Studies of Human Cord Blood Dendritic Cells: Evidence for Functional Immaturity

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We have isolated low-density, nonadherent, nonphagocytic, HLA-DR+ve cells with the morphology of dendritic cells (DCs) from the cord blood of full-term newborn infants. Relative to adult DCs, cord blood DCs were poor stimulators of the mixed leukocyte reaction when either adult or cord blood mononuclear cells (MNCs) or T lymphocytes were used as responder cells. In contrast, cord blood T cells and MNCs responded normally to allogeneic adult DCs. Cord blood DCs performed poorly as accessory cells for T-lymphocyte mitogenic responses at suboptimal concentrations of concanavalin A (Con A) and phytohemagglutinin A or at optimal concentrations of mitogen and low numbers of DCs. Addition of recombinant interleukin-2 (rIL-2) or recombinant interferon-γ (rIFN-γ) to cord blood DC–T-cell cultures containing a suboptimal concentration of Con A potentiated the proliferative response. In contrast, rIL-2 and rIFN-γ exerted little effect on the proliferative response of adult T cells cultured with Con A and DCs. Flow cytometric studies showed that levels of intercellular adhesion molecule-1 (ICAM-1; CD54) and major histocompatibility complex (MHC) class I HLA-ABC and class II HLA-DR antigens on cord blood DCs were significantly lower than those on adult blood DCs. These findings suggest that the relative inefficiency of cord blood DCs in the activation of T cells may be related to their low cell surface expression of MHC and cell adhesion molecules. The demonstrated impairment of cord blood DC function could be of importance in understanding the immunologic relationship between the fetus and mother and could contribute to the susceptibility of newborns to infection.

CONSIDERABLE evidence has been advanced that the human neonatal immune system differs functionally from that of the adult. Although often contradictory, the majority of studies have indicated that cord blood T lymphocytes,[1,2] B lymphocytes,[3] and monocytes[4] are deficient in a variety of in vitro functional assays or express different levels of certain cell surface antigens than do the corresponding cells of adult blood. The relative inability of neonatal T cells to elaborate interferon-γ (IFN-γ)[5,6] is paralleled by the low frequency of cells expressing the CD45RO (memory) isoform of the leukocyte common antigen.[7] Because most cord blood T lymphocytes bear the CD45RA isoform characteristic of naive T cells, it is probable that their activation requires presentation of antigen by lymphoid dendritic cells (DCs).[8] The existence of DCs in human cord blood was suggested by earlier studies,[9] and immunostimulatory DCs have been propagated in vitro from $CD34^+$ cells purified from human cord blood.[10,11]

We assessed DC function in the newborn by measuring the accessory cell–dependent,[12] T cell–proliferative response to the mitogenic lectins concanavalin A (Con A) and phytohemagglutinin A (PHA) and the capacity of cord blood DCs to stimulate the mixed leukocyte reaction (MLR). The functional and phenotypic attributes of cord blood DCs were compared with those of the corresponding cell population isolated from adult peripheral blood. Our findings indicate that, as accessory cells for T-cell responses, cord blood DCs are functionally inferior to adult blood DCs and that this may be at least partly explained by their lower expression of HLA-ABC, HLA-DR, and intercellular adhesion molecule-1 (ICAM-1, CD54).

MATERIALS AND METHODS

Blood samples. Small aliquots (3 to 5 mL) of cord blood were obtained from the blood bank at British Columbia's Children's Hospital from samples routinely taken at delivery from healthy full-term male and female newborns but not required for clinically indicated laboratory studies. Samples were processed within 24 hours of birth. Adult peripheral blood was donated by healthy male and female laboratory personnel (25 to 51 years of age). In both groups, EDTA or heparin were used as anticoagulants.

Isolation of DCs and T cells. Blood was mixed 1:1 with RPMI 1640 medium (StemCell Technologies Inc, Vancouver, British Columbia, Canada) containing 25 mmol/L HEPES, L-glutamine, 40 μmol/L nonessential amino acids, 40 μmol/L Na pyruvate, 100 U/mL penicillin, 100 μg/mL streptomycin, 2 μg/mL gentamycin, 40 ng/mL fungizone (GIBCO BRL, Burlington, Ontario, Canada), and 5×10^{-5} mol/L 2-mercaptoethanol. The diluted blood was layered over Ficoll-Hypaque (Pharmacia, Baie D'Urfé, Quebec, Canada) and centrifuged (30 minutes at 650*g*), and the mononuclear cell (MNC) fraction at the interface was collected and washed twice with medium. To obtain T cells, MNCs were mixed (1:50) with sheep red blood cells pretreated with *Vibrio cholerae* neuraminidase (GIBCO BRL, 2 U/mL, 45 minutes, 37°C) for 10 minutes at 37°C, centrifuged, and incubated on ice for a further 60 minutes.[13] Cells were gently resuspended, layered over Ficoll-Hypaque, and centrifuged. Erythrocytes in the cell pellet were lysed with 0.14 mol/L NH_4Cl to obtain rosetting T cells (ER+ve). These cells were washed twice with medium and incubated overnight at 37°C, 5% CO_2 in 10 mL of medium containing 10% heat-inactivated fetal calf serum (FCS; GIBCO BRL) in 10 × 100 mm plastic Petri dishes. Nonrosetting (ER−ve) cells from the gradient interface were washed with medium

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and cultured separately overnight. Nonadherent cells were then carefully layered over 3-mL columns of hypertonic metrizamide (Sigma Chemical Co, St Louis, MO; 14.5 g plus 100 mL of RPMI 1640 containing 10% FCS) and centrifuged at 600*g* for 10 minutes at room temperature.[14] T cells were recovered from the cell pellet, whereas DCs were obtained at the gradient interface. As a source of macrophages, plastic-adherent cells from the overnight culture were obtained by gently scraping using a rubber policeman after incubation on ice for 1 hour to facilitate detachment.

Characterization of cord blood DC. A combination of techniques was used to characterize cord blood DC, although their low frequency precluded complete characterization of all preparations. To assess phagocytic activity, cells were incubated in 24-well plates in medium with fluorescent plastic beads (Fluoricon particles, Pandex, Mundelein, IL) at 37°C, 5% CO_2 overnight. Cells were then washed and resuspended several times in the same well. The proportion of cells that took up beads was determined by examination under the fluorescence microscope. Indirect immunofluorescence staining was performed on DC cytospin preparations using a murine antihuman HLA-DR monoclonal antibody (IgG_1, clone L243; Becton Dickinson, Mississauga, Ontario, Canada) as the primary reagent and fluorescein isothiocyanate (FITC)-conjugated sheep antimouse IgG $(Fab')_2$ (Sigma) as the second antibody. Cells were examined under the fluorescence microscope.

