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HIV-1 Infection of Placental Cord Blood Monocyte-Derived Dendritic Cells

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Abstract

Dendritic cells (DC), the most potent antigen-presenting cells (APC), have been implicated as the initial targets of HIV infection in skin and mucosal surfaces. DC can be generated in vitro from blood-isolated CD14⁺ monocytes or CD34⁺ hematopoietic progenitor cells in the presence of various cytokines. In this study, we investigated whether monocytes obtained from placental cord blood are capable of differentiation into dendritic cells when cultured with a combination of cytokines—granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-4 (IL-4), and tumor necrosis factor- α (TNF- α). We then examined HIV infection, HIV receptor (CD4, CCR5) expression, and β -chemokine [macrophage inflammatory protein-1 α and -1 β (MIP-1 α , MIP-1 β)] production by placental cord monocyte-derived dendritic cells (MDDC) as compared to that of autologous cord monocyte-derived macrophages (MDM). Monocytes isolated from placental cord blood differentiate into DC after 7 days in culture with the mixture of cytokines, as demonstrated by development of characteristic DC morphology, loss of CD14 expression, and gain of CD83, a marker for mature DC. Mature cord MDDC had significantly lower susceptibility to M-tropic ADA (CCR5-dependent) envelope-pseudotyped HIV infection in comparison to autologous placental cord MDM, whereas there was no significant difference in virus replication in cord MDDC and MDM infected with murine leukemia virus envelope-pseudotyped HIV (HIV receptor-independent). This limited susceptibility of cord MDDC to M-tropic HIV infection may be due to lower expression of CD4 and CCR5 on the cell membrane and higher production of MIP-1 α and MIP-1 β . These data provide important information toward our understanding of the biological properties of cord MDDC in relation to HIV infection.

INTRODUCTION

DENDRITIC CELLS (DC) are the most potent antigen-presenting cells (APC) of the immune system. Unlike other APC, such as macrophages and activated B lymphocytes, which activate memory T cells only, DC process and present antigens to CD4⁺ naïve T cells participating in

primary immune responses (1–4). DC also induce cytotoxic T lymphocyte responses (5–7). DC populate most tissues in the body, especially the skin and squamous mucosal epithelium, and they display different maturation states according to the local microenvironment (8). DC in the skin and mucosal surfaces, known as Langerhans cells or immature DC, are considered the initial targets of HIV infection acquired by nonparenteral routes (9,10). Although many studies have demonstrated that DC can be infected, the role of DC as HIV reservoirs and the extent of productive infection in DC are still unclear. DC may be dysfunctional, depleted, and/or infected with HIV in vivo and after exposure to HIV in vitro (11–14). On the other hand, other studies indicate that DC from HIV-infected individuals are functionally competent and not infected with HIV (15,16). Three populations of cells with DC morphology have been reported to be present in peripheral blood, yet only one of these populations is susceptible to HIV infection in vitro, a population that is morphologically similar to mature DC, but does not share the same T cell-stimulatory activity (17). Nevertheless, all types of DC pulsed with HIV in vitro can support vigorous HIV replication when cultured subsequently with T lymphocytes (18). Granelli-Piperno et al. demonstrated that only the initial round of HIV replication takes place in the DC, and they then migrate to the lymphoid tissue in a state of arrested replication. Upon contact between such DC and T cells, viral replication is triggered in DC, leading to productive infection of T cells in the vicinity (19). Thus, HIV may exploit the normal pathway of antigen uptake and presentation by DC to gain access to CD4⁺ T cells in lymphoid tissue (20).

DC constitute less than 0.5% of immune cells in the blood, which makes them difficult to purify in useful quantities (21). Recently, a method has been developed for the in vitro generation of DC from CD14⁺ monocytes (22–25) or CD34⁺ hematopoietic progenitor cells (26–29) from adult and placental cord blood using granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-4 (IL-4), and tumor necrosis factor- α (TNF- α). The DC generated from both sources display the phenotypic and functional characteristics of mature DC found in vivo (30), indicating that these in vitro-generated DC may be useful for studying the in vivo biological functions of DC.

