Feline immunodeficiency virus infection is enhanced by feline bone marrow-derived dendritic cells

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In the pathogenesis of feline immunodeficiency virus (FIV) infection, feline dendritic cells (feDCs) are thought to play an important role. As with DCs in other species, feDCs are believed to transport virus particles to lymph nodes and transfer them to lymphocytes. Our investigation has focused on the ability of feDCs to influence the infection of syngeneic peripheral blood mononuclear cells (PBMCs) and allogeneic thymocytes. feDCs were derived from bone marrow mononuclear cells that were cultured under the influence of feline interleukin-4 and feline granulocyte–macrophage colony-stimulating factor. By using these feDCs in co-culture with resting PBMCs, an upregulation of FIV replication was shown. An enhancement of FIV infection was also detected when co-cultures of feDCs/feline thymocytes were infected. To obtain this enhancement, direct contact of the cells in the co-culture was necessary; transwell cultures showed that the involvement of only soluble factors produced by feDCs in this process is not likely. These feDCs were also able to induce the proliferation of resting thymocytes, which might explain the enhanced FIV replication observed. Together, these data suggest that feDCs have abilities similar to those shown for simian and human DCs in the interaction with leukocytes. This system is suitable for further investigations of the interplay of DC and T cells during FIV infection in vitro.

INTRODUCTION

Feline immunodeficiency virus (FIV) is a lentivirus that causes an AIDS-like syndrome in cats (Pedersen et al., 1987). An important stage in the pathogenesis of FIV is the earliest phase, in which the virus is introduced into the cat. The infection will occur mainly via bite wounds, so in most cases, the place of entry will be the skin (Yamamoto et al., 1989). In the early stages of a human immunodeficiency virus type 1 (HIV-1) or simian immunodeficiency virus (SIV) infection, macrophages and dendritic cells (DCs) play an important role in the uptake and dissemination of introduced virus particles. When DCs encounter an immunological stimulus, they migrate to search for antigen-specific T cells (Banchereau & Steinman, 1998). Reports of an association of FIV with cells that showed characteristics of DCs suggest an involvement of this cell type in the dissemination of the FIV particle to lymphoid organs (Bingen et al., 2002; Obert & Hoover, 2002; Toyosaki et al., 1993). Besides this first mechanism, another contribution of DCs to lentivirus infection of the host can be observed. Virions are presented efficiently to T cells when associated with macrophages or DCs. For HIV-1 and SIV infections, this has been shown (Cameron et al., 1992; Gummuluru et al., 2002; Pope et al., 1994). For FIV, this is unexplored; however, as FIV replicates both in vivo and in vitro in CD4+ and CD8+ T lymphocytes (Brown et al., 1991) and DCs can stimulate the proliferation of both cell types (Banchereau et al., 2000), we can assume that feline DCs (feDCs) will also play an important role in the pathogenesis of FIV infections.

Until recently, feDCs had not been characterized, which hampered research on this cell type. feDCs share many characteristics with DCs found in other species. They are non-adherent in vitro and show processes that can be regarded as dendrites. feDCs were shown to express CD1a and major histocompatibility complex class II (MHC II) and have the ability of stimulating allogeneic T cells (Bienzle et al., 2003; Freer et al., 2005; Sprague et al., 2005). Our goal was to evaluate the role of feDCs in FIV infections in vitro. This study has focused on the influence of feDCs on FIV infections in allogeneic thymocytes and syngeneic peripheral blood mononuclear cells (PBMCs).