Flow cytometry. T cells and DCs were analyzed for cell surface expression of a variety of leukocyte markers by fluorescence-activated cell sorting (FACS) analysis. Purified T cells (1 to 2 × 10^5/tube) were incubated for 1 hour on ice with directly labeled murine anti-CD3 (IgG_1, clone SK7)-phycoerythrin (PE), anti-CD19 (IgG_1, clone 4G7)-PE, anti-CD4 (IgG_1, clone SK3)-FITC, or anti-CD8 (IgG_1, clone SK1)-FITC monoclonal antibodies (Becton Dickinson) in phosphate-buffered saline (PBS) containing 2% FCS and 0.03% NaN_3. PE- and FITC-labeled IgG_1 (clone MOPC-21) isotype control monoclonal antibodies were purchased from Sigma. Cells were washed twice with the assay buffer and fixed in 1% *p*-formaldehyde in PBS. DCs (1–5 × 10^4 cells/tube) were stained with murine antihuman MHC Class I HLA-ABC (IgG_{2a}, clone B9.12.1,[15] AMAC, Inc., Westbrooke, ME), antihuman MHC class II–DR-specific (IgG_{2b}, clone B8.12.2,[16] AMAC, Inc), or antihuman ICAM-1 (IgG_1, clone 15.2,[17] Boehringer Mannheim, Laval, Quebec, Canada) monoclonal antibodies. Murine control monoclonal antibodies were MRC OX7 (IgG_1 antirat Thy-1,[18] kindly supplied by Dr R.W. McMaster, Department of Medical Genetics, University of British Columbia), MRC OX26 (IgG_{2a}, antirat transferrin receptor,[19] Serotec Canada Ltd, Toronto, Ontario) and MOPC-141 (IgG_{2b}, Sigma). After primary staining, cells were washed twice with PBS, incubated with FITC-labeled antimouse IgG $(Fab')_2$ for 30 minutes, washed and fixed. Cells were analyzed on an EPICS C flow cytometer (Coulter Diagnostics, Hialeah, FL) with a logarithmic fluorescence scale of three decades. The flow cytometer was gated to exclude dead cells and debris. Staining intensity is expressed as mean channel fluorescence, which was derived using a linear scaling program (1–256 channels). Specific fluorescence was calculated by the subtraction of the mean channel fluorescence obtained with the isotype-matched control from that for the human leukocyte-specific monoclonal antibody.

In a separate set of experiments, metrizamide interface cells were characterized with PE-labeled mouse monoclonal antibodies (Sigma) to CD3 (IgG_1, clone UCHT-1), CD14 (IgG_{2a}, clone UCHM-1), and CD19 (IgG_1, clone SJ25-C1). Expression of HLA-DR by these cells was evaluated with the FITC-labeled monoclonal antibody 13 (IgG_{2a}, clone 9-49, Coulter Immunology, Hialeah, FL). PE- and FITC-labeled isotype-matched antibodies were obtained from Sigma. Cells were stained as described above and analyzed on a Coulter XL flow cytometer with a logarithmic scale of four decades.

Mitogen stimulation assays. T cells (2–4 × 10^4/well) were incubated with irradiated (2,000 rad) autologous DCs in 96-well microtiter plates in RPMI 1640 medium containing 10% FCS at 0.2 mL/well in triplicate or quadruplicate cultures. Mixing experiments were performed by substituting allogeneic adult or cord DCs in the cultures. Control cultures contained T cells with or without mitogen, or T cells and DCs without mitogen. PHA and Con A were purchased from Sigma. In some experiments, cultures were supplemented with recombinant human IFN-γ (rIFN-γ; Boehringer Mannheim) or recombinant interleukin-2 (rIL-2; Amgen, Thousand Oaks, CA). Proliferative responses were assessed by the addition of 0.25 μCi of methyl-^{3}H-thymidine (^{3}H-TdR; Du Pont Canada Inc, Mississauga, Ontario) to each well for the final 24 hours of 96-hour cultures at 37°C, 5% CO_2. Cultures were collected onto glass fiber disks using a PHD cell harvester (Cambridge Technologies, Watertown, MA) and the incorporated radioactivity measured by liquid scintillation spectroscopy using a Beckman LS6800 scintillation counter (Beckman Instruments Canada Inc, Toronto, Ontario). The kinetic profile of the proliferative response of adult and cord blood T cells incubated with Con A (2 μg/mL) with or without autologous DC was determined in an 8-day culture. Cells were harvested daily 6 hours after the addition of 1 μCi of ^{3}H-TdR per well. In a separate experiment, proliferative responses to Con A (0 to 8 μg/mL) by cord blood and adult blood MNC preparations (1 × 10^6/mL) were determined.

Bioassay of IL-2. Levels of IL-2 in culture supernatants were measured by assessing the proliferative response of the murine IL-2–dependent CTLL-2 cell line[20] (American Type Culture Collection, Rockville, MD) using a colorimetric assay.[21] Briefly, cells were maintained in RPMI 1640, 5% FCS with rIL-2 (50 U/mL), washed with medium, and seeded at 2 × 10^4/well into microtiter plates containing 5% FCS and culture supernatants (final dilution 1:4). Known concentrations of rIL-2 were used to generate the standard curve. After 24 hours of culture, 20 μL of a 5-mg/mL solution of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; Sigma] was added for an additional 3 hours. One hundred microliters of medium was removed, and 150 μL of acidified isopropanol was added to the wells and vigorously mixed to solubilize the formazan crystals. Color intensity was measured at 590 nm using a Titertek Multiskan (Flow Laboratories Ltd, Mississauga, Ontario, Canada). Supernatant IL-2 concentrations were interpolated from the standard curve.

MLR. MLR assays were performed using cord blood or adult blood MNC (3.4 × 10^5/well) or purified T cells (2 × 10^5/well) cultured in RPMI 1640, 10% FCS. Responder cells were incubated for 5 days with irradiated autologous or allogeneic DCs prepared from cord or adult blood in quadruplicate flat-bottomed (MNCs) or round-bottomed (T cells) wells of microtiter plates at 0.2 mL per well. Proliferative responses were assessed by the addition of 0.25 μCi ^{3}H-TdR for the final 24 hours of culture.

Statistical analysis. Where appropriate, mean values were compared using Student's *t*-test. *P* values of $<.05$ were regarded as significant. Data are reported as means ± SEM.

