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Deficient IL-12(p35) Gene Expression by Dendritic Cells Derived from Neonatal Monocytes

Stanislas Goriely, Benoît Vincart, Patrick Stordeur, Johan Vekemans, Fabienne Willems, Michel Goldman, and Dominique De Wit

To gain insight into the defects responsible for impaired Th1 responses in human newborns, we analyzed the production of cytokines by dendritic cells (DC) derived from cord blood mononuclear cells. We observed that neonatal DC generated from adherent cord blood mononuclear cells cultured for 6 days in the presence of IL-4 and GM-CSF show a phenotype similar to adult DC generated from adherent PBMC, although they express lower levels of HLA-DR, CD80, and CD40. Measurement of cytokine levels produced by neonatal DC upon stimulation by LPS, CD40 ligation, or poly(I:C) indicated a selective defect in the synthesis of IL-12. Determination of IL-12(p40) and IL-12(p35) mRNA levels by real-time RT-PCR revealed that IL-12(p35) gene expression is highly repressed in stimulated neonatal DC whereas their IL-12(p40) gene expression is not altered. The addition of rIFN-γ to LPS-stimulated newborn DC restored their expression of IL-12(p35) and their synthesis of IL-12(p70) up to adult levels. Moreover, we observed that neonatal DC are less efficient than adult DC to induce IFN-γ production by allogeneic adult CD4+ T cells. This defect was corrected by the addition of rIL-12. We conclude that neonatal DC are characterized by a severe defect in IL-12(p35) gene expression which is responsible for an impaired ability to elicit IFN-γ production by T cells. The Journal of Immunology, 2001, 166: 2141–2146.

It is well established that the immune system of the newborn is functionally different from that of the adult, resulting in an increased susceptibility to intracellular pathogens and poor responses to vaccine Ags (1). Neonatal T cell responses are usually characterized by an impaired synthesis of IL-2 and IFN-γ whereas their synthesis of Th2-type cytokines can vary, depending on the species and the Ag considered (2–4). Indeed, vaccinal responses of newborn mice are clearly Th2 skewed under conditions inducing Th1-type responses in adult animals (5). Although human newborns also display Th2-type responses upon in utero contact with environmental allergens (6), a recent study showed the induction of IFN-γ-secreting cells in children vaccinated at birth with the Mycobacterium bovis bacillus Calmette-Guérin vaccine (7).

Inherent T cell defects certainly contribute to the immunological immaturity of the newborn as indicated by impaired tyrosine phosphorylation (8) and hypermethylation of specific sites in the promoter region of the IFN-γ gene (9). Because myeloid dendritic cells (DC) are essential for the priming of naive T cells as well as for their differentiation into Th1 cells through the synthesis of IL-12 (10), we hypothesized that a DC defect could contribute to the impaired immune responses of the human newborn. Herein, we approached this question by analyzing DC generated from adherent cord blood mononuclear cells (CBMC) cultured in the presence of GM-CSF and IL-4. Our finding that newborn DC are profoundly deficient in the synthesis of the bioactive dimeric form of IL-12(p70) led us to analyze their expression of IL-12(p40) and IL-12(p35) at the gene level and to determine its relevance to the observed defect.

Materials and Methods

Culture medium and reagents

Culture medium consisted of RPMI 1640 (BioWhittaker Europe, Verviers, Belgium) supplemented with 2 mM L-glutamine (Life Technologies, Paisly, U.K.), gentamicin (20 µg/ml), 50 µM 2-ME, 1% nonessential amino acids (Life Technologies), and 10% FBS (BioWhittaker). Recombinant IL-4 (24 × 10^3 IU/mg) and recombinant GM-CSF (Leucomax, 16 × 10^3 IU/mg) were kindly provided by Schering-Plough (Kenilworth, NJ). LPS from Escherichia coli (0128:B12) was purchased from Sigma (Bornem, Belgium). Recombinant human IL-12 and recombinant human IFN-γ were purchased from R&D Systems (Abingdon, U.K.) and BioSource Europe (Nivelles, Belgium), respectively. Poly(I:C) was purchased from Sigma.