METHODS

Cells and virus. Bone marrow was obtained from femurs of specific-pathogen-free (SPF) cats. By clipping the femur with pincers, the bone marrow was exposed. After rinsing the femur cavity with PBS/EDTA, the cell suspension was strained through a 70 μm filter (Cell Strainer; BD Falcon), centrifuged for 10 min at 600 g to remove excessive bone-marrow fat and resuspended in 20 ml PBS/EDTA. Bone-marrow mononuclear cells (BMMCs) were isolated by density-gradient centrifugation (1.077 g l−1, Lymphoprep; Axis-Shield PoC AS) for 30 min at 1500 r.p.m. Cells were washed with Iscove's
modified Dulbecco’s medium (IMDM) and stored at −80 °C using DMSO/fetal calf serum (FCS) until use. After thawing, BMMCs were plated in six-well dishes (Costar; Corning Inc.) with IMDM containing Glutamax I (Sigma), 10% heat-inactivated fetal bovine serum (FBS; Hyclone), 100 U penicillin ml⁻¹, 100 μg streptomycin ml⁻¹, 50 μM 2-mercaptoethanol, 10 ng recombinant feline (rfe) granulocyte–macrophage colony-stimulating factor (GM-CSF) ml⁻¹ (R&D Systems) and 10 ng rfe interleukin-4 (IL-4) ml⁻¹ (R&D Systems). After 24 h, non-adherent cells were removed by gentle rinsing with pre-warmed medium. Non-adherent cells were discarded and the remaining adherent cells were cultured further for 6 days in the presence of cytokines. Fresh cytokines were added on day 3. On day 6, the newly formed non-adherent cells derived from the culture were harvested and used for further experiments.

Macrophages were derived by the same procedure as fDCs, except for the addition of rfe GM-CSF and rfe IL-4. The cells obtained were adherent and multinucleated after a culture period of 6 days. Removal of the adherent macrophages took place by rinsing the cells with PBS/EDTA.

PBMCs were derived by isolating them from heparinized blood samples via density-gradient centrifugation (1.077 g l⁻¹; Lymphoprep; Axis-Shield PoC AS) for 30 min at 1500 r.p.m. Cells were washed with culture medium and stored at −80 °C using DMSO/FCS until use.

Thymocytes derived from SPF cats were stimulated with concanavilin A (5 μg ml⁻¹) and kept in culture with recombinant human IL-2 (100 units ml⁻¹) as described previously (Egberink et al., 1990). Thymocytes were maintained in IMDM containing Glutamax I (Sigma), 10% heat-inactivated FBS (HyClone), 100 U penicillin ml⁻¹, 100 μg streptomycin ml⁻¹ and 50 μM 2-mercaptoethanol. Two days after removal of concanavilin A, the thymocytes were regarded as stimulated (Ts). For the experiments with resting thymocytes (Tr), concanavilin A was removed from the thymocytes 9 days before the experiments were performed.

FIV was propagated on thymocytes for 5 days and designated FIV thymocytes (Tr). Concanavilin A was removed from the thymocytes Two days after removal of concanavilin A, the thymocytes were harvested and used for further experiments.

RESULTS

Flow cytometry, functional properties and morphology of fDCs. Functional and morphological characteristics of the cells were assessed as described by Bienzle et al. (2003) with some minor modifications. Antibodies specific for CD1a (Fe1.5F4), CD1c (Fe5.5C1), CD1b (Ca16.3E10) and MHC II (42.3) were used for fluorescein isothiocyanate-labelled secondary antibodies (Becton Dickinson). DCs or macrophages that had been cultured for 6 days were collected by centrifugation for 5 min at 1200 r.p.m., washed twice with FACS buffer (PBS, 1% fetal bovine serum, 0.1% sodium azide) and incubated consecutively with antibodies and conjugates for 60 min at room temperature. Between each step, the cells were washed twice with FACS buffer. Finally, the cells were washed twice with FACS buffer and resuspended in PBS containing 2% paraformaldehyde and stored at 4 °C until analysis. For each sample, 50,000 cells were analysed, employing a FACScalibur flow cytometer (Becton Dickinson) and the Windows-based WinMDI software (J. Trotter, The Scripps Research Institute, La Jolla, CA, USA). In all of these procedures, isotype- and secondary antibody-matched controls were included.

Non-specific esterase activity was detected by using an esterase kit (Sigma) with α-naphthyl acetate as substrate.

