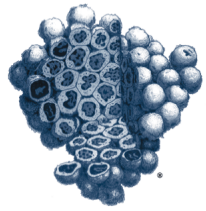

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Selective Generation of Different Dendritic Cell Precursors from CD34+ Cells by Interleukin-6 and Interleukin-3

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Key Words. Interleukin-6 · Interleukin-3 · Myeloid dendritic cell precursors · Plasmacytoid dendritic cells

ABSTRACT

There is a growing interest in generating dendritic cells (DCs) for using as vaccines. Several cytokines, especially stem cell factor (SCF) and FLT3-ligand (FL), have been identified as essential to produce large numbers of myeloid precursors and even to increase DC yield obtained by the action of granulocyte-macrophage colony-stimulating factor (GM-CSF) and tumor necrosis factor alpha (TNF-α). However, there are few studies on the effect of the early-acting cytokines, commonly used to expand CD34+ progenitor cells, on DC generation. We report here that in the absence of serum, SCF, FL, and thrombopoietin (TPO) plus interleukin-6 (IL-6) and SCF, FL, and TPO plus IL-3 were able to generate CD14+CD1a− and CD14−CD1a+ myeloid DC precursors from CD34+ cells, but IL-6 had an inhibitory effect on the generation of CD14−CD1a+ cells. Both DC precursors differentiated into mature DCs by GM-CSF, IL-4, and TNF-α, and DCs obtained from both types of culture exhibited equal allostimulatory capacity. CD1a+ DCs generated could be identified on the basis of DC-specific intracellular adhesion molecule–grabbing nonintegrin (DC-SIGN) expression, a novel C-type lectin receptor expressed on dermal DCs but not on Langerhans cells. In addition, the inclusion of IL-3 to the culture medium induced the appearance of CD13− cells that differentiated into plasmacytoid DC (DC2) on the addition of TNF-α, allowing the identification of developmental stages of DC2. Like true plasmacytoid DCs, these cells secreted interferon-α after TLR9-specific stimulation with a specific CpG nucleotide. ***Stem Cells*** *2004;22:725–740*

INTRODUCTION

Dendritic cells (DCs) represent heterogeneous populations of rare antigen (Ag)-presenting cells that play crucial roles in the elicitation of T cell–dependent immunity. DCs originate in bone marrow, and their precursors migrate via the blood stream to almost all organs of the body, where they capture Ag and present it processed to CD4+ and CD8+ naive T cells, thereby initiating primary cellular immune responses. DCs, in addition to their role in innate immunity, induce and regulate adaptive immune responses [1–5]. The distinct capacity of DCs to induce immunity versus tolerance or Th1 versus Th2 responses depends on their maturation stage [6], signals that induce or inhibit DC maturation [7], and the lineage origin of DCs [8]. Ontogenically, DCs are heterogeneous and derive from lymphoid or myeloid lineages [9]. In humans, a subpopulation of CD34+Lin−CD45RA+ expressing CD10

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gives rise to T, B, and NK cells as well as DCs [10]. These lymphoid progenitors may home to the thymus and lack myeloid cell markers [11, 12]. These cells, also named plasmacytoid T cells, which correspond to the $Lin^-CD4^+CD11c^-$ blood DC precursors, express high levels of α-subunit of interleukin-3 (IL-3) receptor (CD123) [13] and are IL-3–dependent and granulocyte-macrophage colony-stimulating factor (GM-CSF)–independent [14].

In vitro studies with $CD34^+$ progenitor cells have revealed two other subtypes of DCs belonging to myeloid lineage that emerge independently. One pathway involves $CD1a^+CD14^-$ cells that give rise to epidermal Langerhans cells (LCs). The other pathway includes bipotent $CD1a^-CD14^+$ cells that can be induced to differentiate either into monocyte (Mo)-derived or interstitial/dermal DCs and macrophages [15–18].

GM-CSF and tumor necrosis factor alpha (TNF-α) were found to be an efficient cytokine combination for in vitro generation of myeloid DCs from $CD34^+$ progenitor cells [19], but the addition of stem cell factor (SCF) to the culture system led to higher production of DCs by expanding progenitor cells [20] and also colony-forming unit DCs [21]. More recently, FLT3-ligand (FL), another early-acting cytokine that induces the proliferation and survival of primitive hematopoietic progenitor and stem cells and shares similar receptors to SCF, has been demonstrated to enhance the expansion of DC precursors from $CD34^+DR^-$ progenitor cells and to maintain their long-term production [22]. When administered to mice and humans, it expands the number of both myeloid and lymphoid DCs [23, 24]. In most of these in vitro studies reported so far, the culture media used for inducing DC generation from $CD34^+$ cells was supplemented in addition to cytokines with serum or plasma. Strobl et al. [25] demonstrated that the basic cytokine combination of GM-CSF plus TNF-α and SCF in the absence of serum or plasma induced only low percentages and low total yield of $CD1a^+$ cells from $CD34^+$ cord blood cells, but the addition of plasma or transforming growth factor (TGF)-β1 to cytokine cocktail strongly induced cell proliferation and differentiation of $CD1a^+$ DC. TGF-β1 is one of the cytokines present in serum and plasma produced by many cell types that inhibits the expansion of immature $CD34^+CD38^-$ progenitor cells [26] but in the presence of GM-CSF [27] or erythropoietin plus IL-3 and SCF [28] induces the proliferation of lineage-committed progenitor cells. Moreover, the effect of TGF-β1 on DC generation is dependent on the presence of GM-CSF and TNF-α [25]. It has also been reported that TGF-β1 is important for the formation of LCs [29]. In addition, FL cooperates with TGF-β1, GM-CSF, TNF-α, and SCF in the in vitro induction of DC/LC in serum-free culture conditions, being unable to generate $CD1a^+$ cells upon omission of TGF-β1 [30].

The growing interest in the use of DCs as cancer vaccines has obliged many groups of researchers to generate DCs ex vivo, but despite the progress achieved in this field, the results contrast with the extensive amplification of myeloid progenitors obtained from $CD34^+$ progenitor cells with early-acting cytokines with or without GM-CSF. We have previously described that in the presence of SCF, FL, thrombopoietin (TPO), and IL-6 or IL-3 in serum-free culture conditions, it is possible to expand $CD34^+$ cells and colony-forming units, maintaining or even increasing slightly long-term culture-initiating cells [31]. However, the potential of generating DC precursors in this type of culture has not been extensively studied. To date, Arrighi et al. [32], using FL, TPO, and SCF in the presence of serum for some weeks, were able to induce a great expansion of DCs from $CD34^+$ cord blood cells, but taking into account the effects of serum addition to the cultures, it remains to be determined whether early-acting cytokines have substantial effects on DC precursor generation.