RESULTS

Characterization of DCs and T cells. Fractionation of cord blood MNCs (n = 60) by T-cell rosetting, overnight adherence, and a metrizamide density gradient yielded a cell population with the morphological appearance of DCs, representing an average of 0.5% (range, 0.1% to 1.5%) of the Ficoll-Hypaque interface MNC number with an estimated purity of 57% (range, 41% to 69%). The same isolation procedure gave 1% (0.2% to 2.0%) DC-like cells from MNCs prepared from the blood of 10 different adults (includ-

ing multiple repeats) with an estimated purity of 60% (50% to 65%). Cell viability was greater than 95% by Trypan blue dye exclusion. The majority of the cord blood low-density, nonadherent cells obtained from the interface of metrizamide gradients were irregularly shaped, sometimes with veils around the cell, a barely visible nucleus, and projections that slowly extended and retracted, features similar to those observed for adult peripheral blood DCs isolated via the same technique. Essentially all plastic adherent cord blood cells took up fluorescent beads, indicating that the overnight culture had removed phagocytic cells. Sixty-five ± 10% (n = 6) of the cord blood nonadherent, metrizamide interface cells were nonphagocytic. Immunofluorescent staining of cord blood metrizamide interface cells with an anti–HLA-DR monoclonal antibody labeled 61.3% ± 3.7% (n = 3) of these cells, with the majority of these cells having the morphological appearance of DCs.

^{3}H-TdR incorporation by purified cord blood or adult T cells with or without Con A was less than 1,000 cpm. Addition of rIL-2 (200 U/mL) or rIFN-γ (500 U/mL) to cultures containing T cells alone or T cells and Con A (2 μg/mL) did not increase ^{3}H-TdR incorporation significantly above these background levels. However, addition of purified autologous DCs to T cells in the presence of Con A stimulated incorporation of the ^{3}H label in both adult and cord blood cultures, although the response in the adult cultures was characteristically much greater (see below). Plastic-adherent cells prepared from adult MNCs functioned poorly as accessory cells for T-cell responses to Con A (2 μg/mL), supporting proliferative responses 8% to 20% of that obtained using the same concentration of DC (data not shown).

FACS analysis. The majority (80% to 90%) of the ER+ve cells isolated from adult and cord blood for use in this study were CD3$^+$ and contained fewer than 5% cells that expressed the B cell–restricted marker CD19. Cell surface CD3 labeling intensity (mean channel linear fluorescence) was comparable for adult blood (mean 45.7 ± 1.9, n = 4) and cord blood (mean 50.9 ± 5.9, n = 5) T-cell preparations. Adult and cord blood T-cell preparations were composed of CD4$^+$ and CD8$^+$ cells in a ratio of approximately 1.5:1.

Flow cytometric studies demonstrated that metrizamide interface cells prepared from cord blood (n = 6) contained cells that expressed CD3 (3.1% ± 1.5%), CD14 (45.5% ± 5.9%), CD19 (2.7% ± 1.4%), or HLA-DR (67.6% ± 6.0%). The same low-density fraction from adult blood (n = 3) was comprised of cells that expressed CD3 (6.4% ± 4.0%), CD14 (45.4% ± 5.9%), CD19 (4.5% ± 4.4%), or HLA-DR (74.8% ± 8.8%).

Flow cytometric analysis of large metrizamide interface cells (70% to 85% of total cells) for surface expression of HLA-ABC, HLA-DR, and ICAM-1 indicated that these antigens were expressed with a significantly greater frequency and higher density on cells prepared from adult peripheral blood than on the corresponding cells isolated from cord blood (Table 1). FACS analyses of these cells isolated at different times from the same adults gave highly reproducible results with respect to both percentage of positivity and mean channel fluorescence intensity values. Characteristic monoclonal antibody staining patterns of adult peripheral blood and cord blood nonadherent, ER-ve, metrizamide gradient interface cells (DCs) are presented in Fig 1.

Table 1. Results of FACS Analyses for the Nonadherent, ER-ve, Metrizamide Interface Cells (DC) Isolated From Cord Blood and Adult Blood

Cell Surface Antigen	DC Source	n	% Positive	Mean Channel Fluorescence
HLA-ABC	Adult blood	6	93.3 ± 1.7*	125.9 ± 9.7†
	Cord blood	12	84.4 ± 3.1	94.7 ± 5.0
HLA-DR	Adult blood	7	89.1 ± 2.1†	101.9 ± 10.2†
	Cord blood	17	70.2 ± 3.3	56.2 ± 3.7
ICAM-1	Adult blood	5	86.8 ± 2.1†	54.9 ± 6.1†
	Cord blood	6	28.5 ± 12.6	18.8 ± 8.6

Dead and small cells have been gated out, and results obtained for cells with large forward scatter are given. Mean ± SEM percentage of positive cells and linear fluorescence values were obtained by subtraction of the result for the isotype-matched control monoclonal antibody. Mean fluorescence values for control antibodies ranged between channels 40 and 60. n = individuals analyzed.

* $P < .05$, † $P < .005$ for adult blood/cord blood DC comparisons.

MNC proliferative response to Con A. The culture of cord and adult MNC preparations with increasing amounts of Con A (0.5 to 8 μg/mL) demonstrated that, compared with the corresponding cells prepared from adult blood, cord blood MNCs responded poorly to concentrations of Con A of ≤2 μg/mL (Fig 2). When cord blood MNCs were cultured with Con A at 4 μg/mL, ^{3}H-incorporation was equivalent to that of adult blood MNCs; with Con A at 8 μg/mL, ^{3}H-incorporation by cord blood MNCs was significantly greater than that of adult MNCs.