Cells

Human cord blood was obtained from the placenta of normal full-term deliveries at the obstetric department of the Erasme Hospital, Brussels. Adult PBMC were isolated from buffy coats obtained from local routine blood donations. Mononuclear cells were obtained from all samples by centrifugation over Ficoll-Hypaque gradients (Nycomed, Oslo, Norway).

Generation of DC from peripheral blood

DC were generated from PBMC or from CBMC, as described by Romani et al. (11). Briefly, PBMC and CBMC were resuspended in culture medium and allowed to adhere onto six-well plates or large Falcon flasks for larger number of cells. After 2 h at 37°C, nonadherent cells were removed and adherent cells were cultured in 3 ml or 20 ml of medium containing GM-CSF (800 U/ml) and IL-4 (500 U/ml). Every 2 days, 800 U GM-CSF and 500 U IL-4 were added. After 6 days of culture, nonadherent cells corresponding to the DC-enriched fraction were harvested, washed, and used for subsequent experiments. As previously reported (12), the DC-enriched fraction obtained according to this protocol routinely contains >90% of DC as assessed by morphology and FACS analysis.
Flow cytometric analysis

For immunophenotyping, cells were washed in PBS supplemented with 0.5% BSA and 10 mM Na3 and incubated for 30 min at 4°C with one of the following murine mAbs: PE-conjugated anti-HLA-DR IgG2b mAb, PE-conjugated anti-CD40 IgG1 mAb (BioSource International, Camarillo, CA), PE-conjugated anti-CD86 IgG2b mAb (PharMingen, San Diego), PE-conjugated anti-CD80 (B7-1) IgG1 mAb, PE-conjugated anti-CD14 IgG2b mAb, PE-conjugated anti-CD54 IgG2b mAb, PE-conjugated anti-CD1a IgG2a mAb (Dako, Prosan, Belgium), and PE-conjugated anti-CD83 IgG2a mAb (ImmunoDiagnostics, Mountain View, CA), PE-conjugated anti-CD1a IgG2b mAb (PharMingen, San Diego), PE-conjugated anti-CD86 (B7-2) IgG2b mAb (PharMingen, San Diego), PE-conjugated anti-CD40 IgG1 mAb (BioSource International, Camarillo, CA), PE-conjugated anti-CD80 (B7-1) IgG1 mAb, PE-conjugated anti-CD14 IgG2b mAb, PE-conjugated anti-CD54 IgG2b mAb, PE-conjugated anti-CD1a IgG2a mAb (Dako, Prosan, Belgium), and PE-conjugated anti-CD83 IgG2a mAb (ImmunoDiagnostics, Mountain View, CA). As controls, cells were stained with corresponding isotype-matched control mAbs. Analysis was done using a FACScalibur flow cytometer (Becton Dickinson).

Cytokine determination

ELISA kits were purchased from BioSource Europe for quantification of TNF-α, IL-6, and IL-8. The detection limit for these three assays was 20 pg/ml. IL-12(p70) production was measured by ELISA kits provided by Endogen (Woburn, MA; detection limit 2 pg/ml). IFN-γ, IL-5, and IL-12(p40) concentrations were measured by two-site sandwich ELISA systems using Abs from BioSource Europe (Cytoscreen; detection limit for these three assays was 10 pg/ml). IL-10 and IL-2 were detected by sandwich ELISA systems using Abs from PharMingen, San Diego, and Endogen. For determination of IL-2 levels, anti-IL-2 receptor mAb (used as 1:200 dilution of ascitic fluid) was added in cultures (13).

Cell stimulations

DCs generated from adult PBMC or CBMC were cultured at a final concentration of 4 × 10^5 cells/ml with or without LPS (1 μg/ml) in 24-well plates. After 24 h of culture, supernatants were recovered for determination of cytokine levels.

