Effects of feline immunodeficiency virus on feline monocyte-derived dendritic cells infected by spinoculation

G. Freer,† D. Matteucci,† P. Mazzetti, F. Tarabella, V. Catalucci and M. Bendinelli

During type 1 human immunodeficiency virus infection, not only can dendritic cells (DCs) prime T cells against the virus, but they can also infect them in trans. Feline AIDS is caused by feline immunodeficiency virus (FIV) and is considered a model for the human illness because the two diseases have many features in common. Little is known about the interaction of feline DCs with FIV; therefore, this study attempts to tackle such an issue. Infection of feline monocyte-derived DCs (MDDCs) was attempted by spinoculation with FIV strains Petaluma (FIV-Pet) and M2. FIV-Pet was released rapidly in the supernatants of both infected MDDCs and activated T cells after spinoculation. It is shown that FIV-Pet was produced by MDDCs by monitoring viral content in the supernatants of infected MDDCs, by intracellular staining for p25 and by showing its cytopathic effect. Although activated T cells were better substrates for FIV replication, leading to prolonged viral shedding, both immature MDDCs and MDDCs matured with lipopolysaccharide supported virus production, mostly during the first 2 days after infection. At later times, FIV induced syncytium formation by MDDCs. Concerning the FIV receptors, MDDCs were shown to be CD134-negative and CXCR4-positive, a phenotype compatible with permissiveness to FIV-Pet. These results also suggest that maturation is not hampered by FIV infection and that virus exposure itself does not induce MDDC maturation. It is also shown that infected MDDCs can infect activated PBMCs efficiently in trans. It is concluded that MDDCs can be infected by FIV, although infection does not appear to influence their functionality.

INTRODUCTION

Dendritic cells (DCs) are the only antigen-presenting cells that can prime naïve T cells by efficiently uptaking and processing antigen and subsequently undergoing a process that allows them to migrate to lymph nodes and express/upregulate surface molecules involved in T-cell immunity (Sallusto & Lanzavecchia, 2002). In vitro, DCs are infected by different viruses, including type 1 human immunodeficiency virus (HIV-1) and simian immunodeficiency virus (SIV) (Granelli-Piperno et al., 2006; Pope, 1998). HIV-1 replicates in human immature DCs, and these infected cells suppress mixed leukocyte reaction (MLR) and fail to mature (Granelli-Piperno et al., 2004). Also, infected DCs may be pivotal in AIDS pathogenesis by infecting T cells with HIV-1 in trans (McDonald et al., 2003) and by not performing as mature DCs after infection (Geijtenbeek et al., 2000; Steinman et al., 2003).

Feline immunodeficiency virus (FIV) is a non-primate lentivirus that is studied as a model for HIV (Sparger, 2006). It infects domestic cats, leading to an immune deficiency that closely resembles human AIDS. The present study is a first step towards understanding the interactions that FIV establishes with feline DCs, for which culture protocols have recently been described (Bienzle et al., 2003; Freer et al., 2003; Sprague et al., 2005). In particular, we investigated whether feline monocyte-derived DCs (MDDCs) can be infected in vitro with two different strains of FIV, FIV-Pet (FIV-Pet), a clade A tissue-culture-adapted strain (Pedersen et al., 1987), and FIV-M2, a primary clade B strain. We show that spinoculation improves FIV infection of MDDCs and other cell types and that MDDCs allow FIV replication to an extent that depends on virus strain and dose, independently of their maturation stage. FIV did not interfere with maturation or antigen-presentation capacity of MDDCs, in contrast to HIV-1. On the other hand, similarly to HIV-1, infected immature MDDCs (iMDDCs) transmitted FIV actively to T cells.

METHODS

Media and reagents. RPMI 1640 with 2 mM l-glutamine, 1 % non-essential amino acids and 50 μg gentamicin ml⁻¹ was used. Feline
recombinant interleukin (IL)-4 and granulocyte–macrophage colony-stimulating factor (GM-CSF) were purchased from R&D Systems. Recombinant human IL-2 was purchased from Roche.

