CD134 and CXCR4 expression corresponds to feline immunodeficiency virus infection of lymphocytes, macrophages and dendritic cells

F. Reggeti, C. Ackerley and D. Bienzle

1Department of Pathobiology, University of Guelph, Guelph, ON N1G 2W1, Canada
2The Hospital for Sick Children, Toronto, ON M5G 1X8, Canada

The lymphotropic lentiviruses feline immunodeficiency virus (FIV) and human immunodeficiency virus (HIV) enter cells by sequential interaction with primary receptors CD134 or CD4, respectively, and subsequently with chemokine receptors. The host-cell range for FIV is broader than that for HIV, but whether this is a function of receptor expression is unknown. Lack of reagents specific to feline molecules has limited detection and analysis of receptors and their interaction with viral components. Here, the expression of CD134 and CXCR4 on feline T and B lymphocytes, dendritic cells (DCs) and macrophages was examined and the kinetics of FIV replication were assessed. Quantification of CD134 mRNA by real-time PCR indicated expression in all leukocytes, with significantly more transcripts in CD4+ lymphocytes than in other leukocytes. Antibodies against human CD134 bound inconsistently to feline leukocytes. CXCR4 was detected with antibody clone 12G5 on the surface of monocyte-derived cells only, but gene transcripts were present in all cells, with the highest copy number in lymphocytes. CXCR4 expression decreased and CD134 expression increased with cell activation in lymphocytes. A subtype B biological isolate of FIV infected DCs, macrophages and lymphocytes, with the highest replication in CD4+ lymphocytes, whilst cloned FIV P14 infected all cells, but replicated less efficiently. Although viral replication was lower in DCs and macrophages than in lymphocytes, DCs expressed specific receptors and were infected productively with FIV, as indicated by viral ultrastructure and DNA detection. These results may implicate altered function of DCs in the induction of specific immunity against FIV.

INTRODUCTION

Infection of cells with lentiviruses involves a precise sequence of interactions of viral envelope components with cellular receptors, culminating in fusion of the viral envelope with the host-cell membrane (Hernandez et al., 1996). The primate lentiviruses human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) bind initially to CD4, which results in conformational changes in envelope gp120, exposure of a highly conserved site for interaction with chemokine receptors CCR5 or CXCR4 and fusion of gp41 with the host-cell membrane (Doms, 2000). The feline lentivirus feline immunodeficiency virus (FIV) has a similar pattern of receptor usage; however, CD134 rather than CD4 is the initial binding partner, and subsequent interaction with CXCR4 permits cell entry (Shimojima et al., 2004). CD134 has a similar pattern of receptor usage; however, CD134 rather than CD4 is the initial binding partner, and subsequent interaction with CXCR4 permits cell entry (Shimojima et al., 2004). CD134 is a member of the tumour necrosis factor (TNF) receptor family, with predominant expression on memory CD4+ T cells in humans and mice (al Shamkhani et al., 1996; Gramaglia et al., 1998; Paterson et al., 1987). Hence, selective expression of this receptor may direct FIV infection to a lymphocyte compartment essential for memory immune responses, and subsequent signalling via CXCR4 may induce cellular changes favouring viral integration and replication (Willett et al., 1997a, b). Domain 1 of CD134 binds to the FIV envelope and is distinct from domain 3, interacting with its natural ligand (de Parseval et al., 2005). Recent work has shown that incubation of soluble CD134 with FIV gp95 induces a conformational change resulting in exposure of the CXCR4-binding site and cryptic neutralization epitopes in the envelope (de Parseval et al., 2006). Thus, a similar two-step mechanism imparting specificity and potential cell-functional changes appears to be utilized by FIV and HIV.