The ability to generate large numbers of DC from blood CD14⁺ monocytes has made it possible to study characteristics of DC and their interaction with HIV. However, little information is available on DC generated from CD14⁺ monocytes from placental cord blood, and, in particular, the functional and phenotypic alterations that are associated with HIV infection during cord monocyte differentiation into DC. Although peripheral blood-isolated DC express the HIV receptors CD4 and CCR5 (31–34), there is little information about the expression of these receptors on monocyte-derived DC (MDDC), particularly MDDC from placental cord blood. CD4 is a primary HIV receptor on macrophages and T lymphocytes. CCR5 has been identified as a major co-receptor for HIV entry into macrophages (35,36). Subjects with a genetic mutation in CCR5 (CCR5-32) are highly resistant to HIV infection (37). The natural ligands for CCR5, macrophage inflammatory protein-1 α (MIP-1 α) and MIP-1 β , inhibit infection by interfering with HIV binding to the CCR5 receptor (38). Thus, the expression of CD4 and CCR5 and production of β -chemokines by cord MDDC and MDM directly influences HIV infection of these cells.

In this study, we demonstrated that monocytes from human placental cord blood can differentiate into DC when cultured with GM-CSF, IL-4, and TNF- α . Then, using placental cord blood MDDC, we investigated the susceptibility of these cells to HIV infection and the role of HIV receptors (CD4, CCR5) and β -chemokines (MIP-1 α , MIP-1 β) in HIV infection of these cells as compared to autologous cord monocyte-derived macrophages (MDM).

MATERIALS AND METHODS

Monocyte isolation and culture

Monocytes were isolated from placental cord blood from healthy, full-term neonates according to a previously described technique (39). In brief, cord blood mononuclear cells were separated over Ficoll-Paque (Pharmacia, Uppsala, Sweden) at $1500 \times g$ for 45 min and incubated with Dulbecco's modified Eagle medium (DMEM) in gelatin-coated flasks for 45 min at 37°C in 5% CO₂. Nonadherent cells were removed by washing with DMEM. Purified monocytes were detached with ethylenediamine tetra-acetic acid (EDTA) and resuspended in DMEM supplemented with 10% fetal bovine serum (FBS), 2 mM glutamine, penicillin (100 U/ml), streptomycin (100 μ g/ml), and nonessential amino acids. Monocytes were greater than 98% pure as assessed by flow cytometry using a monoclonal antibody (mAb) against CD14 (Leu-M3) and low-density lipoprotein specific for monocytes and macrophages (38). Freshly isolated monocytes were plated in 48-well culture plates at a density of 0.25×10^6 cells/well in DMEM with 10% FBS. All placental cord blood samples were identified as HIV antibody negative by enzyme-linked immunosorbent assay (ELISA) (Coulter Immunology, Hialeah, FL).

In vitro generation and culture of cord MDDC

Cord DC were generated from placental cord monocytes by a 5-day culture in media containing GM-CSF (800 U/ml) (StemCell Technologies, Vancouver, British Columbia) and IL-4 (100 U/ml) (R&D Systems, Minneapolis, MN). To induce the maturation of MDDC, day-5 cultured cells were treated with TNF- α (100 U/ml) (R&D Systems, Minneapolis, MN). The cultures were refed with fresh medium and the cytokines every 2–3 days after TNF- α treatment, and cell differentiation was monitored by light microscopy and flow cytometry using mAbs against CD83 and CD14 markers.