**Animals, cells and culture conditions.** Specific-pathogen-free (SPF) female cats were bought from IFFA Credo. Heparinized venous blood was obtained from lightly anaesthetized 12–36-month-old cats kept in our animal facility under conditions required by European Community Law. MDDCs were obtained as described previously from 35 ml blood (Freer et al., 2005). Peripheral blood mononuclear cells (PBMCs) were obtained by centrifugation over Ficoll-Paque for 30 min at 550 g. Cells isolated were washed in aprogenic saline, counted and resuspended at 3 × 10^6 cells ml^-1. Aliquots (1 ml) were distributed in 24-well plates and 3% autologous plasma was added [instead of fetal calf serum (FCS) in order not to add xenogeneic proteins; Freer et al., 2005]. After 24 h, non-adherent cells were removed, whereas adhering cells were washed twice, then 0.5 ml medium containing 3% autologous plasma, 10 ng IL-4 ml^-1 and 50 ng GM-CSF ml^-1 was added. After 2 days, fresh IL-4 and GM-CSF were added in 100 μl medium. After culturing MDDCs for a total of 5 days, cells were supplemented with fresh cytokines alone (mMDDCs) or together with 20 ng lipopolysaccharide (LPS) ml^-1 [from *Escherichia coli* 0127:B8 (Sigma)] to obtain mature MDDCs (mMDDCs) and cultured for another 2 days unless otherwise stated. MDDCs were defined by their side scatter/forward scatter (SSC × FSC) profile, which was shown to include CD14^+ , CD1a^+ , CD4^- , major histo-compatibility complex (MHC) class II^+ , B7.1^+ cells, as described previously (Freer et al., 2005); contaminating T cells ranged between 5 and 15% of gated live cells. The IL-2-dependent MBM line was grown in 3% pooled plasma from normal SPF cats, instead of in FCS (Matteucci et al., 1995).

**FIV preparation and titration.** FIV-Pet and FIV-M2 stocks were 0.43 μm-filtered supernatants from virally infected FL4 cells (Yamamoto et al., 1991) and freshly infected MBM cells (Matteucci et al., 1995), respectively. They were LPS-free by the *Limulus* amoebocyte lysate assay (PBI International) and ranged in titre between 10^4 and 10^5 50% tissue culture infecting doses (TCID50) ml^-1. Titration of infectivity was carried out by inoculating 100 μl 10-fold dilutions onto 10^6 MBM cells in quadruplicate wells, determining the p25 content by ELISA (Matteucci et al., 1996) after 7 days and calculating TCID50 according to Reed & Muench (1938).

**Infection of primary feline cells.** MDDCs were generated as described above. Activated blasts resulted from non-adherent PBMCs cultured in the presence of concanavalin A (ConA; Sigma), 5 μg ml^-1, for 48 h. At the time of infection, medium was removed and the TCID50 stated of FIV-Pet or FIV-M2 was added to wells. For infection at ambient gravity, cells and viruses were incubated for 2 h in a humidified incubator at 37 °C. For spinoculation, before the 2 h incubation, virus-inoculated plates were centrifuged to 1600 g at 35 °C for 45 min. In both cases, cells were washed twice and 0.5 ml fresh medium containing IL-4 and GM-CSF was added.

**Intracellular staining for p25.** At the times indicated, MDDCs or other cell types were fixed in PBS, 1% paraformaldehyde for 20 min at 4 °C. Cells were then washed and incubated in fluorescence-activated cell sorting (FACS) buffer (PBS, 0.2% BSA, 0.01% NaN3) containing 0.5% saponin and 1 μg biotinylated anti-FIV p25 murine monoclonal antibody (mAb) DF10 in a final volume of 50 μl for 1 h at room temperature. Cells were then washed in FACS buffer containing 0.1% saponin and stained with streptavidin–fluorescein isothiocyanate (FITC) (Vector Laboratories). For FIV-transfer experiments, a mixture of DF10 hybridoma supernatant and 0.1 μg mAb PAK3-2C1 (AbD Serotec) ml^-1 was used. FITC-conjugated goat anti-mouse IgG polyclonal antiserum (Sigma) was used as a secondary antibody. Cells were then analysed by FACS (flow cytometric) analysis.