Response of adult and cord blood T cells to mitogen. Distinct differences in the patterns of proliferation were observed when T cells purified from adult and cord blood were cultured with a constant number of DCs and varying concentrations of Con A or PHA (Fig 3). Thus, cord blood T cells proliferated weakly in response to low concentrations of either mitogen but gave proliferative responses that were equivalent to that observed for adult T cells when cultured with Con A at 10 μg/mL or with PHA at ≥10 μg/mL. When either a high (10 μg/mL) or a low concentration of mitogen (Con A at 2 μg/mL; PHA at 1 μg/mL) was added to T cells and titrated DCs, distinct differences in proliferative responses were again observed for adult and cord blood DC–T cell mixtures (Fig 4). Relative to the corresponding cell cultures, cord blood T cells proliferated weakly with the low concentration of Con A and PHA, even at the highest number of autologous DCs added. With the higher concentration of mitogen (10 μg/mL), cord and adult T cell–proliferative responses were comparable at DC numbers of ≥5 × 10^3 per well. At lower DC concentrations, cord blood DCs were much less effective than were adult DCs in providing accessory activity for T cell–proliferative responses, even at the higher concentration of Con A or PHA. Proliferative responses to Con A in adult DC–T cell cultures were strongly

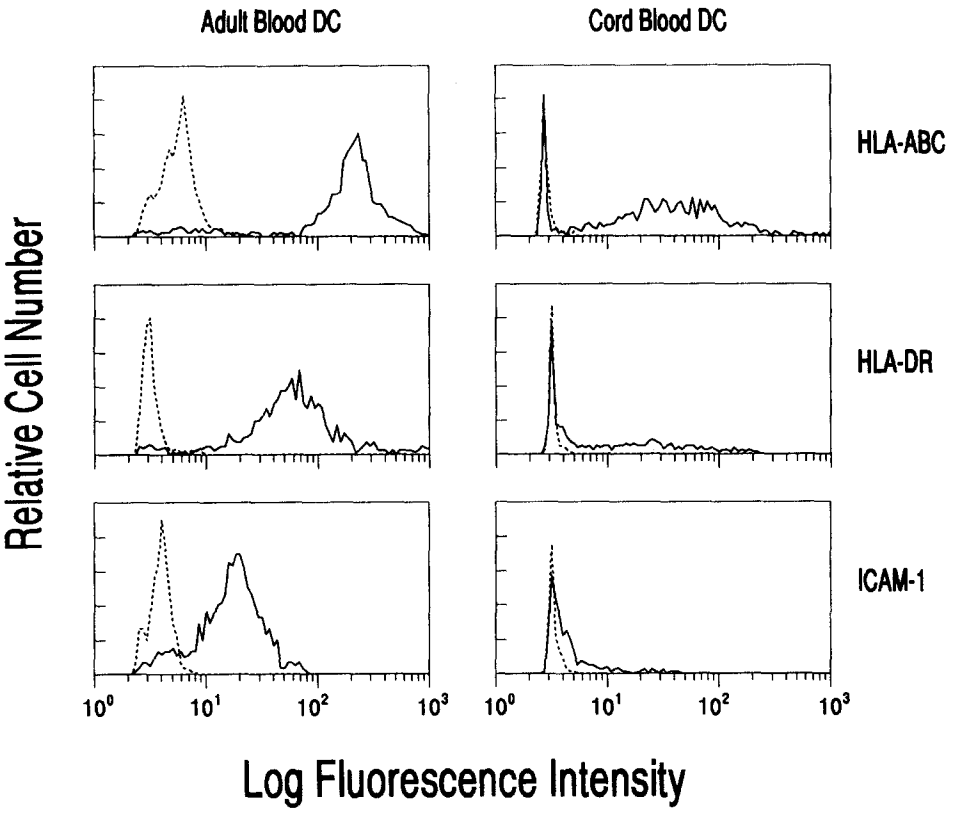

Fig 1. Characteristic FACS profiles of metrizamide interface cells isolated from adult blood and cord blood for their expression of HLA-ABC, HLA-DR, and ICAM-1 molecules. Background staining obtained with isotype-matched monoclonal antibodies is indicated by broken lines.

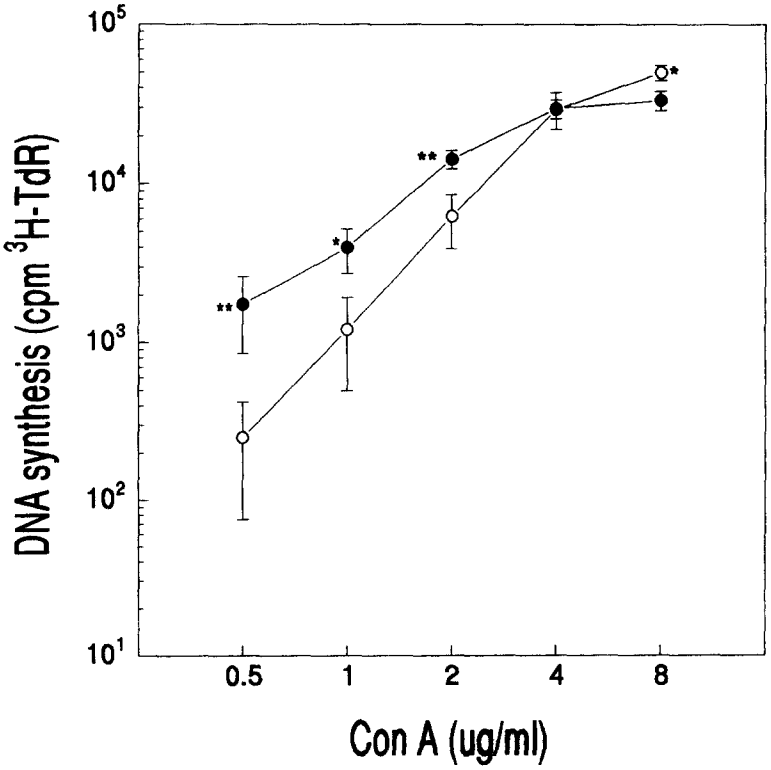

Fig 2. Proliferative response of four adult blood (●) and seven cord blood (○) MNC preparations cultured with a concentration gradient of Con A. ^{3}H-incorporation in the absence of Con A was 128 ± 19 cpm for adult and 285 ± 58 cpm for cord blood MNC. $^{**}P < .025$, $^{*}P < .05$.

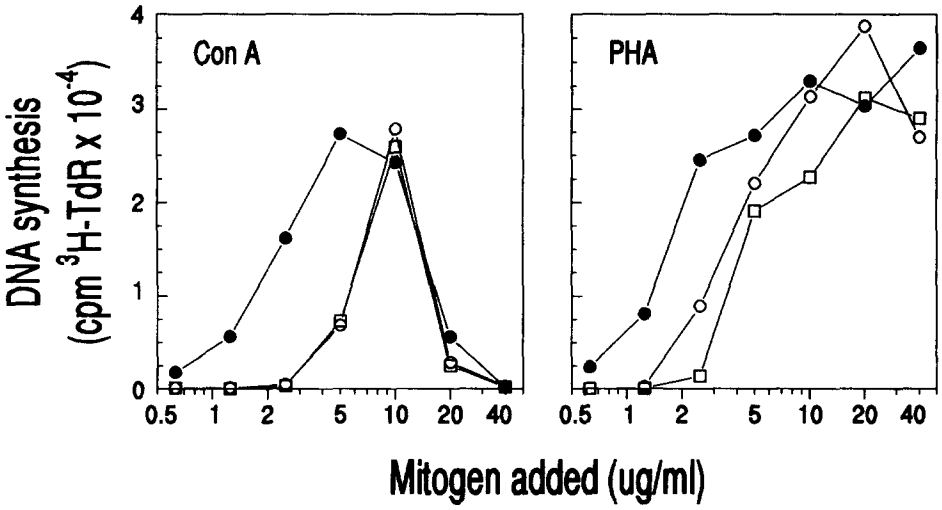

Fig 3. Proliferative response of adult blood (●) and cord blood (□, ○) T cells cultured with 1 × 10^4 individual-matched DC and concentration gradients of Con A or PHA. Error bars have been omitted for clarity of presentation. SEMs were less than 10% of mean cpm values.

inhibited by the addition of an anti–HLA-DR monoclonal antibody (data not shown).