The aims of this study were to expand $CD34^+$ cord blood cells in serum-free culture medium with SCF, FL, TPO, and IL-6 or IL-3 for a short period of time to generate hematopoietic progenitor cells, avoiding their maturation, and to study possible differences induced by IL-6 and IL-3 on the generation of myeloid DC precursors. Moreover, we asked whether the presence of IL-3 at the beginning of the culture was able to induce precursors of lymphoid DC. We report here that cytokines used were able to generate $CD1a^+CD14^-$ and $CD1a^-CD14^+$ DC precursors, but their proportions were differently regulated by IL-6 and IL-3. These DC precursors became mature functional DCs. In addition, we have analyzed DC-specific intracellular adhesion molecule–grabbing nonintegrin (DC-SIGN) expression on generated cells and used it to discern the different myeloid DC subsets. Finally, this type of culture allowed us to identify the developmental pathway of functional plasmacytoid DCs.

Materials and Methods

Purification of $CD34^+$ Cord Blood Cells and Monocytes

After informed consent from the mother, umbilical cord blood samples were collected from normal full-term deliveries by standard procedures. Erythrocyte depletion was achieved by centrifugation in the presence of hydroxyethyl starch (GRIFOLS S.A., Barcelona, Spain). Mononuclear cells (MNCs) were obtained by Ficoll-Hypaque (Amersham Biosciences, Uppsala, Sweden) density gradient centrifugation. $CD34^+$ cells were isolated by immunomagnetic cell separation using two columns (MACS system, Miltenyi Biotec, Bergisch Gladbach, Germany) in accordance with the manu-

facturer's recommendations. Mos from cord blood were obtained by plastic adherence for 90 minutes at 37ºC, plating MNCs at approximately 2×10^6 cells/cm^2 in medium composed of Iscove's modified Dulbecco's medium (IMDM; Gibco BRL, Life Technologies, Paisley, U.K.) supplemented with 10% heat-inactivated fetal calf serum (FCS; BioWhittaker, Walkersville, MD), 50 µg/mL gentamicin, and 2.5 µg/mL fungizone (Gibco BRL). Flow cytometric analysis of purified cell fractions was performed using FACSCalibur equipment (Becton, Dickinson, San Jose, CA). Purity of the CD34$^+$ and CD14$^+$ cell populations obtained was greater than 90% and 75%, respectively.

Primary Cultures of CD34$^+$ Cells with Early-Acting Cytokines and Further Induction into Dendritic Cells

Isolated CD34$^+$ cells were cultured in 24-well plates at 4×10^4 cells/ml in serum-free medium composed of X-VIVO 15 medium (BioWhittaker) supplemented with 1% bovine serum albumin, 200 µg/ml transferrin, 40 µg/ml low-density lipoproteins (Sigma, St. Louis), 10 µg/ml insulin, 0.1 mM 2-mercaptoethanol, 50 µg/ml gentamicin, and 2.5 µg/ml fungizone (Gibco BRL). Cultures were carried out in the presence of purified human recombinant cytokine SCF (50 ng/ml), TPO (10 ng/ml), FL (50 ng/ml), and IL-6 or IL-3 (20 ng/ml each), named in the text as STF6 and STF3, respectively. The cultures were incubated at 37ºC in 5% carbon dioxide and 95% air in fully humidified atmosphere for 7 days, and cytokines were replaced every 3 days. Afterward, the expanded cells were harvested and replated at 5×10^5 cells/ml per well and cultured for 7 days in the presence of SCF and FL (50 ng/ml each) plus GM-CSF and IL-4 (100 ng/ml each). TNF-α (20 ng/ml) was added to the culture for the last 48 hours to induce terminal maturation of DCs. In some experiments, cells derived from CD34$^+$ cells incubated with STF3 combination were incubated with SCF, FL, IL-3, and TNF-α. Flow cytometric analyses were performed after CD34$^+$ cell expansion and before and after addition of TNF-α. All of the cytokines used were purchased from R and D Systems (Minneapolis). For each growth condition, cell expansion was calculated by dividing the number of viable cells obtained at the end of the culture by the number of viable plated initial cells. Viability was assessed by trypan blue exclusion.

Generation of Monocyte-Derived Dendritic Cells

Mos were cultured in 24-well plates at 5×10^5 cells/ml for 7 days in IMDM supplemented with 10% heat-inactivated FCS, 50 µg/ml gentamicin, and 2.5 µg/ml fungizone and GM-CSF plus IL-4 (100 ng/ml each). TNF-α (20 ng/ml) was added to the culture on the fifth day to induce terminal maturation of DCs. Flow cytometric analyses were performed before and after addition of TNF-α.

Cytology Analysis

Cells were cytocentrifuged onto slides (1×10^5 cells per slide), fixed, stained for 15 minutes with May-Grünwald Giemsa solution (Merck, Darmstadt, Germany), and rinsed with distilled water. Samples were examined under a Nikon E400 microscopy. Staining was performed on freshly isolated CD34$^+$ cells, as well as on immature and mature derived DCs.

Flow Cytometry Analysis

Monoclonal antibodies (mAbs) directly conjugated either to fluorescein isothiocyanate (FITC), phycoerythrin (PE), allophycocyanin (APC), or peridinin chlorophyll protein (PerCP) were used in this study for cell-surface staining. FITC-labeled anti-CD7 and anti-Lineage cocktail, including anti-CD3, anti-CD19, anti-CD20, anti-CD16, anti-CD56, and anti-CD14; PE-labeled anti-CD80 and anti-CD123; APC-labeled anti-CD86 and anti-CD11c; and PerCP-labeled anti–HLA-DR were purchased from Becton, Dickinson. FITC-labeled anti-CD45, anti-DC-SIGN, and anti-CD14; PE-labeled anti-CD34, anti-CD14, anti-CD40, anti-CD1a, anti-CD13, and anti-CD45RA; and APC-labeled anti-CD34, anti-CD83, and anti-CD4 were from Pharmingen (San Diego). Flow cytometry was performed on a FACSCalibur flow cytometer (Becton, Dickinson) equipped with two lasers using the CellQuest software (Becton, Dickinson). The cells were labeled according to standard protocols. For discarding nonviable cells in the analysis, 7-amino-actinomycin D was used. Matched labeled isotypes were used as controls.

FITC-Dextran Assay

To evaluate the capacity for uptake of soluble Ags from the culture medium, cells were incubated with 1 mg/ml FITC-dextran (Molecular Probes, Eugene, OR) at 37°C or at 4°C (internalization control) for 1 hour. Uptake was stopped by adding ice-cold phosphate-buffered saline containing 1% FCS, followed by four washes in a refrigerated centrifuge. Cells were then analyzed by flow cytometry.

Mixed Leukocyte Reaction

To test their allogeneic stimulatory activity, CD34$^+$-derived cells were preinactivated with 50 mg/ml mitomycin-C and tested as stimulators in mixed leukocyte reaction (MLR). Cells were resuspended in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated FCS, 50 µg/ml gentamicin, and 2.5 µg/ml fungizone and seeded at graded doses in round-bottom 96-well plates. Peripheral

blood MNCs of healthy donor adults (>80% purity of $CD3^+$ cells) were used as responder cells. Allogenic $CD3^+$ T cells 1×10^5 were added to each well, and the coculture was maintained for 6–7 days at 37°C in a 5% carbon dioxide humidified atmosphere. Cells were pulsed with 1 μM 5'-bromo-2'deoxy-uridine (BrdU) for the last 24 hours, and BrdU incorporation into DNA was determined using the colorimetric immunoassay, Cell Proliferation ELISA, BrdU (Roche Molecular Biochemicals, Mannheim, Germany) according to the manufacturer's instructions.