For the CD40-mediated activation, 3T6 cells transfected with the gene encoding the CD40 ligand (3T6-CD40 ligand transfectants) were used to induce CD40 triggering on DC. Untransfected 3T6 cells were used for control cultures (data not shown). 3T6 cells (5 × 10^5) were cocultured with 2 × 10^5 DC/well (24-well culture plate) in 1 ml of culture medium. Poly(I:C) was used at a final concentration of 20 μg/ml. After 3 days of incubation, supernatants were collected for determination of cytokine levels. For RT-PCR experiments, cells were incubated for a period of 8 h.

Quantification of IL-12 p35, IL-12 p40, and β-actin mRNA levels by real-time PCR

At the end of cell culture, total cellular RNA was extracted by using the TriPure reagent (Roche Diagnostics, Brussels, Belgium) according to the manufacturer’s instructions. Reverse transcription was then conducted as follows: 8 μl of water containing 500 ng of total RNA was added to 2 μl of oligo(dT) primer (0.5 μg/μl) and incubated at 65°C for 10 min. Samples were chilled on ice and 10 μl of RT mix containing the following components were added: 1) 4 μl of RT buffer (250 mM Tris-HCl (pH 8.3), 375 mM KCl, and 15 mM MgCl2); 2) 2 μl of deoxynucleotide triphosphate mix (10 mM each); 3) 0.2 μl of BSA (1 mg/ml); 4) 0.5 μl (25 U) of human placental ribonuclease inhibitor (RNAguard; Amersham Pharmacia Biotech Benelux, Roosendaal, The Netherlands); 5) 1 μl (200 U) of Moloney murine leukemia virus reverse transcriptase (Life Technologies); and 6) 0.3 μl of H2O. The samples were then incubated at 37°C for 60 min. The real-time PCR was conducted on a Lightcycler apparatus (Roche Diagnostics) with a dual-labeled fluorogenic probe in a 20-μl final volume containing: 1) 1 μl of cDNA; 2) 2 μl of sense and 3 μl of antisense primer (6 pmol/μl each), 3) 2 μl of master hybridization probes reagent (Roche Diagnostics), 4) 5 μl of 25 mM MgCl2, 5) 1 μl of bioluminescent probe (4 pmol/μl), 6) 0.32 μl of TaqStart Ab (Clontech, Palo Alto, CA), and 7) H2O up to 20 μl. After an initial denaturation step at 95°C for 30 s, temperature cycling was initiated. Each cycle consisted in 95°C for 0 s and 60°C for 20 s, the fluorescence being read at the end of this second step (F1/F2 channels). A total of 45 cycles was performed. The oligonucleotide sequences were used for IL-12 p35 and p40, respectively: sense primers, 5'-CTCCTGGGACACCCCTGTGTGAC-3' and 5'-CGGATCTGCGCC-3'; antisense primers, 5'-GGTGAAGGCGATGGAAATCTTCC-3' and 5'-GCCACATGGTCACAGGGA3'; probes were 5'-FAM-6'-FAM CCAAGACCTCTGGCAAT(6FAM)-3', and 5'-6'(FAM) C GGGCCAGGAGCCTACTAGTTGC(6FAM)-3'. The real-time PCR for β-actin was performed in the same way, except for the following reagents: 2 μl of cDNA, 4 μl of 25 mM MgCl2, and a 1-μl

Table I. Phenotype of adult and neonatal DC

<table>
<thead>
<tr>
<th></th>
<th>CD40</th>
<th>CD80</th>
<th>CD86</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult</td>
<td>362</td>
<td>93</td>
<td>141</td>
</tr>
<tr>
<td>Neonatal</td>
<td>164</td>
<td>100</td>
<td>54</td>
</tr>
</tbody>
</table>

* DCs were generated from PBMC from adult donors or CBMC as described in Materials and Methods.

** Cells were analyzed by flow cytometry for the expression of HLA-DR, CD11c, CD40, CD54, CD80, CD86, CD1a, and CD44 molecules.