HIV infection and luciferase assay

Recombinant luciferase-encoding HIV virions pseudo-typed with envelope (Env) from either M-tropic ADA (CCR5-dependent), T-tropic NL-43 (CCR5-independent), or murine leukemia virus (MLV) (HIV receptor-independent) were used to study susceptibility of cord MDDC and MDM to HIV infection. The HIV Envdeleted luciferase reporter plasmid PNL-Luc-E⁻R⁺ (Aaron Diamond AIDS Research Center, New York, NY) was co-transfected into 293T cells (American Type Culture Collection, Manassas, VA) along with plasmids containing the genes encoding either ADA, NL-43, or MLV Env (Aaron Diamond AIDS Research Center), as previously described (40). Virus-containing supernatants were collected 48 h post-transfection and frozen at -70°C. Supernatants from cord MDDC and MDM cultures were removed and the cells were washed once with DMEM to remove cytokines before HIV infection. Cord MDDC and MDM (at days 7, 10, or 13 in culture) in

48-well plates (0.5×10^6 cells/well) were then infected for 6 h with 200 μ l of the pseudotyped virus (20 ng of p24 protein) in the presence of polybrene (8 μ g/ml). The cells were washed three times with DMEM to remove the input virus and the polybrene and were cultured in the absence of cytokines. At 72 h post-infection, the cells were lysed in 200 μ l of 0.5% Triton-X-100 in PBS. Luciferase activity was determined, after 50 μ l of each lysate was mixed with an equal volume of luciferase substrate (Promega, Madison, WI), in a microtiter plate luminometer (Dynex Technologies, Chantilly, VA). Results are expressed in relative light units (RLU).

Flow cytometry analysis

Cell-surface marker expression was evaluated by double immunofluorescence staining with the following fluorescein isothiocyanate (FITC)- or phycoerythrin-labeled mAbs: CCR5 (R&D Systems, Minneapolis, MN), CD4, CD14, and CD83 (PharMingen, San Diego, CA). Isotype-matched mouse immunoglobulin G (IgG) (PharMingen) was used as the control. Approximately 1×10^5 cells were suspended in 100 μ l of 1 $\times$ phosphate-buffered saline (PBS) and incubated with the antibodies for 45 min at 4°C. The cells were then washed with 1 $\times$ PBS and fixed with 1% paraformaldehyde. Fluorescence was analyzed on a FACS Caliber flow cytometer (Becton Dickinson, San Diego, CA).

RNA extraction

Total cellular RNA was extracted from cord MDDC and MDM (0.5×10^6 cells) using Tri-Reagent (Molecular Research Center, Cincinnati, OH). Briefly, the total RNA was extracted by a single-step, acid guanidinium thio cyanate-phenol-chloroform method. After centrifugation at $13,000 \times g$ for 15 min, the RNA-containing aqueous phase was precipitated in isopropanol. RNA precipitates were washed once in 75% ethanol and resuspended in 20 μ l of RNase-free water.

Polymerase chain reaction analysis for CD4 and CCR5

Total cellular RNA was reverse-transcribed using Reverse Transcription System (Promega, Madison, WI) with specific primers for CCR5 (antisense), CD4 (anti-sense), and β -actin (antisense) for 1 h at 42°C, and the resulting cDNA was used as a template for PCR amplification. PCR amplification was performed with one-tenth of the cDNA for 35 cycles with AmpliTaq Gold (Perkin Elmer, Branchburg, NJ) in a GeneAmp PCR system 2400 (Perkin Elmer-Cetus, Norwalk, CT). The PCR reaction mixture contained 0.2 mM dNTPs, 20 pM of CD4 or CCR5 primers, and 1.5 U of AmpliTaq Gold in 1 $\times$ reaction buffer (Perkin Elmer). PCR amplification consisted of heat inactivation of AmpliTaq Gold for 8 min at 95°C, followed by 35 cycles of 94°C for 30 sec, 55°C for 30 sec, and 72°C for 30 sec and elongation at 72°C for 7 min, using the specified CCR5 primers: 5'-CAAAAAGAAGGTCTTCATTACACC-3' (sense), and 5'-CCTGTGCCTCTTCTCTCATTTTCG-3' (antisense); and CD4 primers: 5'-GTGAACCTGGTGGTGATGAGAGC-3' (sense), and 5'-GGGCTACATGTCTTCTGAAACCGGTG-3' (antisense). PCR amplification for β -actin consisted of heat inactivation of AmpliTaq Gold for 8 min at 95°C, followed by 30 cycles of 95°C for 30 sec, 55°C for 30 sec, and 72°C for 30 sec and elongation at 72°C for 7 min. The