**Flow-cytometric analysis.** Live-cell staining was carried out in 50 μl RPMI, 0.2% BSA, 0.1% NaN3 on ice for 30 min. The mAbs used were anti-MHC class II (42.3; Dr Peter F. Moore, University of California, Davis, CA, USA), anti-feline B7.1 (B7.1.66, a kind gift from Dr Wayne Tompkins, North Carolina State University, Raleigh, NC, USA; Tompkins et al., 2002); a murine anti-feline CD134, provided as supernatant, a kind gift from Dr Brian Willett, University of Glasgow, UK; anti-human CXCR4 (44701) and its isotype control (R&D Systems). The negative control for all IgG1 isotype mAbs was IgG1 (Freer et al., 1998). An FITC-conjugated goat anti-mouse IgG antiserum (Sigma) was used as a secondary antibody where needed. Cells were fixed in PBS, 1% paraformaldehyde for 20 min on ice. At least 1 × 10^5 live-gated events were acquired by the CellQuest software with a FACSscan flow cytometer (Becton Dickinson). Dead cells and lymphocytes were excluded by light-scatter properties.

**MLR.** MDDCs were grown as described above for 5 days and either infected with FIV-Pet (4750 TCID50) for 24 h or not infected and were then induced to mature or not with 20 ng LPS ml^-1 for 24 h. MDDCs (1 × 10^6 or 3 × 10^6) were added to 10^5 allogeneic PBMCs in 96-well plates in triplicate (stimulator : responder, 1 : 100 or 1 : 33, respectively) and cultured for 4 days in RPMI 1640. Human serum, 10%, was added to decrease background incorporation according to standard protocols (Matteucci et al., 1996). Proliferation was assessed by adding 1 μCi (37 kBq) [methyl-3H]thymidine (Amersham Biosciences) per well 18 h before harvesting the cells.

**FIV transmission assays.** iMDDCs obtained from 3 × 10^6 PBMCs in 24-well plates were spinoculated with 450 TCID50 FIV-Pet, incubated for 2 h at 37 °C and washed twice. Non-adherent PBMCs, precultured 48 h in ConA and washed, were then added at numbers of 5 × 10^6 per well. As controls, ConA-activated PBMCs were (i) spinoculated with the same virus dose, (ii) grown in the presence of the same virus dose without washing or (iii) left uninfected. All cultures were incubated in medium containing IL-4, GM-CSF and 20 ng IL-2 ml^-1 for 48 or 96 h, at which times cells and supernatants were analysed for p25 by FACS analysis and ELISA (Matteucci et al., 1996), respectively.

**Statistical analyses.** Statistical analyses were performed by using Student’s t-test.

**RESULTS**

**Infection of iMDDCs by FIV.** To assess whether iMDDCs could be infected by FIV, we first exposed them to various doses of FIV-Pet or FIV-M2 at 37 °C for 2 h. The cultures were then examined at different times for p25 accumulation in the supernatants by ELISA and inside cells by FACS analysis. The results obtained were inconsistent (data not shown). Because spinoculation has been shown to enhance HIV-1 infection of susceptible cells (O’Doherty et al., 2000), we tried infection of iMDDCs by centrifugal inoculation. Three different doses (2375, 4750 and 9500 TCID50) of FIV-Pet or FIV-M2 were used to infect iMDDCs generated from 3 × 10^6 PBMCs. This number of PBMCs yielded between 1 × 10^5 and 2 × 10^5 iMDDCs per well. As a control, the feline T-cell line MBM (2 × 10^5 cells per well) was also...
infected with 4750 or 9500 TCID$_{50}$ FIV-Pet, using the standard protocol for spinoculation. All cultures were washed twice and evaluated for p25 content in the supernatants, as a measure of virus production, immediately after the final wash (day 0 in Fig. 1) and after 2, 3 and 4 days. Spinoculated iMDDCs produced abundant virus, whereas infection at ambient gravity led to little, if any, virus production (Fig. 1a, b). Virus levels had already peaked in the spinoculated cultures at day 2, then remained stable or declined slightly over the subsequent 2 days. Virus production in some experiments was also assessed after 6 days, with no further increase detectable in virus yield (data not shown). Because infection was carried out at day 5 of culture, it is likely that duration of virus production was limited by a reduction in MDDC synthesis that occurred in the cultures over the subsequent few days. In keeping with this possibility, after approximately 10 days in culture, both infected and uninfected MDDCs were noticed to turn from large and loosely adherent into small and adherent cells, suggestive of a significant change in their properties. Viral yields increased markedly with input dose for both FIV-Pet and FIV-M2.