Design of the infection experiments. All experiments were performed in triplicate.

PBMCs, thymocytes and fDCs were counted by trypan blue in a Glastic Slide 10 (Hycon Biomedical Inc.). Cells (5 x 10⁶) were added to each well of a round-bottomed 96-well plate (Costar). The numbers of PBMCs or thymocytes were equal in co-cultures and monocultures. The wells contained either only thymocytes, PBMCs or fDCs, or a co-culture of fDCs and thymocytes or fDCs and syngeneic PBMCs in a fDC : Tr or fDC : PBMC ratio of 1 : 10 (or 1 : 100 in the experiment in Fig. 3). To each well, IMDM complete medium was added containing 0, 10 or 100 TCID₅₀ FIV Utrecht 113. The final volume of each well was 200 μl.

After a 2 h incubation period at 37 °C, cells were washed twice with IMDM complete medium and incubated for 6 days on medium containing recombinant human (rhu) IL-2. The infection was evaluated by determining p24 antigen production in the supernatant using an ELISA as described previously (Egberink et al., 1992).

Transwell cultures. All tests were performed in quadruplicate. Concanavilin A stimulation of thymocytes was terminated 9 days prior to the start of the infection experiments and the obtained cells were regarded as Tr. Tr and fDCs were combined in a transwell system. Tr (5 x 10⁶) or fDCs (5 x 10⁶) were incubated with 100 TCID₅₀ FIV Utrecht 113 for 2 h in the lower chamber of a round-bottomed 96-well plate (Costar). After two washes with IMDM, 5 x 10⁶ fDCs were added to the transwell upper chamber (Falcon inserts 0.4 μm; Becton Dickinson) of the well containing thymocytes (Tw fDC/Tr) and 5 x 10⁶ Tr were added to the transwell upper chamber of the well containing fDCs (Tw Tr/fDC). Controls consisting of 5 x 10⁶ Tr or 5 x 10⁶ fDCs and co-cultures of both cell types (fDC : Tr ratio, 1 : 10) were maintained without cytokines. All cultures were incubated for 6 days at 37 °C and 5% CO₂ in a total volume of 200 μl.

At day 6, supernatant was harvested for p24 antigen detection by using an ELISA (Egberink et al., 1992).

Proliferation assay. Incorporation of [³H]thymidine into replicating cells was tested in 96-well microtitre plates (Costar). The tests were performed in triplicate and were repeated twice. DCs were incubated with Tr or with freshly thawed syngeneic PBMCs derived from the same cat, which had never been concanavilin A-stimulated, in a ratio of 1 : 10 (5 x 10⁴ fDC : 5 x 10⁵ PBMC or Tr). As controls, monocultures of 5 x 10⁴ Tr, 5 x 10⁵ PBMCs and 5 x 10⁵ fDCs were used. All cells were incubated for 6 days at 37 °C and 5% CO₂. Eighteen hours before ending the incubation period, 0.4 μCi (14.8 kBq) [³H]thymidine in 30 μl IMDM was added to each well.

The plates were stored at −20 °C until harvesting. Cells were harvested onto glass-fibre filters and the incorporation of [³H]thymidine was measured by liquid scintillation counting over a period of 60 s.

Statistical analysis. Statistical analysis was performed by using a two-sample t-test (two-sided).

RESULTS

Generation and evaluation of fDCs

After 6 days culture, BMMCs subjected to rfe IL-4 and rfe GM-CSF stimulation (indicated as fDCs hereafter) showed a morphology distinct from that of the cells cultured without cytokines (considered to be macrophages). fDCs were non-adherent, showed an irregular surface and contained characteristic processes (Fig. 1). Macrophage-like morphology became visible after 5–7 days. Generally, these cells were strongly adherent, flat, large and multinucleated. Non-specific esterase activity was abundant in
macrophage cultures, whereas in feDC cultures, it was hardly noticeable.