The kinetic profiles of adult and cord blood T cell–proliferative responses to Con A (2 μg/mL) in the presence of autologous DCs over an 8-day period were similar, increasing to maximum levels of ^{3}H-incorporation after 3 to 4 days and then declining to approximately 25% of maximum values in the adult cultures and to background levels in the

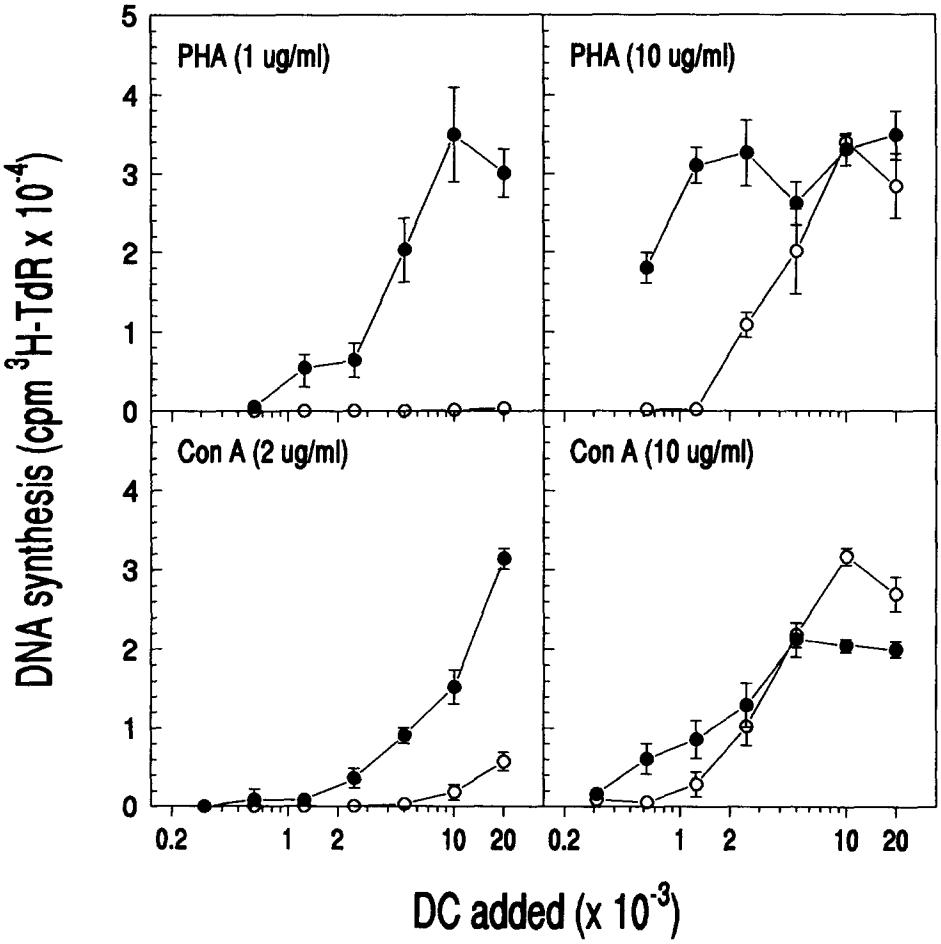

Fig 4. Proliferative response of adult blood (●) and cord blood (○) T cells cultured with titrated, individual-matched DC at two different concentrations of Con A and PHA. One of two representative experiments is shown except for experiments with Con A at 2 μm/mL, which was repeated 19 times with highly similar results.

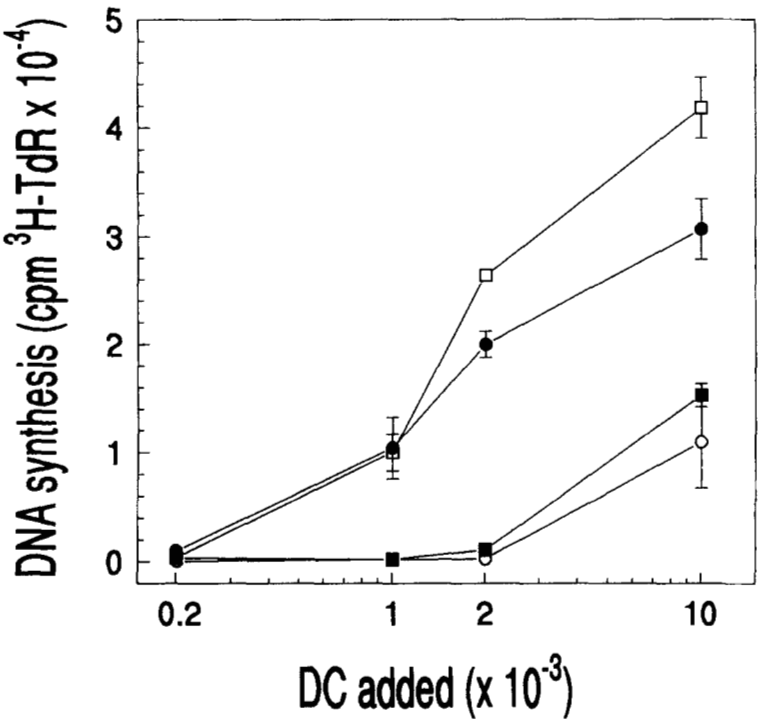

Fig 5. Proliferative response of cord blood T cells cultured with Con A (2 μg/mL) and either autologous cord blood DC (○) or allogeneic adult DC (□) and adult T cells cultured with either autologous DC (●) or allogeneic cord blood DC (■) and the same concentration of Con A. ^{3}H-incorporation in the absence of Con A was less than 1,000 cpm for all cell culture combinations. Two additional experiments gave similar results.

cord blood cultures after 8 days. Maximum observed ^{3}H-incorporation by 4 days was 93,400 ± 11,754 cpm in the adult and 9,957 ± 4,354 cpm in the cord blood cell cultures. Supernatants from adult T cell–DC cultures containing Con A (2 μg/mL) generated readily measurable levels of IL-2 within 24 hours of the start of the culture, increasing to maximum (13 ± 7.3 U/mL, three experiments) levels after 3 days. In contrast, the corresponding cord blood T cell–DC supernatants did not contain levels of cytokines that could support the proliferation of the CTLL-2 indicator cell line.