oligonucleotides were synthesized by Integrated DNA Technologies, Inc. (Coralville, IA). Each sample was matched with β -actin as the control to monitor the amount and integrity of RNA. PCR samples were analyzed by electrophoretic gel separation, using 3% NuSieve agarose gel for the separation of the CCR5 product and 2% NuSieve agarose gel for the separation of the CD4 and β -actin products.

β -chemokine detection

Supernatants were collected from cord MDDC and MDM cultures at days 7, 10, and 13 and stored at -70°C . β -Chemokine ELISA kits for MIP-1 α and MIP-1 β were provided by Endogen, Inc. (Cambridge, MA). The assay was performed as instructed in the protocol provided by Endogen. In brief, 50 μl of supernatants was added to antibody-coated wells and incubated for 1 h at room temperature. The plate was washed with the provided buffer solution and incubated with 100 μl of Biotinylated Antibody Reagent for 1 h at room temperature. The plate was washed again, treated with 100 μl of prepared streptavidin-horseradish peroxidase (HRP) solution, and incubated for 30 min at room temperature. After an additional wash, 100 μl of TMB substrate solution was added to each well, and the color was allowed to develop at room temperature for 30 min. The reaction was stopped by the addition of 100 μl of stop solution to each well. The plate was read on a microplate reader (ELX800, Bio-Tek Instruments, Inc., Winooski, VT).

RESULTS

Differentiation of placental cord monocytes into DC

We first determined whether placental cord CD14⁺ monocytes could differentiate into DC when cultured with GM-CSF, IL-4, and TNF- α . We used light microscopy to observe morphological changes and flow cytometry to examine alteration of CD14 and CD83 marker expression. Purified cord monocytes cultured without cytokines for 7 days differentiated into macrophages as expected (Fig. 1A). Cord monocytes cultured with GM-CSF, IL-4, and TNF- α exhibited a DC morphology by day 7 (Fig. 1B). Analysis of surface marker expression of cord MDDC at day 7 showed a loss of CD14 expression (<1%) and expression of CD83 (>95%), an indicator of DC maturation (25,41) (Fig. 2). In contrast, macrophages at day 7 were highly positive (>84%) for CD14 expression and negative (<2%) for CD83 expression (Fig. 2). We concluded that cord CD14⁺ monocytes could be used for in vitro generation of mature MDDC for our experiments.

HIV infection of cord MDDC and MDM

To determine the susceptibility of cord MDDC to HIV infection, as compared to autologous cord MDM, cord MDDC and MDM were infected with recombinant luciferase-encoding HIV virions pseudotyped with ADA (M-tropic and CCR5-dependent) Env and NL-43 Env (T-tropic) at days 7, 10, and 13 post-isolation. Cord MDDC showed limited susceptibility to HIV ADA infection, whereas cord MDM were readily infected with the virus as demonstrated by luciferase activity (Fig. 3). Both cord MDDC and MDM were resistant to HIV NL-43 infection (Fig. 3). To investigate whether low susceptibility of cord MDDC to HIV ADA infection is due to a block of virus entry, we used an MLV Env-pseudotyped HIV (HIV receptor-independent) to infect cord MDDC and MDM. There was no significant

difference in luciferase activity between MLV Env-pseudotyped HIV-infected cord MDDC and autologous cord MDM (Fig. 3), indicating that the relative resistance of cord MDDC to HIV ADA infection occurs at the viral entry level.