A marked difference in virus production was observed depending on virus strain, in that FIV-Pet constantly led to much higher levels of p25 in supernatants than FIV-M2, regardless of infecting dose. The overall trend of virus production was consistent when MDDCs from different cats were analysed. Spinoculation also accelerated virus production by MBM cells, relative to infection at ambient gravity. However, the amounts of p25 eventually accumulated by these cells were comparable, regardless of the infection protocol, and were not as dependent on input virus dose (Fig. 1c).

**FIV production by iMDDCs compared with primary T cells**

Activated primary T cells are known to be highly permissive to FIV. We therefore considered it of interest to compare FIV production after spinoculation by iMDDCs, resting PBMCs and ConA-activated PBMCs with an intermediate dose of FIV-Pet (4750 TCID$_{50}$). Fig. 2(a) shows that FIV capsid antigen was already detectable at 8 h after infection of iMDDCs and reached a plateau at 24 h. These

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**Fig. 1.** FIV production by infected iMDDCs compared with the MBM T-cell line. (a, b) iMDDCs from 3 x 10$^6$ PBMCs were either spinoculated (empty symbols) or incubated at ambient gravity (filled symbols) with 9500 TCID$_{50}$ (○, ●), 4750 TCID$_{50}$ (□, ■) or 2375 TCID$_{50}$ (○, ●) of FIV-Pet (a) or FIV-M2 (b) (plotted at different scales). (c) MBM cells were either spinoculated (empty symbols) or incubated at ambient gravity (filled symbols) with the two highest quantities of FIV-Pet [9500 TCID$_{50}$ (○, ●) or 4750 TCID$_{50}$ (□, ■)]. At the times from infection indicated, culture supernatants were analysed for p25 content by ELISA. Values from a typical experiment (out of four performed) represent the mean ± SD of triplicate cultures from an individual cat. Normal cell supernatant $A_{450}$ was <0.050 at all times tested.

**Fig. 2.** FIV production by infected iMDDCs and, for comparison, resting and ConA-activated PBMCs. (a) iMDDCs generated from 3 x 10$^6$ PBMCs (▵, ▲) or from 6 x 10$^6$ PBMCs (□, ■) or (b) 3 x 10$^6$ PBMCs, either resting (○, ●) or stimulated with ConA 2 days earlier (○, ●), were spinoculated (empty symbols) or incubated at ambient gravity (filled symbols) with 4750 TCID$_{50}$ FIV-Pet. At the times from infection indicated, culture supernatants were analysed for p25 content by ELISA. Values from a typical experiment (out of three performed) represent the mean ± SD of triplicate cultures from an individual cat. Normal cell supernatant $A_{450}$ was <0.038 at all times tested. Data in (a) and (b) are plotted at different scales.
kinetics were comparable to those of resting PBMCs, but differed markedly from those of ConA-activated PBMCs, which showed larger amounts of p25 in supernatants at all times tested and continued to accumulate virus at least up to 48 h post-infection (Fig. 2b). Of note, the differences in kinetics between iMDDCs and activated PBMCs were not due to different cell numbers in the cultures, as infecting iMDDCs pooled from two wells had no appreciable effects on FIV yield or kinetics (Fig. 2a).

Expression of p25 and formation of syncytia by infected iMDDCs

To confirm that iMDDCs were infected by FIV, we made use of flow cytometry to detect the FIV capsid antigen p25 directly inside these cells, gated by size and complexity. Fig. 3(a) shows the results of a typical experiment where iMDDCs collected separately from two representative cats were spinoculated with 4750 TCID$_{50}$ FIV-Pet and, 2 days later, were fixed, permeabilized and stained for p25. FACS analysis was performed on $1 \times 10^4$ cells gated by SSC×FSC. The proportion of iMDDCs that stained positive ranged between 5 and 12%. In comparison, similarly infected MBM cells used as control were 15% positive. Infected iMDDCs also formed syncytia after longer times from infection (6 days) (Fig. 3b). Collectively, these results confirmed that iMDDCs can be infected by FIV.