FIV strains are classified into subtypes A–E, based on envelope sequences (Bachmann et al., 1997; Sodora et al., 1994), and field isolates infect and replicate in CD4+ and CD8+ T lymphocytes, B lymphocytes and monocyte-derived macrophages (MDMs) (Dean et al., 1996). In vivo, subtype-specific differences in pathogenicity have been inconsistently identified, but in vitro cell tropism and replication efficiency vary. Cloned FIV P34 (Talbott et al.,
1989) replicates poorly in peripheral blood mononuclear cells (PBMCs), whereas FIV PPR did not infect Crandell feline kidney (CRFK) cells (Phillips et al., 1990). FIV P14 (Olmsted et al., 1989) replicated efficiently in both cell types (Dean et al., 1999). Ability to infect CRFK cells relates to viral envelope sequences that can mediate cell fusion without initial interaction with CD134 (de Parseval et al., 2004a). Differential cell tropism may be due to viral Env–receptor interactions, function of transcriptional activators, cellular availability of nucleotides and/or specific long terminal repeat (LTR) sequences (Bendinelli et al., 1995). In vivo, feline CD4+ T lymphocytes contain the highest proviral burden during acute infections, whilst B lymphocytes become the main reservoir at later stages of infection (Dean et al., 1996; English et al., 1993). CD8+ lymphocytes were infected to the lowest extent at any stage of infection (Dean et al., 1996). FIV infected peritoneal and blood MDMs (Brunner & Pedersen, 1989; Dow et al., 1999) and was identified in proximity to follicular dendritic cells (DCs) in lymph-node sections (Hurtrel et al., 1994; Toyosaki et al., 1993). Myeloid-derived DCs are essential for initiating specific immune responses, but may also be natural targets of lentiviruses (Lekkerkerker et al., 2006). DC cell-surface molecules, such as DC-SIGN, heparan sulfate proteoglycans and mannose receptors, may bind non-specifically to lentiviruses and reduce requirements for interaction with specific receptors (de Parseval et al., 2004b). Whether feline DCs are productively infectable by FIV, what receptors may be involved and what functional consequence infection may have are as yet unknown. Hence, in this study, the hypothesis that feline DCs express specific receptors for FIV and are infectable, like primary lymphocytes, was examined.

**METHODS**

**Cell preparations.** Healthy cats were sedated with medetomidine (Dormitor; Pfizer) and ketamine (Ketalar; Bimeda-MTC) and blood was withdrawn by jugular venipuncture into EDTA, acid/citrate/glucose and heparin anti-coagulated tubes for cytometry, quantitative PCR and cell culture, respectively. For macrophage and DC cultures, 12–15 ml bone marrow was aspirated from the proximal humerus or the iliac crest into anti-coagulated syringes. All animals were FIV-negative by proviral PCR (Reggeti & Bienzle, 2004) and all protocols were approved by the Institutional Animal Care Committee.

PBMCs were separated by gradient centrifugation (Ficoll-Paque Plus; Amersham Biosciences) at 600 g for 30 min and washed with PBS. Aliquots of approximately 10 × 10^6 PBMCs were incubated with 1 μg mAb against feline CD4, CD8 (Southern Biotech) or CD21 (Serotec) or CD14 (TU¨ K4; Ancell) were added. Unlabelled (44717) or FITC-conjugated (12G5) CXCR4 antibody was used, and binding of unlabelled antibodies was detected with FITC-conjugated rat anti-mouse IgG (Serotec). Controls in each experiment consisted of cells incubated with isotype-matched IgG plus secondary antibody, and of single staining isotype-matched IgG plus secondary antibody. Cells were washed twice with flow buffer (PBS, 1% FBS, 0.1% sodium azide) and blocked with 0.5% goat anti-mouse IgG to prevent non-specific antibody binding. Cells were incubated with 20 μl pooled cat serum to block non-specific Fc receptor binding and washed, and antibodies against CXCR4 (clones 12G5 and 44717; R&D Systems) or CD134 (BerAct35; Ancell) were added. Unlabelled (44717) or FITC-conjugated (12G5) CXCR4 antibody was used, and binding of unlabelled antibodies was detected with FITC-conjugated rat anti-mouse IgG (Serotec). For dual staining, cells were blocked after the first stain with 0.5 μg purified mouse IgG, washed and then incubated with PE-labelled anti-CD4, CD8 (Southern Biotech), CD21 (Serotec) or CD14 (TU¨K4; DakoCytomation). All incubations were at 6–8°C for 30 min. Controls in each experiment consisted of cells incubated with isotype-matched IgG plus secondary antibody, and of single staining for each cell marker in dual-stain experiments to compensate for the different fluorescence markers.

Macrophages and DCs were washed with flow buffer, incubated with pooled cat serum to block non-specific Fc receptor binding and stained for co-expression of either CXCR4 or CD134 with CD14, as described above.