A selection of antibodies to discriminate macrophages from DCs was used. Antibodies specific for CD1a, CD1c, MHC II and CD11b stained the cells of the feDC cultures, indicating the presence of these surface markers (Fig. 2). The macrophages expressed MHC II, whereas other markers were low (CD11b) or absent (CD1a and CD1c).

**FIV infection of feDC/thymocyte co-cultures**

The feDCs, as defined by morphological and biological properties, were then used for further studies.

The effect of feDCs on FIV infection of thymocytes was evaluated by co-culturing feDCs in different ratios with thymocytes inoculated with a fixed amount of FIV Utrecht 113 (10 TCID$_{50}$). After 6 days culture, the co-cultures showed dispersed clusters of cells, as seen by using a phase-contrast microscope. Supernatants of feDC/thymocyte co-cultures at ratios of 1 : 10 and 1 : 100 were harvested daily for 6 days following infection. Supernatant samples were screened for FIV p24 as a determinant of virus production. Compared with the infected-thymocyte monoculture, a difference in p24 production was already noted at 4 days post-infection in the co-culture with an feDC : T ratio of 1 : 10. This difference became statistically significant at day 5 ($P<0.05$). In the feDC/T co-culture with a 1 : 100 ratio, a statistically significant upregulation of FIV infection was evident at day 6 (Fig. 3; $P<0.01$). The 1 : 10 feDC : T ratio was used for further experiments.

Next, the feDC/T co-cultures were infected with 0.01, 0.1, 1, 10 or 100 TCID$_{50}$ FIV Utrecht 113 to determine the sensitivity of the system. An upregulation of p24 production could be observed when using only 1 TCID$_{50}$ FIV Utrecht 113. However, the results with this amount of virus were not consistent when the experiments were repeated. Infection experiments using 10 or 100 TCID$_{50}$ FIV Utrecht 113 showed similar levels of p24 production (Fig. 4); monocultures of either feDCs or thymocytes differed significantly from co-cultured feDC/thymocytes ($P<0.01$).
feDCs are able to upregulate FIV infection of resting thymocytes

The ability of feDCs to enhance FIV infection in thymocytes was evaluated further for cells from which concanavalin A was withheld for 9 days (Tr). Infection of feDC/thymocyte co-cultures was compared with that of infected, monocultured thymocytes. Results of this experiment are depicted in Fig. 5(a). After the inoculation of the co-culture with 100 TCID<sub>50</sub> FIV Utrecht 113, the cells were incubated for 6 days. The p24 values showed a high production of FIV in the feDC/Tr co-culture ($A_{450} = 0.8 \pm 0.25$), in contrast to the Tr monoculture ($A_{450} = 0.09 \pm 0.1$) (feDC/Tr co-culture vs Tr monoculture, $P < 0.01$). To evaluate the necessity of direct contact of thymocytes and feDCs for the enhancement of FIV Utrecht 113 replication, we used transwell systems. Transwells separate thymocytes physically from feDCs, but
allow soluble factors derived from cells to pass through the membrane. The results are shown in Fig. 5(b). Transwell cultures in which the thymocytes were infected and feDCs were later added to the transwell upper chamber showed similar p24 levels (feDC/Tr, \( A_{450} = 0.085 \pm 0.04 \)) to monocultured Tr, hence no upregulation of FIV infection was detected. The p24 level of feDC monocultures and transwell cultures in which feDCs were infected and Tr were subsequently added to the upper chamber of the transwell system had similar p24 levels (Tr/feDC, \( A_{450} = 0.009 \pm 0.002 \); feDCs, \( A_{450} = 0.009 \pm 0.001 \)) and were regarded as background values. When stimulated thymocytes were evaluated in these experiments, similar results were obtained, although differences were less pronounced (results not shown).