† Data represent median mean fluorescence intensity (25–75th quantiles) of at least eight independent experiments on different donors. Mean fluorescence intensity of corresponding isotype-matched control mAb was subtracted from each value.

* p < 0.05 as compared to adult DC.
We found out that cells generated in the same conditions in GM-CSF and IL-4 have been characterized as immature DC. The differentiation was similar to that observed with adherent adult PBMC, and cells, and assessed by uptake of [3H]thymidine and culture supernatants were collected for determination of cytokine levels.

Experimental standard curves from serial dilutions of standards. These standards consisted in PCR products that included the IL-12 p35 or p40 amplicon and operating standard curves from serial dilutions of standards. These standards were purified following standard procedures. Primer sequences for [3H]thymidine, the serial dilutions were made from a purified plasmid (ATCC clone 77644).

Table II. Cytokine production by adult and neonatal DC

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>DC*</th>
<th>IL-12(p40)</th>
<th>IL-12(p70)</th>
<th>TNF-α</th>
<th>IL-8</th>
<th>IL-6</th>
<th>IL-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium</td>
<td>Adult</td>
<td>38</td>
<td>&lt;2</td>
<td>&lt;20</td>
<td>623</td>
<td>&lt;20</td>
<td>&lt;10</td>
</tr>
<tr>
<td></td>
<td>Neonatal</td>
<td>17</td>
<td>&lt;2</td>
<td>&lt;20</td>
<td>421</td>
<td>&lt;20</td>
<td>&lt;10</td>
</tr>
<tr>
<td>LPS</td>
<td>Adult</td>
<td>24,959</td>
<td>36</td>
<td>64,003</td>
<td>47,368</td>
<td>4,652</td>
<td>594</td>
</tr>
<tr>
<td></td>
<td>Neonatal</td>
<td>5,258</td>
<td>2.8</td>
<td>22,702</td>
<td>107,467</td>
<td>5,070</td>
<td>609</td>
</tr>
<tr>
<td>CD40L</td>
<td>Adult</td>
<td>25,878</td>
<td>159</td>
<td>6,227</td>
<td>16,623</td>
<td>167</td>
<td>114</td>
</tr>
<tr>
<td></td>
<td>Neonatal</td>
<td>20,154</td>
<td>39</td>
<td>7,673</td>
<td>64,250</td>
<td>332</td>
<td>73</td>
</tr>
<tr>
<td>Poly(EC)</td>
<td>Adult</td>
<td>4,602</td>
<td>336</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Neonatal</td>
<td>11,154</td>
<td>77</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

* DC were generated from adult PBMC or CBMC and cultured either in medium alone or in the presence of LPS (1 μg/ml) for 24 h, CD40L transfectants, or poly(I:C) (20 μg/ml) for 72 h.

Cytokine levels in culture supernatants were assayed by ELISA and expressed in pg/ml.

Data represent median (25–75th quantiles) of at least seven independent experiments on different donors.

β-actin kit containing primers and probe (Applied Biosystems; PE, Norwalk, CT).

IL-12 mRNA (p35 or p40) levels were expressed as the absolute number of copies normalized against β-actin mRNA. This was achieved by generating standard curves from serial dilutions of standards. These standards consisted in PCR products that included the IL-12 p35 or p40 amplicon and that were purified following standard procedures. Primer sequence for IL-12 p35 and p40 standard were, respectively: sense, 5′-AGCCTCCTCTCTTGGTGGCTA-3′ and 5′-GCTGGGAGTACCTGACAC-3′; antisense, 5′-TGTGCTGGTTTATCTTTGTTG-3′ and 5′-TGGTGGTATCCCCGTGTG-3′. For β-actin, the serial dilutions were made from a purified plasmid (ATCC clone 77644).

Threshold cycle values (calculated using the Lightcycler software in “arithmetic fit-point analysis”) were converted to a number of mRNA copies by comparison to the respective standard curve.