**Quantitative PCR.** Relative quantification of CD134 and CXCR4 to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was performed in a LightCycler instrument (Roche Diagnostics) by two-step real-time RT-PCR. Total RNA was extracted from fresh or in vitro-activated CD4+, CD8+ and CD21+ lymphocytes and from cultured macrophages and DCs (RNeasy Mini kit; Qiagen). Sufficient non-activated monocytes could not be derived in pure culture; hence, monocyte expression of FIV receptors was assessed only in fresh blood samples by cytometry. Residual genomic DNA was digested with DNase I (RNase-Free DNase; Qiagen) and RNA was eluted into 30 μl RNase-free water. cDNA was synthesized immediately (First Strand cDNA synthesis kit for RT-PCR; Roche Diagnostics) with an oligo(dT)15 primer, according to the manufacturer’s recommendations.
Real-time PCR amplifications were performed with primers CD134-f (5'-AAGGTCGACTTGGTCCAGG-3') and CD134-r (5'-TCCGGCATTCTACAACGAGG-3') for CD134; CXCR4-f (5'-ATGCTGGACACTTGTTAT-3') and CXCR4-r (5'-TGATGGTCTGAAACTGGGATA-3') for CXCR4; and GAPDH-f (5'-AGCCTTCTCCATGGTGGTG-AAGAC-3') and GAPDH-r (5'-CGGAGTCAACGGATTGTTGC-3') for GAPDH. CD134 and CXCR4 primers did not yield PCR products when using genomic DNA templates, due to the presence of introns or reduced efficiency, and amplicon specificity was confirmed by sequencing. Reactions consisted of 20 μl final volume in buffer with 4 mM MgCl₂, 0.5 μM each primer, 2 μl DNA Master SYBR green I (LightCycler FastStart DNA Master SYBR green I; Roche Diagnostics) and 2 μl template. Positive or negative controls consisted of purified PCR product or water in place of the template, respectively. After an initial incubation at 95 °C for 10 min, reactions were cycled 45 times with denaturation at 94 °C for 5 s, annealing at 58 °C for 5 s and extension at 72 °C for 15 s. Amplicons were heated from 65 to 95 °C for melting-curve analysis, and cooled to 40 °C. Fluorescence was acquired at the end of the extension step in the 'single' mode with channel setting F1. The 'second derivative maximum' mode and 'arithmetic' baseline adjustment were chosen. Specificity of PCR was assessed by melting-curve analysis and electrophoresis of amplicons in 1.5% agarose gels with ethidium bromide staining.

DNA standards for relative quantification were prepared by amplification with CD134-, CXCR4- and GAPDH-specific primers. Amplicons were separated by gel electrophoresis, bands were excised and DNA was purified (QIAquick Gel Extraction kit; Qiagen). Purified DNA was sequenced by the BigDye Terminator method (Applied Biosystems) on an ABI Prism 377 XL DNA sequencer (DNA Sequencing Facility, Robarts Research Institute, London, ON, Canada). Tenfold dilutions of each purified DNA sample, covering at least four orders of magnitude, were amplified in duplicate to create the standard curves. The 'fit coefficients' were then calculated for relative quantification with efficiency correction (LightCycler Relative Quantification software, version 1.0; Roche Diagnostics). Unknown samples were amplified with each primer set and a dilution of the respective standard was co-amplified (calibrator) to correct for sample non-homogeneity. Results were based on the 'crossing points' of the amplification curves determined by using the LightCycler software (version 3.5; Roche Diagnostics). Analyses were carried out with RelQuant software and results were expressed as the target : reference ratio of calibrators.

**Viruses.** A plasmid with the complete sequence of FIV P14 was obtained from J. H. Elder (Scripps Institute, La Jolla, CA, USA) through the AIDS Research and Reference Reagent Program. This plasmid was chosen for efficient infection of both lymphocytes and cells of the monocye/macrophage lineage (Dean et al., 1999). Virus stock was prepared by calcium phosphate (Life Technologies) transfection of CRFK cells (ATCC). Transfected cells were maintained in a minimum essential medium containing 10% FBS, 2 mM l-glutamine, 100 U penicillin ml⁻¹, 100 μg streptomycin ml⁻¹, and 5 mM HEPES (all from Life Technologies) at 37 °C in 5% CO₂ and supernatant was collected after 7 days. Virus stock of a primary subtype B isolate, USgaB01, was prepared by two passages in ConA-activated PBMCs. Supernatants containing virus were collected after 7 days, the TCID₅₀ was determined (Lane, 1999) and viruses were stored at −80 °C.