**Ability of feDCs to stimulate FIV infection of stimulated and resting syngeneic PBMC cultures**

As thymocytes are allogeneic to the used feDCs, the question arose as to whether the same phenomenon can be recorded in a syngeneic system. An evaluation of the stimulatory capacity of feDCs on syngeneic PBMC cultures was performed. Syngeneic PBMCs were stimulated twice with concanavilin A, with a 7 day interval to obtain enough PBMCs to perform the test. Analogous to the thymocyte experiments, PBMCs that were deprived of concanavilin A stimulation for 2 days were regarded as stimulated PBMCs; deprivation for 9 days resulted in resting PBMCs. When stimulated or resting PBMCs were combined with syngeneic feDCs, enhancement of FIV infection was detected in both cases (\( P < 0.01 \); Fig. 6). The experiment was repeated on material derived from four other SPF cats, all showing enhancement of FIV infection in the co-cultures compared with the monocultures of stimulated syngeneic PBMCs (results not shown).

**feDC/thymocyte and feDC/PBMC proliferation assay**

In order to evaluate the ability of feDCs to stimulate either syngeneic PBMCs or allogeneic thymocytes directly, resting PBMCs or Tr were exposed for 5 days to feDCs. For the last 18 h, \([\text{H}]\)thymidine was added to the culture and the uptake was subsequently evaluated. Co-cultures of feDC/Tr, as well as feDC/PBMC co-cultures, showed a high level of \([\text{H}]\)thymidine uptake (11 458 ± 598 and 5828 ± 201 c.p.m., respectively) compared with Tr (245 ± 263 c.p.m.) or PBMC (245 ± 66 c.p.m.) monocultures (\( P < 0.01 \)). The feDC monoculture also showed a high uptake of \([\text{H}]\)thymidine (2822 ± 1007 c.p.m.). Results are shown in Fig. 7.

**DISCUSSION**

FIV already serves as an appealing model for the study of lentivirus infections. The pathogenesis of FIV resembles HIV infection in many respects (Bendinelli et al., 1995; Burkhard & Dean, 2003). One of the earliest steps in the pathogenetic process is the interaction of DCs and the lentivirus. As one of the earliest target cells for HIV-1 infection or through the capture of virions, DCs contribute...
to the dissemination of virus, which is transmitted through the mucosa (Lore & Larsson, 2003; Willflingseder et al., 2005). A similar role for feDCs can be expected. However, the early pathogenesis of FIV infection, especially the interaction of FIV with feDCs, has hardly been studied. To investigate this, a system of large-scale, reliable feDC production is necessary. feDC generation from blood and bone-marrow progenitors, together with the characterization of these cells, were described recently (Bienzle et al., 2003; Freer et al., 2005; Sprague et al., 2005). In rats and mice, BMMCs can serve as a source for DCs (Inaba et al., 1992; Talmor et al., 1998). Therefore, we collected feline BMMCs for the cultivation of feDCs. The non-adherent cells that were harvested from BMMC cultures after 6 days fe IL-4 and fe GM-CSF exposure were evaluated and found to be rich in CD11b, MHC II and CD1c. Only a portion of these cells expressed CD1a. In earlier reports in which bone-marrow cells were cultured in a similar way for 6 days, a distinct expression of these cellular markers was obtained (Bienzle et al., 2003). It is likely that our cultures consisted of a population of cells that were partly in the transition stage towards DC and cells that had already reached the immature DC stage. By FACS analysis, the cell populations evaluated seemed to be less uniform. In an attempt to gain a more monomorphic cell population, a magnetic-activated cell-sorting procedure was applied with the recombinant antibodies directed against hu CD14 and hu CD34 (Miltenyi Biotec). CD14 is a monocyte marker that can be used to sort peripheral blood samples (Sprague et al., 2005). Bone-marrow progenitors are CD34+ (Cameron et al., 1996; Pinchuk et al., 1999). Unfortunately, no depleted or sorted cell populations were obtained with these antibodies (results not shown). The expression of CD11b, a myeloid marker, is high on our feDCs, probably because bone marrow was used as monocyte source. Earlier findings of relatively high CD11b expression on murine bone marrow-derived DCs (Inaba et al., 1992), compared with dermal and splenic-derived murine DCs, supports this. Although culture conditions were in essence similar to those used in other studies (Bienzle et al., 2003; Freer et al., 2005; Sprague et al., 2005), differences existed with regard to source (bone marrow), cytokines (rfe) and culture medium (Iscove's) used for the generation of feDCs. These factors might have some effect on the level of cellular marker expression. Antibodies directed against or cross-reacting with feline CD80, CD86 or CD40 to evaluate the maturation state of feDCs are, to the best of our knowledge, not available at the moment. The cells obtained by our procedure can be regarded as feDCs based on morphology, lack of esterase activity, surface-marker profile and origin.