Quantification of Interferon-α Production

Cells derived from STF3-treated cultures and subsequently exposed to TNF-α were plated at 5×10^4 cells/200 μl in flat-bottom 96-well culture plates in the presence of the oligodeoxynucleotide (ODN) in its phosphorothioate form: CpG-ODN 2006, 5'-TCGTCGTTTTGTCGTTTTGTCGTT-3' [33]. ODN was provided by TIB MOLBIOL (Roche Diagnostics S.L.) and used at 2 μM. Cell-free supernatants were collected after 24 and 48 hours and tested for their alpha-interferon (IFN-α) contents by ELISA (IFN-α Human Biotrak ELISA System, Amersham Biosciences).

Statistical Analysis

Results are expressed as the mean ± standard error. The significance of differences between mean values was determined using the Student's *t*-test for paired samples. The statistical analysis was performed with Excel Software (Microsoft, Redmond, WA). Differences were considered significant if $p < .05$.

Results

$CD34^+$ Cord Blood Cells Differentiate into $CD1a^+$ and $CD14^+$ Cells with Early-Acting Cytokines

$CD34^+$ cord blood cells were grown in serum-free medium in the presence of SCF, FL, and TPO containing IL-6 (STF6) or IL-3 (STF3). After 7 days of culture, cells were expanded 12.7 ± 3.9-fold in the presence of IL-6 relative to 25.1 ± 5.8-fold, when IL-3 was added. Both culture conditions led to occurrence of nonadherent round cells (Figs. 1A and 1D). Both cultures were used as source of DCs and are referred in the text as primary cultures. Cell suspensions derived from both primary cultures were 80%–90% $HLA\text{-}DR^+$ and 70%–75% $CD33^+$, but, surprisingly, whereas all cells derived from STF6 treatment were $CD13^+$, approximately 20%–25% of cells from STF3 primary cultures did not express this Ag. $CD34^+$ cells remaining in the culture constituted 36.5 ± 9.8% and 15.5 ± 3.5% of the total cells for STF6 and STF3 primary cultures, respectively. Both treatments used in this study to expand hematopoietic progenitors induced the generation of the myeloid DC precursors $CD34^-CD1a^+$ and $CD34^-CD14^+$ (Fig. 2A). Because CD11c is a marker of myeloid DCs, we determined its expression in primary cultures. As can be seen in Table 1, 10% of the cells obtained from STF6 primary cultures expressed CD11c and approximately 20% when IL-3 was present, this difference being proportional to the different expansion capacity induced by these cytokines. A small percentage of expanded cells were $CD1a^+$, and again the presence of IL-3 induced a greater proportion of this cell subset (Table 1). However, despite the lesser increase in the total cell number produced by the cytokine combination, including IL-6, the percentage of $CD14^+$ cells was greater than that observed for IL-3–containing cultures (Table 1).

To assess whether the differential effect on the generation of DC precursors could be attributable to a specific effect of IL-6 or IL-3, we cultured $CD34^+$ cells with SCF, FL, and TPO and observed that the percentage of $CD1a^+$ and $CD14^+$ cells were 6.3 ± 2.1% and 4.9 ± 1.5%, respectively. Considering that total cell expansion induced by SCF, FL, and TPO was 1.6 ± 0.8-fold increased by addition of IL-6, it can be assumed that IL-6 induced a decrease in the proportion of $CD1a^+$ cells and an increase in that of $CD14^+$ cells similar in absolute numbers to fold expansion induced. However, when IL-3 was added, percentage of $CD1a^+$ cells increased in parallel with cell expansion, but the proportion of $CD14^+$ cells was not significantly affected; therefore, IL-3 prevented the generation of $CD14^+$ cells and IL-6 prevented the generation of $CD1a^+$ cells induced by SCF, FL, and TPO. Nevertheless,

Table 1. Frequency of cell-surface markers induced after ex vivo expansion of cord blood $CD34^+$ cells

Cytokines	$CD11c^+$	$CD1a^+$	$CD14^+$	$CD14^+CD1a^+$	DC-$SIGN^+$
STF6	9.7 ± 2.0	4.1 ± 2.4	7.3 ± 1.2	1.0 ± 0.5	2.1 ± 1.6
STF3	18.5 ± 5.0[a]	11.6 ± 5.7[b]	4.2 ± 1.7[a]	2.1 ± 1.3[b]	2.7 ± 0.9

Cells were cultured with STF3 or STF6 for 7 days. Results are expressed as means ± standard error and represent the mean percentage of 5 to 10 independent experiments. STF3: SCF, FL, TPO, and IL-3; STF6: SCF, FL, TPO, and IL-6. STF6 versus STF3: [a]$p < .005$; [b]$p < .05$.
Abbreviations: DC, dendritic cell; FL, FLT3-ligand; IL, interleukin; SCF, stem cell factor; TPO, thrombopoietin.