For infection of lymphocytes, mitogen-stimulated CD4⁺, CD8⁺ and CD21⁺ lymphocytes were incubated with 2 μg polybrene ml⁻¹ (Sigma-Aldrich) at 37 °C for 1 h. Cells were washed and 100 TCID₅₀ FIV was added at 37 °C for 2 h. Cells were washed to remove unbound viruses, resuspended in complete RPMI medium supplemented with IL-2 and plated at 2 × 10⁶ cells per well in 96-well tissue-culture plates. Macrophages and DCs were infected in an identical manner except for use of 24-well plates, and with cytokines added to DC cultures every third day. Supernatants and cell pellets from all cultures were harvested at 0, 6, 24, 72 and 168 h post-infection, and stored at ~20 °C for p24 determination by ELISA (Idexx) and proviral amplification, respectively. Supernatants were considered FIV ELISA-positive if the A₅₀ was >3 SD above the mean of two FIV-negative culture supernatants included in each assay. Genomic DNA was extracted from cell pellets (QiAamp DNA Blood Mini kit; Qiagen), eluted into 50 μl buffer and stored at ~20 °C. Proviral DNA was amplified by PCR with primers encompassing 1287 bp of the LTR-gag region, as described previously (Regetti & Bienzle, 2004).

**Immunocytochemistry.** USgaB01-infected DCs were washed twice with PBS and centrifuged onto glass slides (Cytocentrifuge; Thermo Shandon) at 250 g for 6 min. The slides were fixed in acetone for 5 min, washed and incubated with anti-p24 (F2710; a gift of H. Kashiwasa, Sankyo, Tokyo, Japan) or an irrelevant antibody. After washing, slides were incubated with horseradish peroxidase-labelled secondary antibodies, and bound antibodies were detected with DAB chromogen (DakoCytomation). Slides were counterstained with haematoxylin and coverslips were added.

**Electron microscopy.** FIV-infected and mock-infected (control) DCs were harvested 5 days post-infection and centrifuged lightly (500 g for 10 min). Following two washes in PBS, the pellets were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, for 1 h. The samples were then rinsed thoroughly with phosphate buffer and post-fixed in 1% OsO₄. They were then washed in distilled water, dehydrated in an ascending series of acetone, infiltrated and embedded in Embed 812-Araldite resin and polymerized overnight at 60 °C. Ultrathin sections were cut and mounted on grids and stained with uranyl acetate and lead citrate. Sections were then examined and photographed under a JEOL JEM 1230 transmission electron microscope (JEOL USA) with a CCD camera (AMT Corporation).

**Statistical analysis.** For receptor expression, data from relative quantification of CD134 and CXCR4 mRNA were analysed with the MIXED procedure, SAS for Windows version 8.2 (SAS Institute). For virus replication, A₅₀ values representing p24 concentrations at different time points were analysed as repeated measures (Everitt, 1995). SAS MIXED procedure was also chosen to compare results from lymphocyte, macrophage and DC infection experiments, as variances were unequal. The summary measure was the slope of the regression lines created from p24 concentrations at different time points for each cell type; thus, the rate of change in p24 production over time was compared. There were no significant effects for experiment or cat; hence, these variables were removed to gain degrees of freedom for experimental error. Logarithmic transformations were performed to meet the criterion of normality for all datasets. Differences were considered significant at P<0.05.

**RESULTS**

**Cell preparations**

Differentiation of adherent bone-marrow cells into DCs with abundant cytoplasm, eccentric nuclei and numerous irregular cytoplasmic projections (Fig. 1a) yielded 2–4 × 10⁶ cells per cat. Macrophages, also derived from adherent bone-marrow cells but without cytokine stimulation, were consistently fewer in number, large, round and with vacuolated cytoplasm (Fig. 1b). Cells with lymphocyte...
morphology and expressing CD4, CD8 or CD21 cell-surface antigens comprised <2% of cells in macrophage and DC cultures (not shown). The purity of positively selected lymphocyte subpopulations, as determined by cytometry with antibodies against CD4, CD8 and CD21, was consistently >97% (Fig. 2) and viability was >95%. Lymphocytes proliferated and clustered in response to mitogen or cytokine stimulation.