DC-T cell cocultures

Responder cells were CD4+ T lymphocytes purified from PBMC of healthy adult donors by a negative selection using a mixture of hapten-conjugated Abs (including anti-CD8, anti-CD11b, anti-CD16, anti-CD19, anti-CD39, and anti-CD56 Abs), MACS microbeads coupled to anti-hapten mAbs, and a MACS column (Miltenyi Biotec, Palo Alto, CA). Purity of CD4+ T cells was >98% as assessed by FACS analysis. Stimulator cells were allogenic DC generated from adherent PBMC or CBMC, washed, and irradiated (3000 rad). Purified CD4+ T cells were then cocultured with DC at a ratio of 10:1. When mentioned, rIL-12 (10 ng/ml) was added at the beginning of the cultures. After 5 days at 37°C, cell proliferation was assessed by uptake of [3H]thymidine and culture supernatants were collected for determination of cytokine levels.

Statistical analysis

Data were compared using unpaired (Mann-Whitney U) or paired (Wilcoxon) nonparametric tests.

Results

Expression of surface markers by DC differentiated from adherent CBMC

In preliminary experiments, we characterized the starting population of plastic-adherent CBMC by flow cytometry. In a typical experiment, these cells consisted of 68% monocytes as indicated by CD14 and CD33 expression, 12% CD3+ T cells, 5% CD19+ B cells, and <1% CD56+ NK cells and CD34+ cells. This composition was similar to that observed with adherent adult PBMC. Plastic-adherent monocytes from adult PBMC cultured for 6 days in GM-CSF and IL-4 have been characterized as immature DC (11). We found out that cells generated in the same conditions from adherent CBMC also expressed surface markers of immature myeloid DC such as CD11c, CD1a, HLA-DR, CD40, CD80, CD86, CD4, and CD54 molecules as determined by flow cytometry (Fig. 1). CD14 and CD83 expression was low or absent on both adult and newborn DC. When compared with adult PBMC-derived DC, the only significant differences were reduced surface expression of HLA-DR, CD80, and CD40 (Table I). The phenotype of DC generated from highly purified cord blood monocytes was similar to that of DC generated from adherent CBMC (data not shown), indicating that the neonatal DC used in ensuing experiments are of monocyte origin as in the case of adult DC derived from PBMC.

Impaired IL-12 production by neonatal DC

In the absence of stimulus, spontaneous production of IL-12(p70), TNF-α, IL-10, and IL-6 was below detection levels both in adult and neonatal DC, whereas their synthesis of IL-8 did not significantly vary. In contrast, spontaneous production of IL-12(p40) was significantly lower in neonatal DC (Table II). In accordance with previous reports, LPS and CD40 ligation strongly stimulate the production of cytokines by DC (14, 15). As shown in Table II, newborn and adult DC produced comparable levels of IL-6, IL-8, TNF-α, and IL-10 in either condition. As far as IL-12(p40) is concerned, levels secreted by neonatal DC were significantly lower upon LPS activation but not upon CD40 ligation (Table II). The most dramatic difference was observed for IL-12(p70) which was produced at much lower levels by neonatal DC than by adult DC under both conditions of stimulation. In an additional experiment using poly(I:C) as DC stimulus, we confirmed that neonatal DC are deficient in the synthesis of IL-12(p70) whereas their production of IL-12 (p40) was similar to that of adult DC in that scheme (Table II).

Allosstimulatory capacity of neonatal DC

To evaluate the consequences of the reduced capacity to produce IL-12, we prepared MLC with DC as stimulators and purified adult CD4+ T cells as responders. First we observed in three independent experiments that neonatal DC were as efficient as adult DC in inducing T cell proliferation (data not shown). However, the profile of cytokines that they elicited was different. As shown in Table III, neonatal DC induced significantly lower levels of IFN-γ and higher IL-10 levels than adult DC. There was a trend toward the

β-actin kit containing primers and probe (Applied Biosystems; PE, Norwalk, CT).
induction of lower IL-2 levels by neonatal DC, although the difference vs adult DC did not reach statistical significance. IL-5 production induced in MLC by neonatal and adult DC was similar.