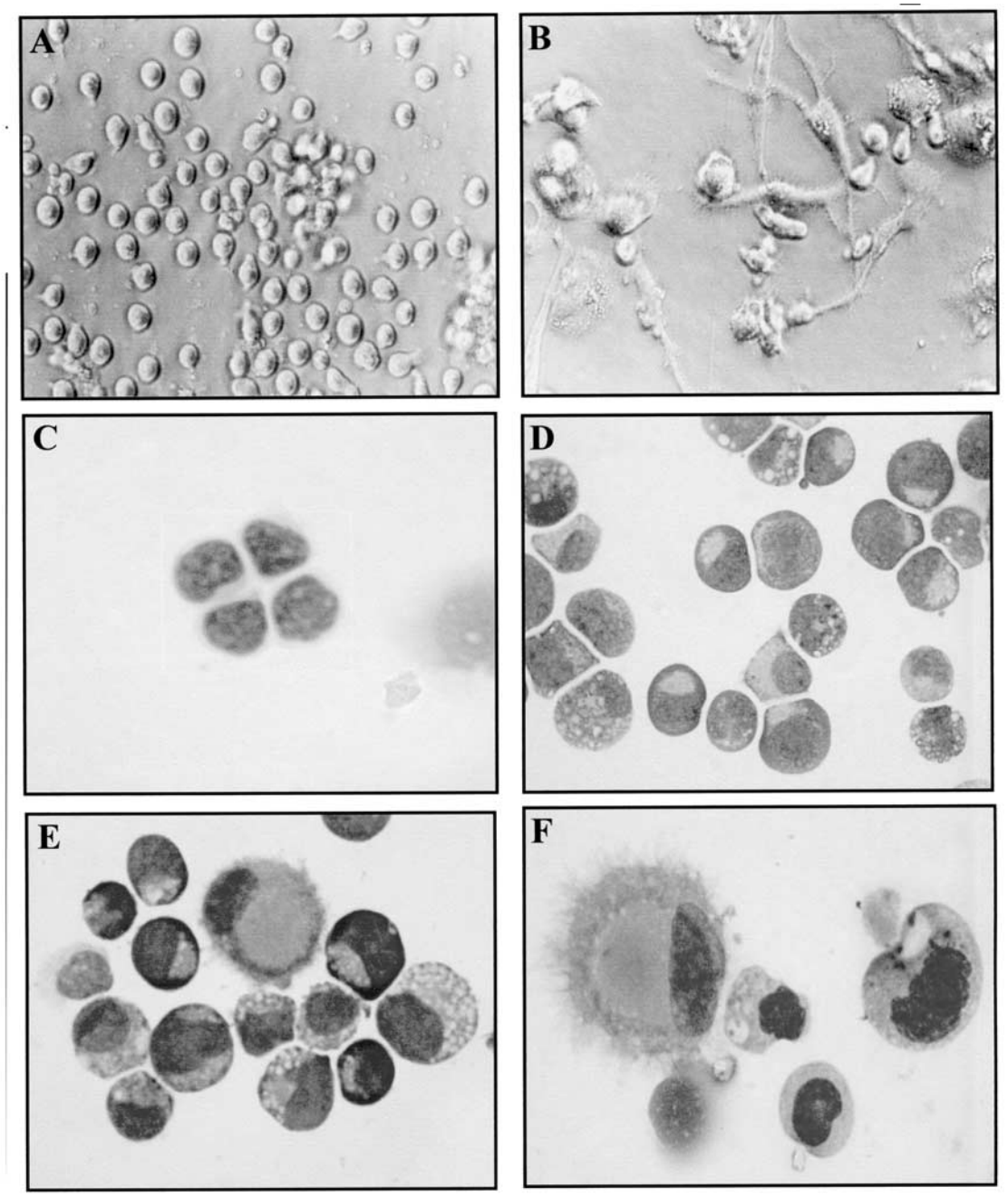

Figure 1. Morphological changes of CD34$^+$ cells induced to differentiate into DCs. Light microscopy of CD34$^+$ cells cultured with stem cell factor, FLT3-ligand, thrombopoietin, and IL-6 (STF6) for 7 days **(A)** subsequently switched to GM-CSF plus IL-4 until day 14 and induced the DC maturation by adding TNF-α during the last 2 days **(B)**. Original magnification × 40. May-Grünwald Giemsa staining of freshly isolated CD34$^+$ cells **(C)**, cultured with STF6 for 7 days **(D)**, cultured with GM-CSF plus IL-4 the following 5 days **(E)**, and cultured 2 days more with TNF-α **(F)**. Original magnification × 50. Abbreviations: DC, dendritic cell; IL, interleukin; TNF, tumor necrosis factor.

this cytokine combination was not additionally used in this study, because it had a low capacity to expand hematopoietic progenitors.

Among CD14$^+$ cells, those expressing CD1a represented 14.0 ± 5.3% for STF6 primary cultures but 50.1 ± 15.6% for STF3 cultures; therefore, the proportion of the intermediate DC precursor, CD14$^+$ CD1a$^+$, obtained in these two primary cultures was also differentially regulated by cytokines (Table 1, Fig. 2B). We assessed the presence of CD11c Ag on the different DC subsets obtained from both type of cultures. Approximately 40% of CD1a$^+$ cells were also CD11c$^+$ in STF6-derived cells and 50% in STF3 cultures; however,