Cell-mixing experiments. When adult DCs were substituted for cord blood DCs, cord blood T cell–proliferative responses to Con A were comparable with those of adult T cells cultured with autologous DCs (Fig 5). The cell densities used in these assays did not generate significant allogeneic T-cell responses in the absence of mitogen. Substitution of cord blood DCs for adult DCs diminished the adult T-cell response to Con A to the level observed in the cord blood DC–T cell cultures.

Effects of rIFN-γ and rIL-2. Addition of rIFN-γ to cord blood DC–T cell cultures containing Con A (2 μg/mL) increased ^{3}H-TdR incorporation up to threefold above that of cultures without added rIFN-γ (Fig 6). rIFN-γ had little effect on the proliferative response of the corresponding adult cell cultures. Addition of rIFN-γ (500 U/mL) to cultures containing up to 4 × 10^4 DCs and Con A (2 μg/mL) significantly increased the proliferative response of cord blood T cells, although ^{3}H-incorporation was still lower than in the corresponding adult DC–T cell cultures (Fig 7). rIFN-γ did not significantly alter the proliferative response of the corresponding adult cell cultures through a range of DC concentrations. Addition of rIL-2 up to 1,000 U/mL stimulated a minor (less than 15%) increase in ^{3}H-incorporation by adult T cell–DC cultures containing Con A, whereas the same concentration of rIL-2 enhanced the cord blood cell response up to twofold (data not shown). When both rIL-2 and rIFN-γ were added to the adult and cord blood cell culture systems, no additional augmentation of proliferation occurred (data not shown).

MLR studies. The proliferative response of adult and cord blood MNCs to allogeneic adult DCs was significantly greater than that to allogeneic cord blood DCs (Fig 8). The response of purified adult T cells to allogeneic adult DCs was also much greater than to allogeneic cord blood DCs (Fig 9). ^{3}H-incorporation by adult T cells cultured with allogeneic adult DCs was still greater than fivefold above background levels at DC:T cell ratios of 1:500 (data not shown). When the converse experiment was performed, cord blood T cells responded strongly to allogeneic adult DCs but weakly to allogeneic cord blood DCs. The proliferative response of adult T cells to allogeneic adult DCs was 6 to 20 times greater than that against the same concentration of plastic-adherent cells prepared from the same individual (data not shown).

DISCUSSION

DCs occupy a crucial position in the initiation of primary immune reponses and are characteristically potent accessory cells for T-cell responses to mitogenic lectins and as stimulators of the MLR.[22] The study of human DCs is confounded by their low yield from all tissues and the unavailability of lineage-specific monoclonal antibodies for their identification. Using a series of techniques, we isolated from cord

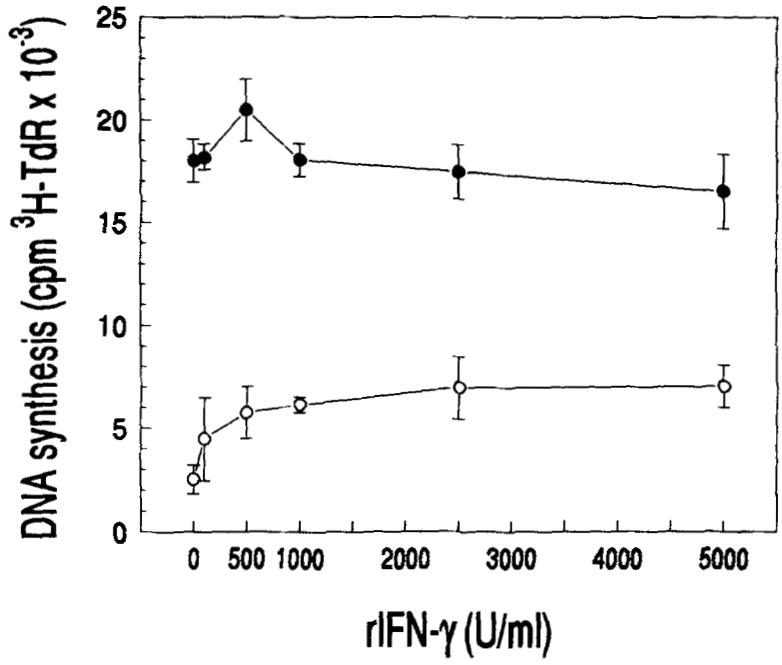

Fig 6. Proliferative response of adult blood (●) and cord blood (○) T cells cultured with 1 × 10^4 individual-matched DC and Con A (2 μg/mL) and a concentration gradient of rIFN-γ. Two additional experiments gave similar results.