Expression of FIV receptors

Expression of CXCR4 was initially assessed by cytometry with antibody 44717, reported to cross-react with feline CXCR4 on B lymphocytes (Willett et al., 2003), and antibody 12G5, reported not to recognize feline CXCR4 (Willett et al., 1997a). In our experiments with samples from six outbred cats, CXCR4 was detected only on rare CD4+ or CD8+ fresh lymphocytes with either antibody, and up to 3% of CD21+ lymphocytes stained with clone 44717 only (Fig. 3). Similar results were obtained after mitogen exposure of lymphocytes (data not shown). More than 60% of monocytes, macrophages and DCs bound antibody 12G5, but not 44717. Expression of CD134 was assessed with antibody BerAct35, which has been variously reported to recognize either human and feline CD134 (Shimojima et al., 2004) or only human but not feline CD134 (de Parseval et al., 2005). In this study, binding of BerAct35 to feline cells was inconsistent among cats and leukocytes, suggesting non-specific interactions (data not shown).

To examine expression of FIV receptors on different leukocytes more definitively, quantitative real-time PCR assays specific for feline CXCR4 and CD134 were designed. Amplicon sequences corresponded to feline CXCR4 and CD134, and reactions were sensitive over eight tenfold dilutions. Results indicated that CXCR4 was expressed in all leukocytes assessed, but expression in lymphocytes was significantly higher than in macrophages or DCs ($P<0.0001$). Mitogen activation induced significant down-regulation of CXCR4 in CD4+ ($P<0.0001$), CD8+ ($P<0.0001$) and CD21+ ($P=0.027$) lymphocytes, and was most pronounced in CD8+ cells (Fig. 4a). Macrophages and DCs expressed CXCR4 transcripts, albeit at a reduced level relative to lymphocytes.

Similarly, CD134 mRNA was amplified from all leukocytes, but expression was 100- to 10 000-fold lower than that of CXCR4. CD4+ lymphocytes contained a higher number of transcripts ($P<0.0001$) than CD8+ and CD21+ lymphocytes, and overall expression in lymphocytes was significantly higher than in macrophages and DCs ($P=0.001$). Mitogen activation consistently increased gene expression in all lymphocyte subsets, but this was significant only in CD8+ lymphocytes ($P<0.0001$) (Fig. 4b). Macrophages expressed detectable CD134 transcripts in three out of six samples only, whereas DCs consistently expressed 50- to 100-fold more copies of CD134 mRNA than macrophages. Differences between macrophages and DCs were not significant ($P=0.067$).

Virus replication

All lymphocyte subsets, macrophages and DCs contained proviral DNA at 6 h post-infection with either USgaB01 or P14, and provirus was amplified consistently thereafter (Fig. 5). Concentration of viral p24 protein in cell supernatants increased significantly over time ($P<0.0001$) for FIV USgaB01, but not for P14 ($P=0.584$), in all cell types (Fig. 6). Although P14-infected cells did not increase viral replication over time, p24 concentration in supernatants was consistently >2 SD higher than in control cultures, and cells contained proviral DNA. Replication of
USgaB01 in CD4⁺ lymphocytes was significantly higher than in other lymphocytes ($P=0.013$) (Fig. 6a). Replication of this virus in macrophages and DCs was indicated by the increment in p24 concentration over time, but differences were not significant ($P=0.488$) (Fig. 6c). Viral replication in DCs was also assessed by immunocytochemistry, which indicated the presence of p24 protein throughout the cytoplasm, with enhanced staining along the cytoplasmic

**Fig. 2.** Positive selection of lymphocyte subpopulations. Immunomagnetically enriched cell fractions contained $<3\%$ other lymphocyte subpopulations.

**Fig. 3.** CXCR4 expression on lymphocytes, monocytes, macrophages and DCs. Cell-surface expression of CXCR4 was compared with antibodies 12G5 (a–f) and 44717 (g–l). Neither antibody bound to CD4⁺ (a, g) or CD8⁺ (b, h) lymphocytes, but 44717 bound to a small proportion of CD21⁺ cells (c, i). Antibody 12G5, but not 44717, detected CXCR4 on monocytes (d, j), macrophages (e, k) and DCs (f, l). Quadrants were set to include $>98\%$ of cells prepared with control reagents.
Further evidence of productive infection was provided by electron microscopic evaluation of infected and mock-infected DCs, which showed clubbing of cytoplasmic projections in infected cells compared with controls. Complete viral particles were identified in infected DCs within large cytoplasmic vesicles in close proximity to the cell membrane, whereas free intracellular particles were localized proximal to cytoplasmic or nuclear membranes. Similar to previous findings in HIV-infected human DCs (Patterson et al., 1995), mature FIV particles containing electron-dense conical cores budding through the cell membrane were apparent (Fig. 8).