Infection of mMDDCs

We also checked whether, following maturation, MDDCs could still be infected with FIV. We had shown previously that LPS treatment induced upregulation of MHC class II and B7.1 expression by feline MDDCs more efficiently than other maturation stimuli and was the only stimulus that made them able to prime alloreactivity (Freer et al., 2005). Thus, day 5 cultures of iMDDCs were matured by incubation with 20 ng LPS ml$^{-1}$ for a further 2 days or left untreated, spinoculated with 4750 TCID$_{50}$ FIV-Pet and finally analysed sequentially for p25 content in the supernatants. In these studies, the virus produced was also titrated for infectivity in MBM cells. Table 1 shows that FIV infectivity became measurable in culture fluids at 24 h post-infection, regardless of whether MDDCs were mature.
or immature, then increased only marginally during the subsequent 24 h. Although p25 determination by ELISA and evaluation of infectivity differ in sensitivity, the results point to the same conclusion: as shown by Fig. 4, mMDDCs supported FIV replication as effectively as iMDDCs and actually released higher amounts of virus ($P<0.01$ at 18 h; $P<0.05$ at later times).

Interestingly, mMDDCs produced nearly as much infectious virus as ConA-activated PBMCs that were spin-infected in parallel as a control, whereas iMDDCs produced less.

**Exposure to FIV does not affect MDDC maturation**

To examine the impact of FIV on the ability of MDDCs to mature, day 5 cultures of iMDDCs were spinoculated with 4750 TCID$_{50}$ FIV-Pet or medium alone and, 1 day later, were treated with LPS to induce maturation. After 24 h, cells were examined for MHC class II and B7.1 expression. As shown in Fig. 5, LPS-treated cells expressed the same levels of both of these maturation markers, whether or not they had been exposed to FIV. It is noteworthy that FIV itself did not induce MDDCs to mature, given that iMDDCs exposed or not to FIV expressed comparable levels of both maturation markers. We also evaluated whether FIV-infected MDDCs matured in terms of ability to prime naïve T cells. Immature MDDCs, spinoculated or mock-infected and treated with LPS or not, exactly as described above, were incubated with $1 \times 10^5$ allogeneic feline PBMCs for 5 days. As can be seen in Fig. 5(c), infected and mock-infected mMDDCs primed MLR with equal efficiency. Thus, in vitro infection with FIV did not affect LPS-induced MDDC maturation appreciably.

**iMDDCs do not express CD134, but do express CXCR4**

Recently, CD134 has been shown to be a primary receptor for fresh viral isolates, such as GL8 and others (Shimojima et al., 2004), but to be dispensable for tissue-culture-adapted FIV-Pet, which can enter cells by binding CXCR4 directly (de Parseval et al., 2004b; Shimojima et al., 2004). We therefore checked whether the difference observed above in the ability of feline MDDCs to support FIV-Pet and FIV-M2 replication could be explained on the basis of differential CD134 expression. We used a novel mAb obtained from mice immunized with recombinant feline CD134 and screened by ELISA against this protein and then with retroviral vector CD134-transduced cells (B. Willett, personal communication). This mAb stained MBM cells, but showed no reactivity against iMDDCs (Fig. 6a, b), indicating that the latter do not express CD134 on the cell surface in a detectable fashion. In contrast, mMDDCs were found to be as positive for CXCR4 as the T cells contaminating the cultures (Fig. 6c).

**Activated PBMCs can be infected in trans with FIV-Pet by iMDDCs**

Human DCs are able to infect CD4$^+$ T cells in trans with HIV-1 by recruiting virus and receptors at the so-called

### Table 1. Infectious FIV released by iMDDCs, mMDDCs and, for comparison, ConA-stimulated blasts at various times of spinoculation

1st and 2nd refer to experiments done. Results are reported as TCID$_{50}$ (ml culture supernatant)$^{-1}$. Titres were obtained from culturing quadruplicates of the same dilution by the method of Reed & Muench (1938).

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<th>Cell type</th>
<th>Infectious FIV released at these time points after infection (h):</th>
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*PBMCs that were ConA-stimulated for 2 days.