Since IL-12 is critically involved in the induction of IFN-γ (10), we tested the effects of the addition of rIL-12 in MLC. As shown in Table III, rIL-12 strongly up-regulated the production of IFN-γ in MLC both in adult and neonatal DC. Indeed, the capacity of neonatal DC to induce IFN-γ production was similar to that of adult DC under this condition, indicating that the impaired synthesis of IL-12 by neonatal DC is involved in their reduced ability to elicit IFN-γ production by T cells. The addition of T cells also resulted in increased production of IL-2 and IL-10 in MLC. The trend toward decreased induction of IL-2 and increased induction of IL-10 by neonatal DC was still observed, although it did not reach statistical significance under this condition.

rIFN-γ stimulates IL-12(p70) synthesis by neonatal DC

It has been shown that the synthesis of IL-12 by adult monocyte-derived DC can be primed by IFN-γ (16). As shown in Fig. 2, addition of exogenous rIFN-γ, even at low concentrations, strongly up-regulated LPS-induced IL-12(p70) production by both adult and newborn DC. Indeed, under the LPS + IFN-γ condition, IL-12(p70) production by neonatal DC reached levels similar to that secreted by adult DC. In control experiments, we found out that IFN-γ alone did not induce IL-12(p70) synthesis either in neonatal or in adult DC.

IL-12(p35) gene expression is defective in stimulated neonatal DC

To further consider the molecular basis of the deficient IL-12(p70) synthesis by neonatal DC, RT-PCR analysis was performed to measure IL-12(p40) and IL-12(p35) mRNA levels. As shown in Fig. 3, IL-12(p40) mRNA levels were strongly up-regulated by LPS in adult and newborn DC. In marked contrast, IL-12(p35) mRNA was not up-regulated upon LPS stimulation in neonatal DC so that IL-12 (p35) mRNA levels were significantly lower in LPS-stimulated neonatal DC as compared with adult cells. Similar observations were made when DC were stimulated by poly(I:C). Indeed, under this condition, tested on six different donors. rIFN-γ strongly up-regulated LPS-induced IL-12(p70) production by both adult and neonatal DC. Indeed, under the LPS + IFN-γ condition, IL-12(p70) production by neonatal DC reached levels similar to that secreted by adult DC. In control experiments, we found out that IFN-γ alone did not induce IL-12(p70) synthesis either in neonatal or in adult DC.

Discussion

The production of IL-12 by DC, triggered by their exposure to microbial products or their interaction with activated T cells, is known to play a critical role in the induction of Th1-type responses (10, 17). The present study indicates that the most striking characteristic of neonatal DC derived from cord blood monocytes is their decreased capacity to produce IL-12. Indeed, their synthesis of bioactive IL-12(p70) was significantly impaired in response to bacterial LPS (15), poly(I:C), a synthetic surrogate of viral dsRNA (18), as well as CD40 ligation which mimics a major signal delivered by activated T cells to DC (14).

Bioactive IL-12 is a heterodimeric cytokine composed of two subunits, p35 and p40, encoded by different genes. Both subunits must be expressed in the same cell to generate the bioactive form of the cytokine. Until recently, IL-12 synthesis in monocyte cells was thought to be mainly regulated at the level of p40 gene expression (19). However, the expression of both subunits was shown to be tightly controlled in human monocytes, the induction of the p35 chain being required for their production of bioactive IL-12(p70) (20, 21). Posttranscriptional events appear critical for the control of the IL-12(p40) chain. Indeed, the impaired IL-12(p40) chain synthesis by LPS-stimulated CBMC was previously shown to be related to decreased p40 mRNA stability (22). We also observed a defect of IL-12(p40) synthesis in neonatal DC at least at the basal state and upon LPS stimulation. Our finding of a normal IL-12(p40) gene expression under these conditions is consistent with a predominantly posttranscriptional control.