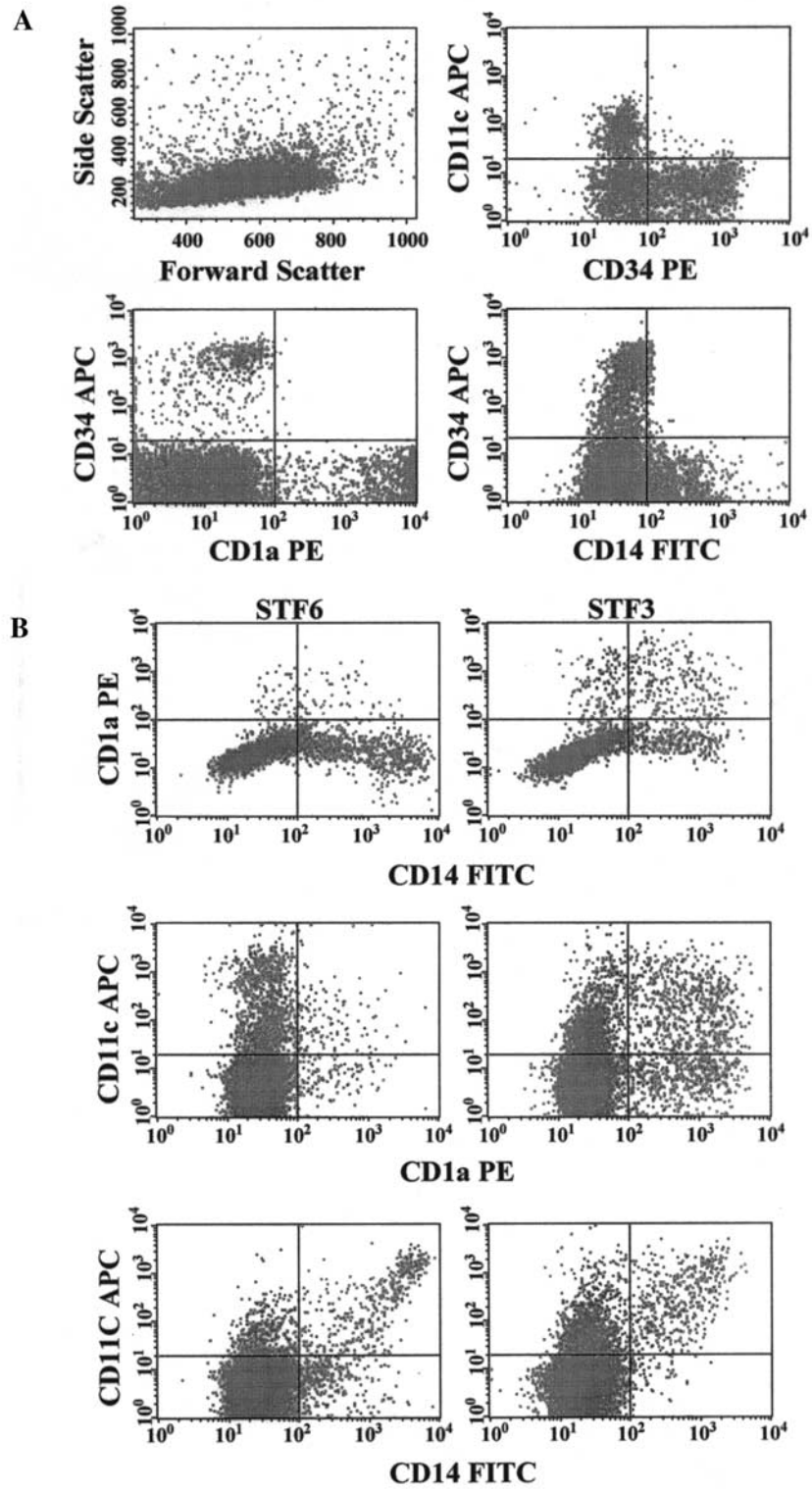

Figure 2. Phenotypic development of CD34+ cord blood cells expanded with early-acting cytokines. CD34+ cells from cord blood were cultured with stem cell factor, FLT3-ligand, thrombopoietin, and IL-6 (STF6) or IL-3 (STF3) as described in Materials and Methods. On day 7 of culture, a representative sample of the cultures was stained with anti-CD14, anti-CD1a, anti-CD34, or anti-CD11c. One representative experiment showing forward scatter and side scatter profile of cultured cells as well as the lack of expression of CD11c, CD14, and CD1a within CD34+ cells derived from (STF3) primary cultures **(A)** and myeloid dendritic cell precursors obtained from both primary cultures **(B)** is given. Abbreviations: APC, allophycocyanin; FITC, fluorescein isothiocyanate; IL, interleukin; PE, phycoerythrin.

most CD14+ cells expressed CD11c, reaching values of 71% and 81% for STF6 or STF3 primary cultures, respectively (Fig. 2B). Moreover, CD14+ CD1a+ cells were CD11c+, thus implying a different time acquisition of CD11c Ag by DC precursors.

It has been described that DC-SIGN is a molecule present in some immature DC subsets; therefore, we wondered whether DC precursors obtained could express it. In fact, as shown in Table 1, a very low percentage of cells were DC-SIGN+, and cytometric analysis demonstrated that 58 ± 9% and 86 ± 9% of the DC-SIGN+ cells expressed CD11c in STF6 and STF3 primary cultures, respectively. In addition, 20%–27% of CD1a+ cells expressed DC-SIGN+, irrespective of the primary culture.

Myeloid DC Precursors Become DCs with GM-CSF Plus IL-4

Cells derived from primary cultures were harvested, washed, and resuspended in serum-free medium containing GM-CSF, IL-4, SCF, and FL to induce generation of immature DCs. After 5 days of culture, twofold cell expansion was obtained with 75% viability, determined by trypan blue exclusion, and only 2% of total cells expressed CD34 in both primary cultures. Most cells became elongated, and veiled cells were also observed. When TNF-α was added to maturate DCs, cell viability was similar, no CD34+ cells were detected, and the morphological changes induced on cultured cells were more noticeable (Fig. 1B). Giemsa staining revealed that after exposure to GM-CSF plus IL-4, approximately 20% of cells increased their size with a large cytoplasm, some of them containing granules and dendritic prolongations (Fig. 1E). The nucleus was eccentric, indented, and sometimes double. After incubation with TNF-α, these effects were more remarkable (Fig. 1F). Based on cytometric analysis of cell size and granularity, two cell populations were observed: R1, containing cells with a low side scatter and resembling primary cultures; and R2, including cells with a high scatter profile and a high expression of HLA-DR, similar to DCs under cytometric criteria (Fig. 3). Percentages of total viable cells gated on R2 after incubation with GM-CSF plus IL-4 were 22 ± 8% and 15 ± 10% for STF6- or STF3-derived cells, respectively. After exposure to TNF-α, this gate included 27 ± 10% and 15 ± 6% of STF6- or STF3-derived cells, respectively. Therefore, phenotypic analysis was performed separately on gates R1 and R2. As shown in Table 2, most cells belonging to R2 expressed CD11c Ag, and approximately 40% of cells were CD1a+, but approximately 15% of CD1a+ cells did not display CD11c yet. Although cell cultures contained IL-4 during this period of time, down modulation of CD14 was not complete, and in fact 12% of cells gated on R2 were CD14+. Of that 12%, 52% of cells derived from STF6 expressed CD1a and 63% of cells derived from STF3 expressed CD1a.

The percentage of DC-SIGN+ cells was similar to that of CD1a+ cells. Based on DC-SIGN expression, two different CD1a+ cell subsets were revealed, CD1a+DC-SIGN− and CD1a+DC-SIGN+, that represented 55% of CD1a+ cells, irrespective of the type of primary culture. In addition, 17%–28% of cells gated on R2 displayed the molecules of costimulation, CD86, CD80, and CD40, and 8%–12% of cells also were CD83+.

Maturation of DCs induced by TNF-α was accompanied by a significant decrease in the percentage of CD14+ cells, which only comprised 4% of cells gated on R2. The analysis of coexpression of DC-SIGN and CD1a confirmed again the presence of two distinct myeloid DC subsets. Thus, only 60% of CD1a+ cells were also DC-SIGN+. Therefore, TNF-α did not alter this selective Ag distribution. As expected, after 2 days of incubation with TNF-α, an overexpression of costimulatory molecules as well as an increase in the content of cells positive for these molecules was observed (Table 2, Fig. 3). However, it is noteworthy that whereas percentages of CD40+ and CD83+ cells were similar in both derived cell cultures, a significant increase in CD80+ and CD86+ cells was observed in STF6 with respect to STF3-derived cells. Additionally, FACS analysis indicated that CD83 was induced on DC-SIGN+ and CD1a+ myeloid DCs. Thus, averaged numbers demonstrated that, irrespective of primary cultures, the proportions of DC-SIGN+ and CD1a+ cells expressing CD83 increased after addition of TNF-α from 13% and 15% to 45% and 75%, respectively.

In contrast to R2, only 20%–30% of the cells gated on R1 exhibited a high expression of HLA-DR, and 12.5% or 16.6% of cells derived from STF6 or STF3 primary cultures expressed CD11c, respectively, a very similar percentage to that observed after 7 days of CD34+ cell expansion. After incubation with GM-CSF and IL-4, the proportions of CD14+ and CD1a+ cells were considerably lower than those present in R2 (Table 2). To assess whether the presence of factors generating immature DCs induced the expression of CD1a on CD14+ cells, as happened with cells gated on R2, we analyzed the coexpression of these two Ags. Only 23% and 37% of CD14+ cells derived from STF6 or STF3 primary cultures, respectively, expressed CD1a. Percentage of DC-SIGN+ cells gated on R1 was slightly increased, but not statistically significant, compared with primary cultures (Table 2). Within the CD1a+ cell subset, 44% and 71% of cells derived from STF6 primary cultures were DC-SIGN+ and CD11c+, respectively, whereas 69% and 59% of cells derived from STF3 primary cultures expressed DC-SIGN and CD11c, respectively. Therefore, this coexpression was similar in both gates R1 and R2. The addition of TNF-α also