Expression of membrane CD4 and CCR5 on cord MDDC and MDM

To study further whether HIV receptors play an important role in the differential susceptibility to HIV infection observed in cord MDDC and MDM, the cells were analyzed by flow cytometry for membrane expression of CD4 and CCR5 at days 7, 10, and 13 post-isolation. Both cord MDDC and MDM showed a steady increase in CD4 and CCR5 expression during in vitro culture from day 7 to day 13 (Fig. 4). Cord MDDC expressed significantly lower levels of CD4 and CCR5 than autologous cord MDM (Fig. 4). CCR5 expression on cord MDDC correlated with their susceptibility to HIV ADA infection (Fig. 5). These results provide compelling evidence that the limited susceptibility of cord MDDC to HIV ADA infection is at least partially due to low levels of CD4 and CCR5 on the cell membrane.

Expression of CD4 and CCR5 mRNA in cord MDDC and MDM

To investigate whether CD4 and CCR5 mRNA expression correlates with membrane expression of these proteins in cord MDDC and MDM, semiquantitative RT-PCR was performed. Total cellular RNA was extracted from equal numbers of cord MDDC and MDM (0.5×10^6 cells) at days 7, 10, and 13 in culture and subjected to RT-PCR analysis. Cord MDDC showed levels of CD4 and CCR5 mRNA comparable to that of autologous cord MDM (Fig. 6), indicating that lower CD4 and CCR5 expression during cord monocyte differentiation into mature DC is attributable to post-transcriptional events. In addition, there was little change in CD4 and CCR5 mRNA expression in both cord MDDC and MDM during the course of culture from day 7 to day 13 (Fig. 6).

β -Chemokine production by cord MDDC and MDM

Because β -chemokines (MIP-1 α , MIP-1 β) are the natural ligands for CCR5, and inhibit HIV entry into macrophages, we investigated whether the differential susceptibility of cord MDDC and MDM to HIV infection is associated with a difference in β -chemokine production. Culture supernatants were collected from cord MDDC and MDM cultures on days 7, 10, and 13 and assayed by ELISA for MIP-1 α and MIP-1 β production. Day-7 cultured cord MDDC and MDM produced similar levels of MIP-1 α and MIP-1 β , whereas day-10 and day-13 cultured cord MDDC yielded significantly higher (>threefold) amounts of MIP-1 α and MIP-1 β than day-10 and day-13 cultured autologous cord MDM (Fig. 7). Because β -chemokines are the natural ligands for CCR5 receptor employed by HIV to enter macrophages, these results suggest that high levels of β -chemokine production by cord MDDC may also be partially responsible for their limited susceptibility to HIV infection as compared to cord MDM.

DISCUSSION

Investigation of the role of DC in HIV infection has been difficult because of their very low frequency in the circulating blood and tissues. Several studies have shown that DC can be

derived from CD14⁺ monocytes of adult peripheral blood (22–25) and from CD34⁺ progenitor cells (26–29) by culturing the cells in the presence of GM-CSF, IL-4, and TNF- α . The ability to generate useful quantities of DC in vitro from CD14⁺ monocytes or CD34⁺ progenitor cells has aided in the extensive study of these cells. Monocytes are the preferential source of DC, because they can be obtained from peripheral blood in high quantities without mobilization, the culture period required is much shorter, and their functional activity is similar to that of hematopoietic progenitor cell-derived DC (42). In the present study, we have shown that DC can also be generated in vitro from CD14⁺ monocytes isolated from placental cord blood, as demonstrated by morphological changes, diminished surface CD14 expression, and development of CD83 (41) on the surface of cord MDDC.