**DISCUSSION**

DCs are heterogeneous cells that are central to host defence against infectious agents through elaboration of cytokines and initiation of specific immunity (Steinman & Hemmi, 2006). Infection of DCs by organisms can limit the defence functions and enhance establishment of infection (Lekkerkerker et al., 2006). HIV, a lymphotropic lentivirus like FIV, is captured by lectin receptors on DCs at mucosal surfaces, translocated to specific cytoplasmic organelles and subsequently transmitted to CD4+ lymphocytes in lymph nodes (Lekkerkerker et al., 2006). In addition, HIV enters DCs through CD4 and chemokine receptor interactions, with genomic integration and full replication modulated by the maturational stage of DCs (Pion et al., 2007). The nature of different DC populations in cats, the only non-primate naturally susceptible to lymphotropic lentiviruses, has not been elucidated fully. However, feline DCs derived from adherent bone-marrow or blood mononuclear cells appear analogous in function and surface-marker expression to human immature myeloid DCs (Bienzle et al., 2003). Hence, to understand better the pathogenesis of FIV, it was important to determine whether feline DCs express specific viral receptors and support FIV replication to a similar extent to lymphocytes.

CXCR4 is highly conserved across species (Willett et al., 1997b), but differential oligomerization and post-translation modifications account for marked variability in expression and function in different human cell types (Sloane et al., 2005). Antibodies generated against human CXCR4 did not bind uniformly to feline CXCR4 (Brelet et al., 1999). In this study, two different CXCR4 antibodies bound either to monocytes and their derivative macrophages and DCs, but not to lymphocytes (clone 12G5), or, conversely, to a subset of CD21+ lymphocytes, but not to...
monocyte-derived cells (clone 44717). The precise epitope recognized by either mAb has not been determined, but both target distinct regions of the extracellular loop of CXCR4 (Brelot et al., 1997) and recognize different isoforms of CXCR4 on T cells, umbilical vein cells and carcinoma cells (Sloane et al., 2005). Heterogeneity of CXCR4 expression has also been identified by differential binding of a panel of mAbs to a T-cell line transfected with human, rat or feline CXCR4 (Baribaud et al., 2001). Similar conformational variability is likely to occur in primary feline cells, explaining the inconsistent binding of antibodies in this study. Other potential causes of variable antibody binding include post-translational glycosylation, sulfation or ubiquitination of CXCR4 interfering with recognition, as has been described for different primary human cells (Farzan et al., 2002; Lapham et al., 2002).

CD134 is not highly conserved between humans and cats, with an overall amino acid identity of 58% and particular variability in regions 2 and 3, containing the putative binding site for antibody BerAct35 (de Parseval et al., 2005). We detected highly variable and non-reproducible binding of this antibody to different feline leukocytes, and considered non-specific interaction to be the most likely cause. These findings were in agreement with previous domain-exchange experiments, which mapped envelope-binding sites and showed lack of antibody binding (de Parseval et al., 2005). However, studies on mutagenesis of region 2 determinants and subsequent cytometric assessment have shown that the antibody bound to feline CD134 and that different FIV strains interact variably, either exclusively with region 1 or in addition with region 2 (Willett et al., 2006). The discrepancy between these findings is as yet unresolved, and antibodies generated specifically to feline antigens may permit more unanimous identification of CD134.

Determination of receptor-gene expression identified CXCR4 in all lymphocytes and monocyte-derived cells, with downregulation after mitogen stimulation. These findings were consistent with widespread expression of CXCR4 in leukocytes, analogous to other species; however, decreased gene expression may not reflect reduced function as a receptor as, in previous experiments, post-translational

Fig. 6. Virus replication in lymphocytes, macrophages and DCs. Viral replication was assessed by quantification of viral p24 in tissue-culture supernatants from FIV USgaB01- and P14-infected lymphocytes (a, b) and macrophages (mφ) and DCs (c, d) over time. Replication of USgaB01 in CD4+ lymphocytes was higher than in CD8+ and CD21+ lymphocytes (P=0.013). Differences between DCs and macrophages were not significant (P=0.488). Replication of P14 did not differ significantly among lymphocyte subpopulations or macrophages and DCs. Each point represents the mean ± SD (error bars) of p24 concentration from six experiments; results are expressed as A650 units. Note different y-axis scales for lymphocytes (a, b) versus macrophages/DCs (c, d).
modification through ubiquitination appeared most important for efficiently supporting HIV infection (Zaitseva et al., 2005). The role of different CXCR4 isoforms in interaction with the FIV envelope remains to be determined, but initial interaction with CXCR4 is likely to be a requisite for pathogenic primary viral isolates.