To propagate FIV efficiently in vitro, the use of mitogens prior to infection of thymocytes or PBMCs is necessary (Egberink et al., 1990; Pedersen et al., 1987). DCs can also fulfil this stimulating role as they are capable of stimulating T cells and transfer retroviruses very efficiently, as was shown for SIV (Kimata et al., 2004; Pope et al., 1997) and HIV-1 (Gummuluru et al., 2002; Pope et al., 1994, 1995). This transfer leads to enhanced virus replication in DC/CD4+ lymphocyte co-cultures infected with HIV-1 (Cameron et al., 1992; Pope et al., 1994, 1995). For DC–T-cell interaction, three mechanisms are proposed (Wu et al., 2002): transmission via a virological synapse, ligand interaction between DC and T cells and an indirect mechanism of DC-mediated stimulation towards T cells, which renders them more susceptible to infection. A study was performed to give a first insight into the interaction of feDCs with syngeneic PBMCs or allogeneic thymocytes when infected with FIV Utrecht 113. The co-cultures that we evaluated were infected as a whole for 2 h and p24 production was compared with that of infected monocultured cells. In control experiments in which feDCs were incubated with FIV Utrecht 113 before the addition of thymocytes, similar results were observed (not shown). The enhancement of FIV Utrecht 113 replication in both PBMCs and thymocytes due to the addition of feDCs was marked. The characteristics of this co-culture system were evaluated by lowering the amount of FIV Utrecht 113 used for infection. Even 1 TCID50 could still lead to a detectable infection in these co-cultures, but not in thymocyte monocultures. However, this low virus amount led to a large variation in p24 production. Co-cultures infected with 10 or 100 TCID50 did not differ in this respect. When feDCs were infected as monocultures, neither 10 nor 100 TCID50 led to detectable p24 production in any of the experiments performed so far. Hence, it seems unlikely that feDCs support FIV Utrecht 113 replication. The upregulation of FIV infection through the addition of feDCs to cultures of PBMCs or thymocytes might also be of benefit when FIV isolation from feline blood cells is used as a diagnostic tool.

FIV Utrecht 113 is capable of replicating in resting thymocytes or resting PBMCs when co-cultured with feDCs. In monocultures of either of these cells, no or low amounts of p24 could be detected. Furthermore, the enhanced replication within resting cells co-cultured with feDCs suggests activation, probably due to the excretion of soluble factors or through cell–cell interactions in which an improved environment for virus replication is created. It became clear from transwell experiments that feDCs were able to enhance replication of FIV in resting thymocytes only when they were cultured in close contact. Enhancement of virus replication is probably mediated via intercellular interactions among feDCs and thymocytes. This is in line with a previous study on SIV in which transwell cultures were used, and only in direct-contact cultures was virus replication detected (Kimata et al., 2004). As rhu IL-2 was always present in the medium of the experiments, it is not likely that this cytokine is involved in the enhancement of FIV Utrecht 113 in our system. Direct contact could result not only in optimal circumstances for the virus to be transported from one cell to the other, but also in stimulation of these cells. Both could lead to an increased amount of virus produced. The possible role of DC-SIGN as an attachment factor (de Parseval et al., 2004) in this process was studied by adding mannan to the co-cultures to a
maximum level of 100 μg ml⁻¹. No blocking of the enhancement was observed (results not shown). This seems to be in line with previous findings (Wu et al., 2002), where no effect of mannan on SIV transmission by macaque DCs and only a limited effect of this compound on HIV transmission by human DCs was detected. However, the lack of inhibition by mannan still does not exclude a role for a feline version of DC-SIGN. It is evident that, for HIV, several DC-SIGN-independent mechanisms of HIV attachment and internalization exist (Gummuluru et al., 2003). Therefore, a more detailed study on the role of attachment factors for FIV on feDCs is needed.