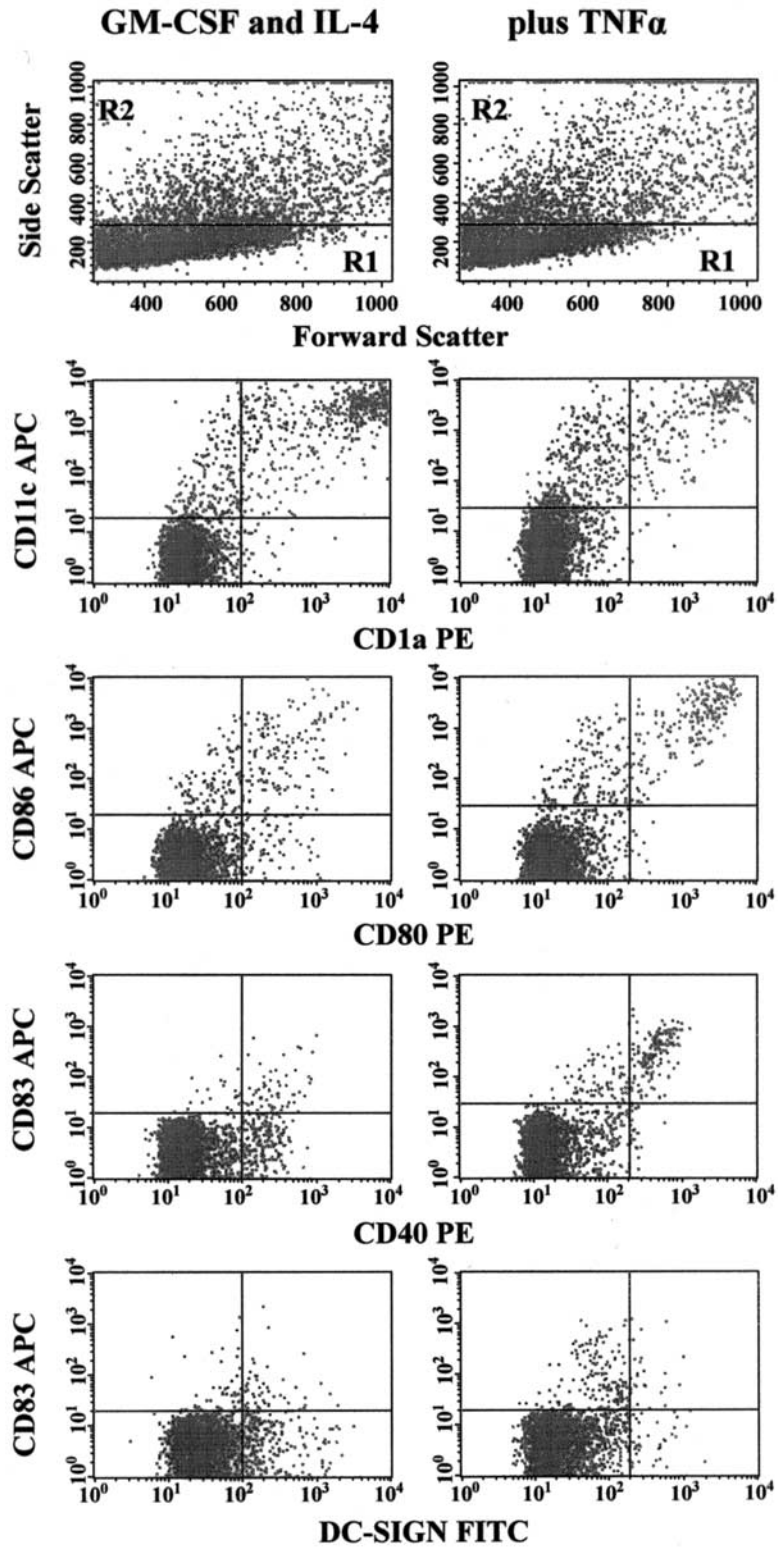

Figure 3. Phenotypic evolution of cells derived from primary cultures incubated with GM-CSF plus IL-4 and with TNF-α. Cells derived from STF3 or STF6 primary cultures were induced to differentiate into DCs by incubation for 5 days with GM-CSF and IL-4 and 2 days more with TNF-α. Forward scatter versus side scatter of cultured cells at both stages of the culture showing regions R1 and R2 is given. Phenotypic analysis from a representative experiment showing different cell-surface molecules at both stages of DC maturation from a sample of primary cultures is shown. Abbreviations: APC, allophycocyanin; DC, dendritic cell; FITC, fluorescein isothiocyanate; IL, interleukin; PE, phycoerythrin; TNF-α, tumor necrosis factor alpha.

Table 2. Phenotypic analysis of R1 and R2 cell populations induced by GM-CSF plus interleukin-4 (IL-4) and matured with tumor necrosis factor alpha (TNF-α)

	STF6		STF3	
	R1	**R2**	**R1**	**R2**
GM-CSF + IL-4				
CD11c	12.5 ± 4.7	66.0 ± 11.2	16.6 ± 10.2	61.8 ± 19.5
CD1a	4.6 ± 2.9	45.2 ± 20.6	2.5 ± 1.4	36.0 ± 14.8
CD14	5.2 ± 2.8	12.3 ± 4.0	3.0 ± 1.7[a]	12.4 ± 1.6
DC-SIGN	5.9 ± 2.6	44.3 ± 11.3	3.9 ± 1.9	29.7 ± 9.8
CD86	2.4 ± 1.3	26.0 ± 5.1	5.7 ± 4.3	16.7 ± 8.1
CD80	3.2 ± 1.3	28.4 ± 7.5	1.6 ± 0.9	17.0 ± 8.4
CD40	2.0 ± 1.3	20.6 ± 6.2	0.8 ± 0.6	16.5 ± 9.3
CD83	1.1 ± 0.7	11.8 ± 3.6	0.7 ± 0.3	7.6 ± 2.4
TNF-α				
CD11c	14.2 ± 5.7	68.0 ± 7.3	18.3 ± 7.9	66.3 ± 15.2
CD1a	4.5 ± 2.9	35.9 ± 23.5	2.6 ± 0.9	30.7 ± 6.9
CD14	1.1 ± 0.8[b]	4.3 ± 0.4	0.6 ± 0.2[b]	4.5 ± 0.1
DC-SIGN	7.8 ± 4.5	32.6 ± 8.2	5.9 ± 3.2	27.6 ± 7.2
CD86	5.2 ± 0.1[b]	44.8 ± 10.2[b]	7.0 ± 2.4	20.2 ± 8.9[a,b]
CD80	7.1 ± 0.7[b]	45.2 ± 9.7[b]	2.3 ± 1.4[a]	20.4 ± 5.1[a]
CD40	6.1 ± 2.1[b]	32.8 ± 11.7[b]	3.5 ± 1.4[a,b]	29.7 ± 12.1[b]
CD83	3.8 ± 1.2[b]	29.6 ± 12.7[b]	2.2 ± 1.2[a,b]	25.7 ± 11.9[b]

Cells were cultured with STF3 or STF6 for 7 days before induction of immature DCs with GM-CSF and IL-4 for 5 days. Maturation was induced by addition of TNF-α. R1 and R2 correspond to gates depicted in Figure 3. Frequency of cells expressing the selected antigen is given. Values are the mean percentage ± standard error corresponding to four to six independent experiments. STF3: SCF, FL, TPO, and IL-3; STF6: SCF, FL, TPO, IL-6.

