.

10. **Endo, Y., Y. Goto, Y. Nishimura, T. Mizuno, T. Watari, A. Hasegawa, T. Hohdatsu, H. Koyama, and H. Tsujimoto**. 2000. Inhibitory effect of stromal cell derived factor-1 on the replication of divergent strains of feline immunodeficiency virus in a feline T-lymphoid cell line. Vet. Immunol. Immunopathol. **74**:303-314.

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

12. **Labeta, M. O., R. Landmann, J. P. Obrecht, and R. Obrist**. 1991. Human B cells express membrane-bound and soluble forms of the CD14 myeloid antigen. Mol. Immunol. **28**:115-122.

13. **Lee, B., M. Sharron, L. J. Montaner, D. Weissman, and R. W. Doms**. 1999. Quantification of CD4, CCR5, and CXCR4 levels on lymphocyte subsets, dendritic cells, and differentially conditioned monocyte-derived macrophages. Proc. Natl. Acad. Sci. U. S. A. **96**:5215-5220.

14. **Mackay, C. R.** 1999. Dual personality of memory T cells. Nature **401**:659-660.

15. **Nicholson, J. K., S. W. Browning, R. L. Hengel, E. Lew, L. E. Gallagher, D. Rimland, and J. S. McDougal**. 2001. CCR5 and CXCR4 expression on memory and naive T cells in HIV-1 infection and response to highly active antiretroviral therapy. J. Acquir. Immune. Defic. Syndr. **27**:105-115.

16. **Qin, S., J. B. Rottman, P. Myers, N. Kassam, M. Weinblatt, M. Loetscher, A. E. Koch, B. Moser, and C. R. Mackay**. 1998. The chemokine receptors CXCR3 and CCR5

mark subsets of T cells associated with certain inflammatory reactions. J. Clin. Invest **101**:746-754.

17. **Richardson, J., G. Pancino, T. Leste-Lasserre, J. Schneider-Mergener, M. Alizon, P. Sonigo, and N. Heveker**. 1999. Shared usage of the chemokine receptor CXCR4 by primary and laboratory-adapted strains of feline immunodeficiency virus. J. Virol. **73**:3661-3671.

18. **Sallusto, F., D. Lenig, C. R. Mackay, and A. Lanzavecchia**. 1998. Flexible programs of chemokine receptor expression on human polarized T helper 1 and 2 lymphocytes. J. Exp. Med. **187**:875-883.

19. **Willett, B. J. and M. J. Hosie**. 1999. The role of the chemokine receptor CXCR4 in infection with feline immunodeficiency virus. Molecular Membrane Biology **16**:67-72.

20. **Willett, B. J., M. J. Hosie, J. J. Callanan, J. C. Neil, and O. Jarrett**. 1993. Infection with feline immunodeficiency virus is followed by the rapid expansion of a CD8+ lymphocyte subset. Immunology **78**:1-6.

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

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

23. **Wu, L. J., W. A. Paxton, N. Kassam, N. Ruffing, J. B. Rottman, N. Sullivan, H. Choe, J. Sodroski, W. Newman, R. A. Koup, and C. R. Mackay**. 1997. CCR5 levels and expression pattern correlate with infectability by macrophage-tropic HIV-1, in vitro. J. Exp. Med. **185**:1681-1691.