To study the possible role of stimulation of PBMCs and thymocytes by feDCs, a proliferation assay was performed. This assay was able to show a strong stimulation of thymocytes by allogeneic feDCs (Fig. 7). In this respect, feDCs have the same capacities as DCs of humans (Cameron et al., 1992), monkeys (O’Doherty et al., 1997), rabbits (Cody et al., 2005) or dogs (Ibisch et al., 2005) in allogeneic systems. In the syngeneic feDC/PBMC system, this stimulation was still present, but less pronounced. An explanation could be the more heterogeneous constitution of a PBMC culture, even when kept in culture for a longer period. Besides, syngeneic cells do not provoke a mixed leukocyte reaction, which will result in lower feDC-mediated PBMC proliferation.

The FIV Utrecht 113 enhancement that occurs in an allogeneic system with DCs from an SPF cat and thymocytes derived from an unrelated SPF kitten is in accordance to previous findings (Cameron et al., 1992) for HIV. This interaction of DCs and allogeneic T cells is, in our view, mediated indirectly: DC-mediated stimulation towards T cells. In the syngeneic system, in which feDCs enhanced FIV infection of PBMCs strongly, the interactions could be more direct and none of the above proposed mechanisms were excluded by our experiments. When syngeneic human DCs were added to T cells, Cameron et al. (1992) could not show any HIV replication. However, this was possibly strain-dependent, as other investigators indicate that only macrophage-tropic strains of HIV-1 (Granielli-Piperno et al., 1998; Petit et al., 2001) or SIV (Kimata et al., 2004; Messmer et al., 2000) were transmitted and replicated efficiently in resting syngeneic peripheral blood leukocytes by immature DCs. Apart from the cellular interplay as the cause of upregulation of FIV, the virus itself can also be of influence. This was illustrated by experiments with HIV (Petit et al., 2001) and SIV (Messmer et al., 2000) in which the accessory gene nef in particular was regarded as important in the ability of these viruses to replicate in co-cultures of immature DCs and syngeneic T cells. Even though no nef-like FIV gene is known at the moment, a gene designated ORF A is suggested to have similarities to nef of HIV-1 (Gemeniano et al., 2003). Further investigation into the function of the product of this ORF A gene as a Nef-like ‘superantigen’ (Torres et al., 1996a, b) in the stimulation of PBMCs is required. The feDCs in this co-culturing system might induce cytotoxicity against T cells, hence inducing apoptosis. Apoptosis induction by human DCs not only depends on the strength of the antigenic stimulation (Langenkamp et al., 2002), but HIV is known to sensitize human CD4⁺ T cells to human DC cytotoxicity (Lichtner et al., 2004). Although extrapolation of these results to feDC–T-cell interaction is difficult, we cannot exclude some effects of apoptosis induced by feDCs on p24 levels in our co-culturing system.

In summary, we have shown that, under the conditions described, feDCs can be generated from bone marrow-derived mononuclear cells. These feDCs are able to enhance FIV Utrecht 113 infection in allogeneic thymocytes and resting syngeneic PBMCs. This enhancement in feDC/thymocyte co-cultures was only detected when direct contact of cells was possible. feDCs were capable of inducing a proliferation in allogeneic thymocytes, which could be one of the explanations for the fact that, even in an allogeneic system, upregulation of FIV Utrecht 113 infection occurs.

REFERENCES


The role of dendritic cells in the