[a] $p < .05$ for STF6 versus STF3.

[b] $p < .05$ for GM-CSF+ IL-4 versus TNF-α.

Abbreviations: DC, dendritic cell; FL, FLT3-ligand; SCF, stem cell factor; TPO, thrombopoietin.

induced a significant increase in percentage of cells expressing costimulatory molecules, which was more remarkable in STF6-derived primary cultures, and a significant decrease in the number of $CD14^+$ cells (Table 2). Taken together, these results indicate that a continuous generation of DCs is taking place; thus we observed DCs at different stages of maturation.

Functional Assays of Myeloid DCs

DCs derived from $CD34^+$ cells were compared with Mo-derived DCs for their capacity for endocytosis examined by uptake of FITC-dextran. Expanded cells generated in the presence of early-acting cytokines were incapable of internalizing FITC-dextran. A slight uptake in GM-CSF plus IL-4–derived cultures was observed, with most of the cells responsible for this moderate uptake belonging to R1 (Fig. 4). Averaged numbers indicated that within R1, 21.7 ± 6.6% and 21.3 ± 1.6% of cells derived from STF3 or STF6 cultures, respectively, internalized FITC-dextran, compared with only 4%–8% of cells corresponding to R2, and these percentages were not modified by incubation with TNF-α (Fig. 4). The low number of cells gated on R2 capable of taking up dextran probably indicated a great proportion of DC in a high maturation stage. In contrast, freshly Mo- and immature Mo-derived DCs showed a high level of FITC-dextran uptake, and, as expected, after exposure to TNF-α for 2 days, mature Mo-derived DCs diminished their ability to internalize FITC-dextran (Fig. 4).

A comparative assessment of the MLR-stimulating potency of both $CD34^+$-derived cultures is shown in Figure 5A. Neither STF3- nor STF6-treated cells were capable of stimulating allogenic lymphocytes from adult peripheral blood (data not shown). When cells derived from primary

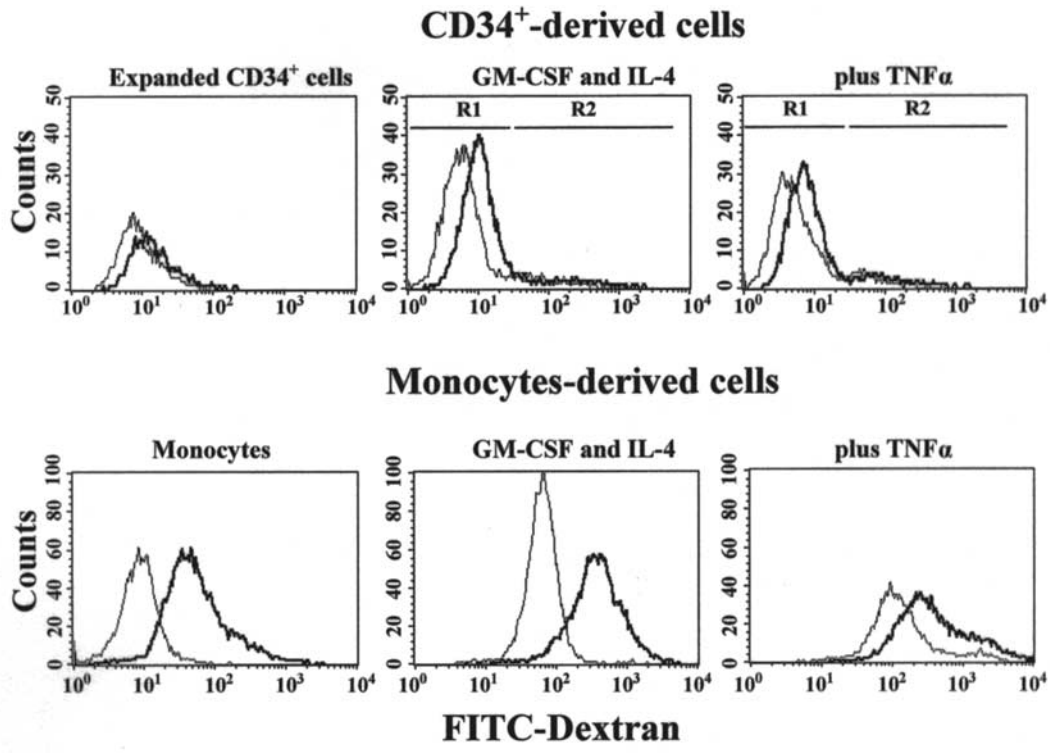

Figure 4. Differential uptake of FITC-dextran by CD34+- and monocyte-derived cells induced to differentiate into myeloid dendritic cells. Endocytosis assays were performed as described in Materials and Methods. CD34+ cord blood cells were incubated with early-acting cytokines for 7 days and additionally cultured with GM-CSF and IL-4 for 7 days, the last 2 days with TNF-α. R1 and R2 correspond to the gates in Figure 3. Monocytes were from cord blood, freshly isolated and cultured for 5 days with GM-CSF plus IL-4 and 2 days more with TNF-α. Thin lines in histograms are negative controls incubated at 4ºC for 1 hour with FITC-dextran. A representative experiment from each type of culture is shown. Abbreviations: FITC, fluorescein isothiocyanate; IL, interleukin; TNF-α, tumor necrosis factor alpha.

cultures were incubated with GM-CSF plus IL-4, a slight allostimulatory capacity was observed, increasing after exposure to TNF-α, thus indicating that mature DCs were obtained. As can be seen in Figure 5A, cells derived from STF6 treatment were more potent stimulators, but not statistically significant, than those derived from primary cultures incubated with STF3 at any cell dose tested, probably because of the higher percentage of cells expressing costimulatory molecules. Because mature DCs are responsible for lymphocyte proliferation and are characterized by the presence of CD83 molecule, we correlated cell proliferation with the cell number expressing CD83 present in the cultures incubated with TNF-α. Independently of early-acting cytokines used to expand CD34+ cells, a very good correlation (R2 = 0.92) between these two parameters was obtained, as observed in Figure 5B, demonstrating that the allostimulatory capacity is closely related to CD83+ cell content.