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**Fig. 4.** Both mature and immature MDDCs produce FIV after infection. iMDDCs (■) or mMDDCs (□) were infected with 4750 TCID$_{50}$ FIV-Pet. At the times from infection indicated, culture supernatants were analysed for p25 content by ELISA. Values from a typical experiment (out of four) represent the mean±so of triplicate cultures from an individual cat. Normal cell supernatant $A_{450}$ was <0.045 at all times tested.
infectious synapse, enhancing the likelihood of productive infectious events (McDonald et al., 2003). Because this seems to be a key role of DCs in the pathogenesis of AIDS, we verified whether FIV could be transmitted by iMDDCs as well. Bearing in mind that (i) feline T cells infected with FIV at ambient gravity do not express FIV for at least 48 h, whereas T cells spinoculated with the virus do (Fig. 2b), and (ii) activated PBMCs infected with low inocula of FIV produce no detectable virus for at least 96 h (unpublished observation), we spinoculated day 5 iMDDCs with 450 TCID₅₀ FIV-Pet, incubated them for 2 h at 37 °C, washed them twice and then added 5 × 10⁵ autologous ConA-activated PBMCs. The mixed cultures were finally incubated for 48 or 96 h. In addition, 5 × 10⁵ ConA-activated PBMCs were left uninfected, spinoculated or just cultured with the same dose of virus. The latter control was meant to exclude the possibility that T cells might simply be infected by virus released by iMDDCs, without necessarily involving active transfer. Cells were stained for p25, and 1 × 10⁴ events gated as shown in Fig. 7 were analysed by FACS analysis. Fig. 7(d) shows that 3.5 % of PBMCs cultured with infected iMDDCs were already positive for p25 after 48 h and that this percentage increased by 3-fold by 96 h post-infection. PBMCs spinoculated with the same virus dose as was used for iMDDCs were 21.4 % positive 48 h after infection and this decreased to 13.4 % at 96 h (Fig. 7b), possibly due to cell death. When FIV was simply added to PBMCs without spinoculation or washing, only background levels of FIV-positive cells could be detected (Fig. 7c). Supernatants from infected cultures exhibited p25 contents that paralleled the FACS analysis results (data not shown). Thus, it could be concluded that iMDDCs improve FIV infectivity for T cells.

**DISCUSSION**

FIV infection of domestic cats is considered one of the best non-primate models to study AIDS (Sparger, 2006). Increasing evidence indicates that DCs are an efficient means by which HIV-1 makes contact with T lymphocytes (Steinman et al., 2003). Indeed, T-cell infection with HIV-1 in trans by DCs seems to be much more efficient than infection by free virions (Arrighi et al., 2004).

We have recently described a protocol to generate feline MDDCs from peripheral blood (Freer et al., 2005). Because not much is known about the interaction of FIV with DCs to date, we tried to infect cultured feline MDDCs with two strains of FIV, namely FIV-Pet of clade A and FIV-M2 of clade B, by the standard protocol used in our laboratory for infecting other cell types. As this led to inconclusive results, we performed iMDDC infection by spinoculation, which had previously been shown to enhance HIV-1 infection of T cells (O’Doherty et al., 2000). iMDDCs inoculated with FIV yielded unequivocal evidence of viral replication. This included accumulation in the supernatant of p25 antigen already after 8 h, which agrees with the timing of HIV-1 replication after spinoculation (O’Doherty et al., 2000). We did not attempt to clarify how spinoculation facilitated FIV infection of MDDCs; however, increased virus binding by depositing virions onto cells, as suggested for HIV-1 (O’Doherty et al., 2000), seems a plausible explanation. Spinoculation not only permitted infection of iMDDCs, but also accelerated FIV appearance in supernatants of infected MBM cells and ConA-activated PBMCs, suggesting that also for FIV, as reported for HIV-1 (O’Doherty et al., 2000), binding without spinoculation is rate-limiting for the appearance of virus. A typical feature of
Fig. 6. Expression of FIV receptors by iMDDCs. (a) MBM cells and (b) iMDDCs were stained with anti-feline CD134. (c) iMDDCs from a different experiment were stained with anti-human CXCR4. Large histogram plot, analysis on gate 1 (iMDDCs); small histogram plot, analysis on gate 2 (T cells). Negative-control mAb was L8D8 (shaded histograms). Top panels, SSC × FSC plots showing the gates of analysis.

Fig. 7. iMDDCs infect ConA-activated PBMCs in trans with FIV-Pet. PBMCs were activated with ConA for 48 h, then: (a) left uninfected; (b) spinoculated with 450 TCID₅₀ FIV-Pet and washed thoroughly; (c) exposed to virus and left unwashed; or (d) cultured with iMDDCs that had been spinoculated with the same dose of virus and washed thoroughly. At 48 and 96 h after infection, cells were stained for p25 and analysed by FACS analysis. Numbers at the top right-hand corner of panels represent percentages of events outside the uninfected T-cell gate at the respective time (i.e. positive events). (e–h) Events were gated for SSC × FSC as shown. (e) T cells infected for 48 h (uninfected and spinoculated were indistinguishable); (f) infected iMDDCs co-cultured with T cells for 48 h; (g) infected T cells for 96 h (uninfected and spinoculated were indistinguishable); (h) infected iMDDCs co-cultured with T cells for 96 h. Plots shown are on FL-1 (p25) × SSC.
spin-infected iMDDCs alone was that p25 yield was dependent on input virus dose and was independent of the number of cells in the cultures. Also, iMDDCs seemed to stop or, at least, slow down virus production very soon, as a plateau of p25 concentration was reached by 24 h post-infection. We suggest that this was in part accounted for by the fact that, with increased time in culture, iMDDCs slowed down their activity, even when given fresh medium and cytokines.