The degree of HIV infection and infectability of peripheral blood DC remains controversial (11–15). In addition, because functional and phenotypic maturation play an important role in HIV infection of DC and MDM, we investigated susceptibility of cord MDDC to HIV infection as compared to cord MDM. To eliminate interindividual differences, cord MDDC and MDM were derived from the same cord blood sample. Cord monocyte populations with a purity >98% (CD14⁺) were used in our study. To minimize possible influence of the cytokines added to the MDDC cultures on HIV infection of these cells, we used luciferase-encoding, pseudotyped variants of HIV that undergo a single cycle of replication. In addition, we removed the cytokines from MDDC culture at the time of HIV challenge and did not treat the cells with those cytokines again after viral infection. The use of M-tropic ADA Env (CCR5-dependent)- or MLV Env (HIV receptor-independent)-pseudotyped HIV allowed us to avoid the long-term culture time (2–3 weeks) necessary for HIV RT assay, during which cord MDDC could revert back to macrophages in the absence of the cytokines (43, our data not shown). Such reversion could affect the outcome of the experiments. Under these conditions, we have demonstrated that mature cord MDDC had significantly lower susceptibility to M-tropic ADA Env-pseudotyped HIV infection, as compared to autologous cord MDM as determined by luciferase assay. Our data support the conclusions of Granelli-Piperno et al. that mature adult blood MDDC do not support HIV replication, whereas MDM show high infectability (44). Our findings and those of Granelli-Piperno et al. are in disagreement with the study by Mallon et al., which showed that both macrophage-tropic and T cell-tropic virus could productively infect MDDC (45). We demonstrated that both cord MDDC and MDM are highly resistant to infection with T-tropic HIV (Fig. 3). The data presented by Mallon et al. show that only some HIV isolates can efficiently infect MDDC, particularly immature MDDC in comparison to MDM (45). In addition, because they failed to show data on HIV infection of MDM at day 8 post-infection, which is a critical time point for measuring RT activity, they could not determine whether there is differential susceptibility to HIV infection between MDDC and MDM at that timepoint (45). Furthermore, it is not clear in their study whether they continued to treat MDDC with cytokines at the time of HIV infection as well as after HIV infection. Because MDDC could revert to macrophages in the absence of cytokines (43), it is possible that HIV replication took place in “reverted” macrophages, which may contribute to the differences between their results and ours.

To determine whether the differential susceptibility to HIV between cord MDDC and MDM occurs at the viral entry level, we also examined HIV infection of cord MDDC and MDM using MLV Env-pseudotyped HIV that does not utilize HIV receptors (CD4 and CCR5) to enter macrophages. There was no significant difference in virus replication between cord MDDC and MDM infected with MLV Env-pseudotyped HIV, indicating that the susceptibility to HIV infection by cord MDDC and MDM is affected by Env-determined early events. Thus, we further investigated membrane expression of HIV receptors on cord MDDC and MDM. Our data show that cord MDDC expressed significantly lower levels of membrane CD4 and CCR5 as compared to autologous cord MDM, although cord MDDC and MDM express comparable levels of CD4 and CCR5 mRNA. We also found that membrane CXCR4 expression was lower in cord MDDC as compared to autologous cord MDM (data not shown). Interestingly, mRNA expression for β -actin, a cell structure gene used as a control gene in our RT-PCR assay, was higher in cord MDDC compared to autologous cord MDM (Fig. 6). This finding is consistent with the study by Hashimoto et al., showing that β -actin gene expression in MDDC was increased, as compared with MDM (46). Thus, to normalize the amounts of cellular RNA extracted from cord MDDC and MDM, we carefully monitored the cell numbers in each well and performed RNA extraction from equal numbers of cord MDDC and autologous cord MDM.

Because β -chemokines are ligands for the CCR5 receptor that are used by HIV to gain entry into monocytes/macrophages, they play a crucial role in HIV infection of these cells. We also examined β -chemokine production by cord MDDC and autologous MDM to determine whether there is differential expression of β -chemokines by cord MDDC and MDM, which may contribute to the observed difference in susceptibility to HIV infection. We demonstrated significantly higher levels of β -chemokine (MIP-1 α , MIP-1 β) production by cord MDDC as compared to cord MDM (Fig. 6). This higher β -chemokine production by cord MDDC may be partially responsible for their limited susceptibility to HIV ADA infection.