The defect in IL-12(p70) synthesis by neonatal DC was much more pronounced than that of IL-12(p40). This was observed for the three conditions of DC stimulation that we tested. In the case

| T | Cytokine production in MLC prepared with adult and neonatal DC* |
|---|---|---|---|---|---|
| DC | rIL-12 | IL-2 | IFN-γ | IL-5 | IL-10 |
| Adult (n = 12) | – | 1,358 (789–1,758) | 4,652 (1,391–8,864) | 193 (132–777) | 69 (32–92) |
| Neonatal (n = 12) | – | 84 (478–1,017) | 1,766 (1,103–3,996)** | 32 (242–518) | 126 (87–139)* |
| Adult (n = 5) | + | 1,930 (1,631–2,046) | 60,698 (52,337–71,925) | 141 (85–537) | 79 (48–182) |
| Neonatal (n = 5) | + | 1,46 (1,075–1,944) | 45,884 (36,066–67,021) | 148 (87–390) | 159 (123–495) |
| * MLC were prepared with CD4+ T cells purified from adult PBMC and allogenic DC derived either from adult PBMC or CBMC. After 5 days of culture in the absence or presence of rIL-12 (10 ng/ml), supernatants were collected for determination of cytokine levels expressed in pg/ml. Data are shown as median (25–75th quantiles) of independent experiments on different donors. ** p < 0.05; *** p < 0.01 as compared to adult DC.
of poly(IC), the average levels of IL-12(p40) secreted by neonatal DC were even higher, although not significantly, than those produced by adult DC, indicating that impaired synthesis of the p40 chain is unlikely to be the main mechanism responsible for their deficient IL-12(p70) synthesis.

Indeed, using a real-time PCR technique, we showed a major defect in the expression of the IL-12(p35) gene in neonatal DC whereas the expression of the IL-12(p40) gene was similar to that of adult DC. Regulation of human IL-12(p35) gene expression is poorly characterized but it has been reported that IL-12(p35) transcript can be initiated from different sites (23, 24). A constitutively active CpG-rich promoter region was identified 5' of the TATA box in EBV-transformed lymphoblastoid cells (24). In human monocytes, priming of IL-12(p35) synthesis by IFN-γ was shown to rely on an alternative TATA-dependent promoter. Our observation that rIFN-γ restored the ability of neonatal DC to express IL-12(p35) suggests that this pathway is operative in those cells. There is compelling evidence that DNA methylation has a repressive action on gene expression (reviewed in Ref. 25). As far as the immune system is concerned, the low capacity of thyocytes, cord blood, and naive adult T cells to produce IFN-γ has been correlated with hypermethylation of CpG sites of the IFN-γ gene (9). We plan to explore in the near future the methylation status of CpG sites upstream of the coding sequence of the p35 gene to determine whether a similar phenomenon could be involved, at least partly, in the deficient IL-12 synthesis by neonatal DC.

The results of our MLC experiments strongly suggest that the impaired IL-12 production by neonatal DC could contribute to the deficient production of IFN-γ observed during most T cell responses induced in newborns. Other DC defects, including the decreased HLA-DR, CD80, and CD40 expression observed in the present study, could be involved in the immunological immaturity of neonates. Along this line, Hunt et al. (26) previously demonstrated that unseparated low-density cord blood cells enriched in DC have a decreased capacity to elicit T cell proliferation. Indeed, we are currently extending the analysis of IL-12 synthesis to freshly isolated cord blood DC.

Our observations might be relevant to the increased susceptibility of newborns to intracellular pathogens but also have important implications for the development of new strategies in early life immunization. Indeed, the demonstration that IFN-γ restores the capacity of neonatal DC to produce bioactive IL-12 suggests that either rIFN-γ or adjuvants inducing IFN-γ in an IL-12-independent manner might overcome the inability of the human newborn to develop efficient Th1-type responses.

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References