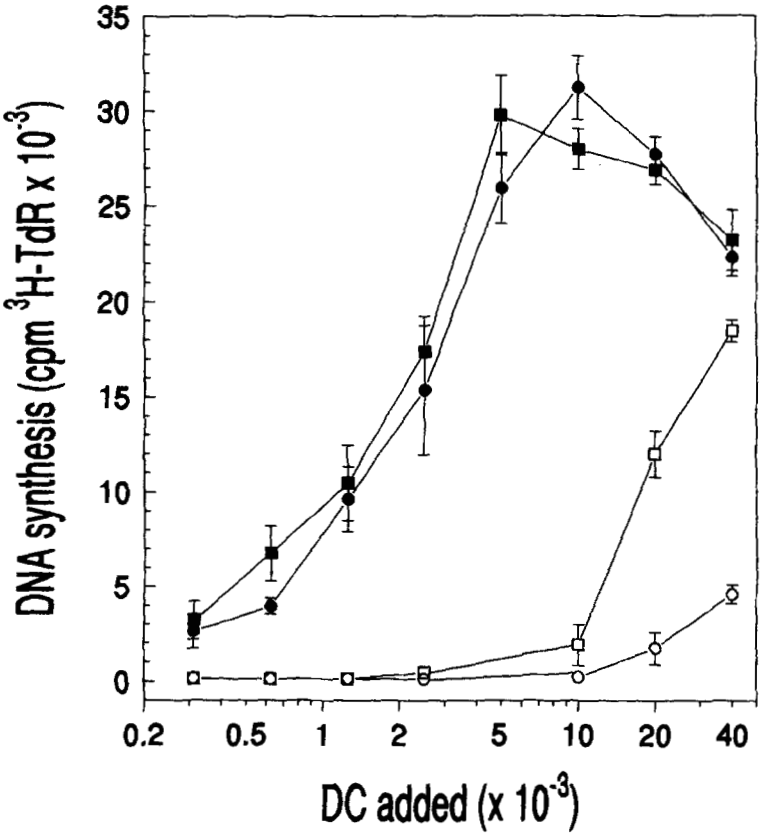

Fig 7. Proliferative response of cord blood T cells cultured with Con A (2 μg/mL) and titrated autologous DC without (○) or with rIFN-γ (500 U/mL, □). Results for adult T cells cultured with titrated autologous DC and Con A without (●) or with rIFN-γ (■) are also presented. An additional experiment gave similar results.

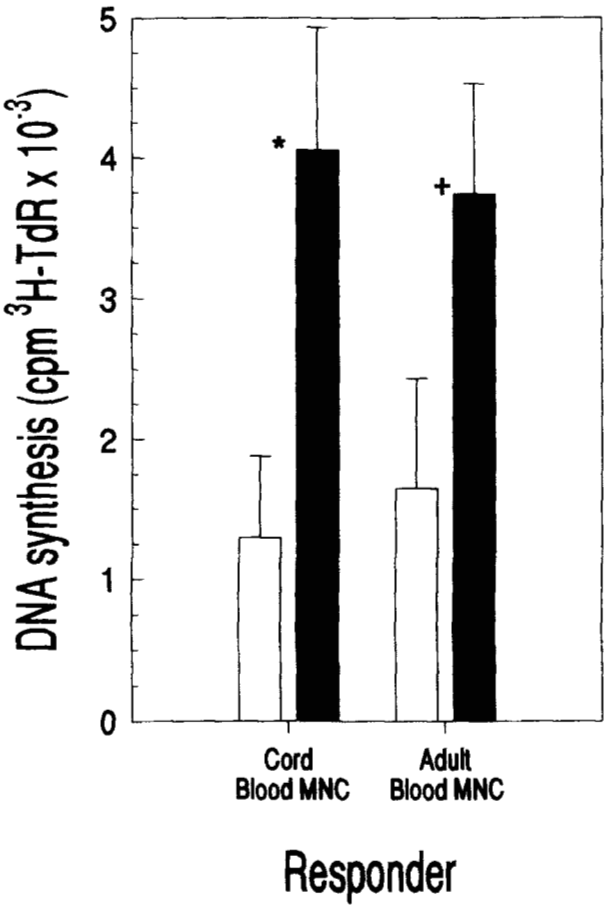

Fig 8. Proliferative responses by 3.4 × 10^5 cord blood (n = 7) and adult blood (n = 6) MNC cultured with 2 × 10^4 allogeneic cord blood DC (n = 8, □) or allogeneic adult blood DC (n = 6, ■) in an MLR. Mean ^{3}H-incorporation for wells containing MNC alone was subtracted from the mean result obtained for cultures containing allogeneic DC and corresponded to 349 ± 65 for cord blood MNC and 1,018 ± 255 for adult blood MNC. *$P < .01$, $^+P < .05$.

blood a low-density, HLA-DR+ve, ICAM-1^{lo}, ER-ve, nonphagocytic, nonadherent fraction containing cells with the morphological appearance of DCs. Estimates of DC purity for cord and adult blood preparations were within the range reported by others using highly similar cell isolation protocols.[21,22] However, categorizing DCs by purely morphological criteria may be somewhat misleading because other leukocytes may display a dendritic or veiled appearance under certain conditions.[23,24] We found that cord blood DCs, relative to DCs isolated from adult blood using the same methodology, were inefficient accessory cells for T-cell mitogenic responses and as MLR stimulators. The immunostimulatory strength of adult DCs was confirmed by the observation that these cells supported T cell–proliferative responses many times greater than those generated when plastic adherent cells were employed, as described previously.[25]

The potency of DC as antigen-presenting cells (APCs) correlates with their constitutive expression of high levels of cell surface MHC class I and II antigens and cell adhesion molecules, including ICAM-1,[22] BB1/B7 (CD80),[26] and leukocyte function–associated antigen-3 (LFA-3).[22] These molecules serve to initiate and stabilize DC interaction with T cells through specific counter-ligands expressed on T cells. The density of MHC class II molecules on APCs correlates with their ability to present antigen to T-cell clones,[27] although expression of high levels of MHC gene products does not necessarily confer immunostimulatory activity.[22] The observation that cord blood DCs are inefficient accessory cells for T-cell replication parallels their relatively low expression of HLA-ABC and HLA-DR molecules. Human cord blood monocytes were shown to express significantly less HLA-DR than adult blood monocytes.[28] Splenic macrophage Ia expression in neonatal mice was considerably lower than that of adult mice and correlated with an impaired capacity to present antigen.[29] However, a recent study suggested that the antigen-presenting function of human cord blood MNCs is comparable with that of adult blood MNCs.[30] Relative to adult blood DCs, cord blood DCs also expressed significantly lower levels of ICAM-1, a molecule involved with the stabilization of intercellular interactions through binding to LFA-1.[31] ICAM-1 is upregulated on a variety of activated leuko-