Differences in CD134 gene expression were more pronounced between different cell types, consistent with predominant expression on CD4+ T cells. It has been suggested that CD134 is expressed preferentially on memory lymphocytes (Shimojima et al., 2004); however, it is unlikely that the 'memory' phenotype is induced fully by in vitro mitogen exposure. Hence, increased expression of CD134 after cell activation may not fully capture fine regulation of cellular changes subsequent to clonal expansion in response to antigen encounter. B lymphocytes, DCs and macrophages also expressed CD134 transcripts, which may in part account for their susceptibility to FIV infection. The role of CD134 in those cells is incompletely understood, but signalling via CD134 probably promotes cell survival. Relative expression of CD134 was 102- to 103-fold lower in B lymphocytes, DCs and macrophages than in resting CD4+ lymphocytes, which paralleled the extent of viral replication. However, as quantification of gene transcripts does not necessarily correlate with stable receptor expression, complete evaluation of the role of FIV receptors will depend on development of specific antibodies for protein detection.

Assessment of viral infection in different leukocytes indicated more efficient replication in lymphocytes than in monocyte-derived cells. Furthermore, CD4+ lymphocytes sustained viral replication more efficiently than other lymphocytes, which is likely to be reflective of higher receptor expression and increased transcriptional activation. Importantly, FIV infected DCs productively in this study. Identification of complete viral particles in different intracellular compartments provided definitive evidence of productive infection of DCs with FIV. Although the presence of viruses within vesicles might have represented viral uptake via binding to a C-type lectin receptor, such as DC-SIGN (de Parseval et al., 2004c), the identification of free intracellular mature viral particles supported production of new viruses. As DCs expressed both CD134 and CXCR4, it is also plausible that fusogenic viruses entered the cells as free particles; however, the presence of viruses budding through the cell membrane suggested active replication and release of new particles. In addition, PCR proviral amplification indicated reverse transcription, and identification of viral p24 in DC culture supernatants and on the surface of the cells provided evidence of early transcription and translation of viral nucleocapsid proteins, further supporting productive infection. Thus, similar to HIV- and SIV-infected DCs (Frank et al., 2002), DC transmission of FIV to lymphocytes may also occur through release of sequestered infectious particles within vesicles and/or production of new viruses. In addition, feline DCs have recently been observed to induce proliferation of allogeneic thymocytes and to enhance replication of FIV, indicating that the stimulating role of DCs renders T cells more susceptible to viral replication (van der Meer et al., 2007). Together, these findings support the central role of feline DCs in transmitting FIV to susceptible CD4+ T-helper lymphocytes during antigen presentation, and expand our understanding of the mechanisms concerning early stages of retroviral infections.

Finally, FIV-infected DCs had stunted dendrites, a larger number of apoptotic nuclei and more cytoplasmic vacuolation than uninfected cells. Effects on the function of DCs in antigen presentation or cytokine production were not assessed, but are of great interest, considering the marked but ineffective immune activation that has been observed soon after FIV infection (Bendinelli et al., 2001).

In summary, CXCR4 and CD134 mRNA expression in lymphocytes and monocyte-derived cells was variable, but consistent with viral cell tropism. Although viral replication

---

**Fig. 7.** DCs contained cytoplasmic FIV p24 protein. Immunocytochemistry of bone marrow-derived DCs 3 days post-infection with FIV USgaB01 detected expression of p24, most notably in close proximity to the cell membrane (a). No staining was identified if the primary antibody was omitted (b). Haematoxylin counterstain. Bar, 10 μm.
in DCs was of lesser magnitude than in lymphocytes, we have shown here that feline DCs express specific viral receptors and are infected productively by FIV.

ACKNOWLEDGEMENTS

We thank the Natural Sciences and Engineering Research Council of Canada, the Pet Trust Foundation at the University of Guelph and the Central University of Venezuela for financial support, and M. E. Clark for assistance with immunochemical assays.

REFERENCES