In summary, we have determined that CD14⁺ monocytes from placental cord blood are a reliable source of MDDC. Highly enriched DC can be generated in great numbers by culturing cord monocytes in the presence of GM-CSF, IL-4, and TNF- α . Our results indicate that the differential susceptibility of cord MDDC and MDM to HIV infection is most likely due to a significant difference in membrane expression of CD4 and CCR5 and in production of MIP-1 α and MIP-1 β . These data provide further information toward our understanding of the biological properties of cord MDDC in relation to HIV infection. Our data indicate that placental cord MDDC show limited susceptibility to HIV infection and could possibly be used as immunotherapeutic agents for HIV-infected patients. As the most potent APC, they could be used to augment T cell-mediated immune responses to achieve better control of HIV in infected individuals.

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FIG. 1.

Morphological changes during culture of CD14⁺ placental cord monocytes. Cord monocytes were cultured in the presence or absence of GM-CSF, IL-4, and TNF- α . **(A)** Morphology of day-7 cord MDM. **(B)** Morphology of day-7 cord MDDC. (Light microscopy; original magnification $\times 400$.)

FIG. 2.

Flow cytometry analysis of membrane expression of CD14 and CD83 on cord MDDC and MDM. Cord monocytes were cultured in the presence or absence of GM-CSF, IL-4, and TNF- α . Then 7-day cultured cord MDDC (solid histogram) and MDM (open histogram) were harvested and stained with FITC- or PE-conjugated monoclonal antibodies against CD14 (**A**) or CD83 (**B**) markers. Staining is representative of five experiments performed with similar results.

FIG. 3.

Comparison of HIV infection of cord MDDC and MDM. Luciferase-encoding HIV virions pseudotyped with either ADA, MLV, or NL-43 Env were used to infect cord MDDC and MDM at days 7, 10, and 13 in culture. Luciferase activity was quantitated in cell lysates 72 h after infection. Results are presented as mean $\pm$ SD of duplicate cultures and are representative of experiments using five cord blood samples.

FIG. 4.

Flow cytometry analysis of membrane CD4 and CCR5 expression on cord MDDC and MDM. Cord monocytes cultured in the presence or absence of GM-CSF, IL-4, and TNF- α for 7, 10, or 13 days were harvested and stained with a mAb against CD4 and CCR5 receptors. Results are shown as the percentage of CD4⁺ or CCR5⁺ cells (mean $\pm$ SE) and represent an average of flow cytometry data obtained from experiments using five cord blood samples.

FIG. 5.

Correlation of membrane CCR5 expression and HIV replication in cord MDDC. Cord monocytes cultured in the presence of GM-CSF, IL-4, and TNF- α were harvested and stained with a mAb against CCR5 at days 7, 10, and 13 post-isolation. MDDC from the same cord blood sample cultured under identical culture conditions were challenged with HIV pseudotyped with ADA Env at the indicated time points. Luciferase activity was quantitated in cell lysates 72 h after infection. Results shown are representative of three experiments.

FIG. 6.

RT-PCR analysis of CD4 and CCR5 mRNA expression in cord MDDC and MDM. Cord monocytes were cultured in the presence or absence of GM-CSF, IL-4, and TNF- α . Total cellular RNA was extracted from equal numbers of cord MDDC and MDM (0.5×10^6 cells) at days 7, 10, and 13 in cultures. D, MDDC; M, MDM. Results shown are representative of experiments with three cord blood samples.

FIG. 7.

Production of MIP-1 α and MIP-1 β by cord MDDC and MDM. Cord monocytes were cultured in the presence or absence of GM-CSF, IL-4, and TNF- α . Supernatants were collected from cord MDDC and MDM cultures at days 7, 10, and 13 in cultures and assayed for MIP-1 α and MIP-1 β production by ELISA. Results are presented as mean +SD of duplicate determinants and are representative of experiments using three cord blood samples.