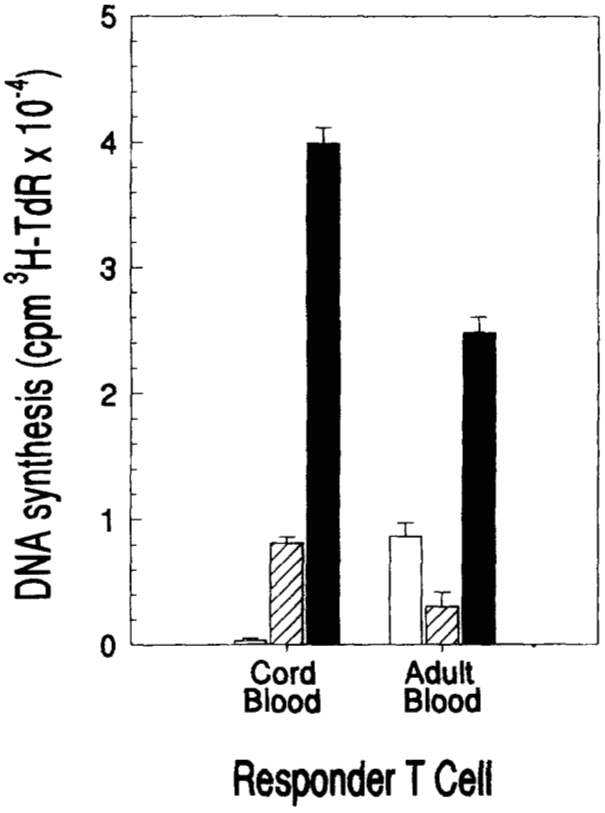

Fig 9. Proliferative responses by 2 × 10^5 cord blood and adult T cells cultured with either 1 × 10^4 autologous DC (□), allogeneic cord blood DC (cross-hatched), or allogeneic adult DC (■) in the MLR. The same panel of DC preparations was tested against the adult and the cord blood T cells. ^{3}H-incorporation in the absence of added DC was 84 ± 20 cpm for cord blood T cells and 110 ± 47 cpm for adult T cells. A second experiment gave similar results.

cytes,[32] although it is constitutively expressed on adult blood DCs.[22] Thus, in contrast to adult DCs, neonatal DCs do not display the dense array of some of the cell surface molecules required for interaction with T-lymphocytes.

The hyporesponsiveness of cord blood T cells to low concentrations of Con A or PHA as described in this study could represent an intrinsic defect in a cellular activation pathway through the T-cell receptor (TCR). Relative to those generated by adult blood MNCs, low proliferative responses to soluble anti-CD3 monoclonal antibody by cord blood MNC and T cells have been observed.[33] This deficiency was not reversible using adult macrophages as accessory cells.[33] However, adult and neonatal T cells proliferated to the same degree when cultured with immobilized anti-CD3 monoclonal antibody[5] in the absence of accessory cells. Upon activation, cord blood T cells release IL-2, whereas adult T cells also synthesize IFN-γ and IL-4[7] and transcribe mRNAs for IL-3, IL-5, IL-6, granulocyte-macrophage colony-stimulating factor (GM-CSF). We showed that cord blood T cells proliferated as strongly as adult T cells when cultured with optimal concentrations of mitogen at higher densities of autologous DCs or with a suboptimal concentration of Con A and adult DCs. Correspondingly, cord blood MNCs responded poorly to low concentrations of Con A but proliferated as well as or even more strongly than adult MNCs at higher concentrations of the mitogen. In addition, cord blood T cells proliferated strongly in an MLR when cultured with allogeneic adult DCs but not allogeneic cord blood DCs. The lectins PHA and Con A act as polyclonal activators of T-lymphocytes and bind to multiple cell surface glycoproteins including the TCR/CD3 complex.[34,35] The density of CD3 on cord blood T cells was equivalent to that on adult T cells, a finding that differs from an earlier study.[2] Thus, the weak proliferative response of cord blood T cell–DC cultures to low concentrations of mitogen, as described in this study, was not related to an intrinsic deficiency of the cord blood T cell. In the response to a low concentration of Con A, cord blood T cells incorporated low amounts of the ^{3}H label, produced no detectable IL-2, but were responsive to exogenous IL-2. This suggests that cord blood DCs do not provide the appropriate cellular signals to maximally activate T cells. At higher concentrations of mitogen, cord blood T cell–proliferative responses were comparable with those of adult T cells with the highest concentrations of DCs. The explanation for this result is unclear, but higher mitogen concentrations may enhance cellular interactions through linkage of T-cell and DC lectin-binding domains.

In a recently published study, cord blood DCs, isolated with a method similar to the one described herein, were evaluated for their ability to elicit primary T-cell responses against the major outer membrane protein (MOMP) of *Chlamydia trachomatis*.[36] Antigen-specific proliferation was detected in fewer than half of the cord blood T-cell cultures containing DCs.[36] IFN-γ was detectable in the majority of the antigen-pulsed cord blood cell cultures but at relatively low levels. In contrast, essentially all experiments testing MOMP-pulsed DCs from nonsensitized, naive adults generated significant T cell–proliferative responses and high levels of supernatant IFN-γ. The capacity of cord blood DCs to promote primary T cell responses and elicit formation of IFN-γ will require further study. In the present investigation, addition of rIFN-γ to cord blood but not to adult blood DC–T cell cultures containing a suboptimal concentration of Con A significantly enhanced cord blood T-lymphocyte–proliferative responses. Although we have no evidence that IFN-γ acted directly on cord blood T cells, an earlier study showed that IFN-γ potentiated the proliferation of mitogen-activated cord blood T cells and dramatically augmented their expression of HLA-DR.[37] Current evidence[22] indicates that IFN-γ does not modify expression of MHC class II molecules by DCs, and one study found that IFN-γ diminished murine splenic DC function.[38] Because CD14$^+$ cells represent the major contaminant of adult and cord blood DC preparations, it is conceivable that rIFN-γ may have influenced cord blood T-cell responses through an interaction with macrophages. Although human blood DCs may weakly express CD14,[24] use of a more stringent DC purification scheme, as recently described,[24,39] would limit the influence of other cell types on T-lymphocyte responses.

Differential effects of various cytokines on the immunostimulatory activity of DCs have been observed.[38,40,41] IL-10, in contrast to its potent inhibitory effect on macrophage activity, did not alter the ability of DCs to support proliferation and IL-2 secretion by antigen-specific murine T-cell clones but did impair IFN-γ release by these T cells.[41] The cytokine profile of differentiating T-lymphocytes may be dictated by the APCs during interaction with the T cell.[41] IFN-γ production could be induced in neonatal T cells cultured with PHA and adult but not cord blood monocytes.[4] It has been postulated that an immunoregulatory circuit may exist in the fetus in which IL-10 has a central role in the modulation of T-cell production of inflammatory cytokines, including IFN-γ.[42] The delineation of the cytokine network that controls fetal DC formation could provide insights into immunological mechanisms that permit tolerance of the fetus and also identify avenues for immunomodulation in episodes of neonatal infection. The identification of GM-CSF and tumor necrosis factor as cytokines that synergistically promote the in vitro production of DCs from early stem cells[43] suggests that these factors may also be important in fetal DC maturation.

The biological and clinical significance of the findings reported herein are uncertain. Survival of the fetus in a semiallogeneic environment may be facilitated by a relatively impotent fetal immune system. On the other hand, because of the limited ability of the newborn DCs to support T-cell responses, the neonate may be especially vulnerable to pathogens. Thus, the functional immaturity of the neonatal DC may be central to the susceptibility of newborns to infection with intracellular bacteria and viruses.

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