A frequent problem with cultured MDDCs is contamination with T cells (Figdor et al., 2004). Our MDDCs contained between 5 and 15 % contaminating T cells (data not shown), but we never noticed a parallel between the number of these cells and the amount of p25 in the cultures. Furthermore, the fact that iMDDCs stained for intracellular p25 similarly to control MBM cells, known to be infected productively by FIV, confirmed that iMDDCs were infected and contributed to viral release in the supernatants. Determination of viral DNA in the infected cultures was not attempted because T-cell contamination, no matter how low, would have been misleading in this kind of approach.

FIV-Pet-infected iMDDCs produced much more p25 than FIV-M2-infected iMDDCs at any time tested. As CD134 has been shown to be the primary receptor for fresh FIV isolates, such as FIV-M2, but to be dispensable for the tissue-culture-adapted FIV-Pet strain (de Parseval et al., 2004b; Shimojima et al., 2004; Willett et al., 2006), we investigated whether iMDDCs expressed this molecule by using a novel anti-feline CD134 mAb (B. Willett, personal communication). The cells showed no evidence of detectable CD134, but expressed CXCR4, which is known to be the molecule used by FIV-Pet to enter cells (Shimojima et al., 2004). Thus, the greater permissiveness of iMDDCs to FIV-Pet relative to FIV-M2 is probably explained by their FIV receptor expression. In addition, as FIV has been shown to bind human DC-SIGN (de Parseval et al., 2004a), the possibility that the feline homologue of DC-SIGN, if it exists, contributes to mediate virus adhesion cannot be excluded.

To our knowledge, this is the first study showing that MDDCs can be infected by FIV and that FIV infection of MDDCs, but also of ConA-activated PBMCs and MBM cells, is favoured strongly by spinoculation. Only 6–10 % of spinoculated iMDDCs became virus-positive, which was a much lower proportion than similarly infected MBM cells and also much lower than that in T-cell cultures spinoculated with HIV-1 (ODoherty et al., 2000). This suggests that DCs are not infected easily by FIV, whereas T cells are well known to be among the most sensitive cells for this virus. However, iMDDCs infected with a low FIV dose infected T cells efficiently in trans, as also reported by others while this manuscript was in preparation (Van der Meer et al., 2007); such transfer had been mainly reported for HIV-1 (Geijtenbeek et al., 2000; McDonald et al., 2003).

In order to prime adequate cellular immune responses, DCs need to undergo maturation and to express increased levels of selected surface molecules (Sallusto & Lanzavecchia, 2002; Steinman et al., 2003). There is evidence that infection with certain retroviruses interferes with DC maturation, with HIV-1- and SIV-mediated inhibition of DC maturation being hypothesized as one of the mechanisms contributing to immune deficiency during AIDS (Fantuzzi et al., 2004; Granelli-Piperno et al., 2006; Patterson et al., 2005; Soderlund et al., 2004). For human DCs, maturation and HIV-1 production have been reported to be mutually exclusive (Cavrois et al., 2006; Granelli-Piperno et al., 2004). In our hands, FIV-exposed cultures of feline MDDCs responded to LPS with increased expression of MHC class II and B7.1 and with an increased ability to elicit MLR as effectively as control cultures. However, as only a minority of cells were infected productively in the cultures, a definitive answer to whether FIV impairs MDDCs will require further studies using cells sorted for FIV positivity.

ACKNOWLEDGEMENTS

This work was supported by grants from the Ministero della Salute-Istituto Superiore di Sanita ‘Programma per l’AIDS’, Rome, Italy. We thank Dr Wayne Tompkins for his generous gift of anti-B7.1 antibody. Special thanks go to Dr Brian Willett for the generous gift of his novel anti-CD134 mAb.

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Frer, G. Freer and others