Primary Cultures Derived from STF3 Also Contain Plasmacytoid DC Precursors

The presence of CD13− cells within STF3 primary cultures led us to consider the possibility that these cultures could also generate precursors of plasmacytoid DCs (pro-DC2). To study this, we carried out the isolation of CD34+ cells from two cord blood units and first characterized phenotypically CD34+ cells. We found that 91 ± 5% of CD34+ cells expressed CD13, 92 ± 3% were HLA-DR+, 33 ± 11% were CD45RA+, 59 ± 7% were CD123+, and 9 ± 5% were CD7+, whereas less than 0.2% were CD1a+ and expression of CD4 was not detected. According to CD45RA and CD7 expression, CD45RA+CD7+, CD45RA−CD7+, and CD45RA+CD7− cells represented 4.5 ± 2.6%, 1.5 ± 0.4%, and 27.7 ± 8.2% of CD34+ cells, respectively. After 7 days of culture with STF3 combination, we examined the presence of pro-DC2 by staining cells with a lineage cocktail of mAb against CD3, CD19, CD20, CD16, CD56, and CD14 and analyzed the expression of selected Ag on Lin−HLA-DR+ cell fraction. In the primary cultures, 20 ± 6% of cells were CD13−, from which 92 ± 2% lacked CD11c expression; 13 ± 1% of cells were CD45RA+; 5 ± 2% were CD4+; 29 ± 5% were CD34+; and 34 ± 5% were CD123+, from which 63 ± 1% were CD11c−. CD7+ cells were not detected. Taking into account that pro-DC2 develops from a CD34+CD45RA+ late progenitor, we performed extensive phenotypic evaluation on

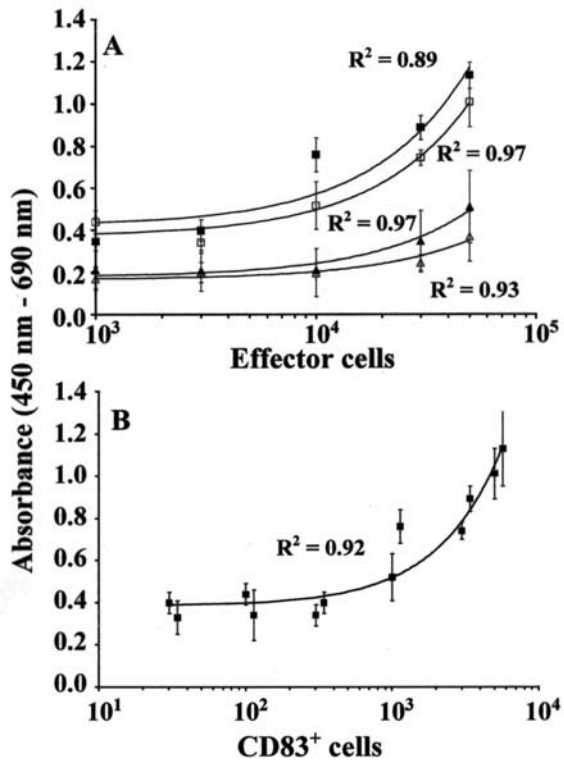

Figure 5. Stimulatory capacity of myeloid DCs derived from $CD34^+$ cells. **(A)**: $CD34^+$ cells from cord blood were expanded in the presence of FLT3-ligand, thrombopoietin, stem cell factor plus IL-3 (STF3), or IL-6 (STF6) and induced to generate immature or mature DCs by addition of GM-CSF plus IL-4 and TNF-α, as described in Materials and Methods. A total of 1×10^5 allogenic $CD3^+$ T cells from adult peripheral blood were incubated with graded doses of $CD34^+$-derived cells generated in culture. Results are presented as mean ± standard error of duplicate samples corresponding to three independent experiments. Cells derived from STF3 cultures (△) or STF6 (▲) were incubated with GM-CSF plus IL-4 for 5 days; cells derived from STF3 cultures (□) or STF6 (■) were incubated with GM-CSF plus IL-4 for 7 days, the last 2 days with TNF-α. **(B)**: 5'-bromo-2'deoxyuridine incorporation is represented versus $CD83^+$ cells present in primary cultures cultured with GM-CSF, IL-4, and TNF-α, independently of the combination of early-acting cytokines used to expand $CD34^+$ cells. Absorbance values correspond to those of **(A)**. Abbreviations: DC, dendritic cell; IL, interleukin; TNF-α, tumor necrosis factor alpha.

$CD45RA^+$ cells. Therefore, 87 ± 1% lacked CD11c, 91 ± 8% were $CD33^+$, 73 ± 2% expressed CD34, and 7 ± 4% were $CD4^+$. Regarding $CD34^+$ and $CD4^+$ cells, 21 ± 3% and 17 ± 2% were $CD45RA^+$, respectively.

IL-3 with TNF-α Promotes the Differentiation into Plasmacytoid DCs

It has been reported that plasmacytoid cells (DC2) die rapidly by apoptosis unless IL-3 has been added; therefore, cells derived from primary cultures were induced to differentiate into DC2 by incubation with IL-3 plus TNF-α, maintaining SCF and FL. Three and 6 days after addition of these cytokines, viability of cells, determined by trypan blue exclusion, was 94% and 91%, respectively, and cells were expanded three and four more times. This cytokine combination induced morphological changes in cultured cells, with the appearance of clusters of cells with dendritic prolongations as well as veiled cells that were more abundant after 6 days (Figs. 6A and 6B). Giemsa staining results showed that DC2 derived from STF3 primary cultures exhibited typical plasmacytoid morphology (Fig. 6C), displaying most cell pseudopods and veiled morphology after 6 days with IL-3 plus TNF-α (Fig. 6D).

After 3 days with IL-3 plus TNF-α, 14 ± 4% of cells expressed $CD4^+$, 17 ± 2% were $CD45RA^+$, and the remaining $CD34^+$ cells constituted 15 ± 5% of the total cells. Three days later, $CD34^+$ cells represented less than 3% of cells, and the percentages of $CD4^+$ and $CD45RA^+$ reached 30 ± 2% and 22 ± 2% of cells, but the proportion of cells coexpressing these two Ags constituted 10% of bulk cells. It is assumed that within $Lin^-HLA\text{-}DR^+$ cells, those with a high expression of CD123 and lacking CD11c are DC2. In our culture conditions, $CD123^+$ cells constituted 30%–35% of cells, from which approximately 40% and 21% did not express CD11c at the third and sixth day after addition of TNF-α, respectively, but the remaining cells exhibited a low expression of CD11c. FACS analysis revealed that only a small proportion of cells expressed CD86 (10 ± 2%), CD40 (3 ± 1%), and CD83 (3 ± 1%), unlike the proportions observed when cells derived from primary cultures were incubated with GM-CSF, IL-4, and TNF-α.

To provide support for the notion that hematopoietic late progenitor cells could give rise to pro-DC2, precursors of DC2 (pre-DC2), and finally DC2, we analyzed by FACS the presence of $CD34^+CD45RA^+$, $CD34^+CD4^+$, and $CD34^+CD123^+$ within Lin^- $HLA\text{-}DR^+$ cell subset. After 3 days with TNF-α, 31 ± 10%, 67 ± 5%, and 63 ± 11% of $CD34^+$ cells remaining in the culture expressed CD4, CD45RA, and CD123, respectively (Fig. 7), thus demonstrating the presence of cells at different stages of DC2 development.

Functional Properties of Plasmacytoid DCs

To investigate the function of DC2 as a stimulator of allogenic $CD3^+$ T cells, its ability to stimulate an allogenic MLR was assessed. As it is shown in Figure 8A, after 6 days in the presence of TNF-α, allostimulatory capacity was slightly increased. Nevertheless, DC2 generated in culture was found to be a less-potent stimulator of allogenic MLR than myeloid DCs. Plasmacytoid DCs are a major source of type I IFN upon exposure to virus and bacteria. It has been demonstrated that oligodeoxynucleotides containing CpG motifs (CpG

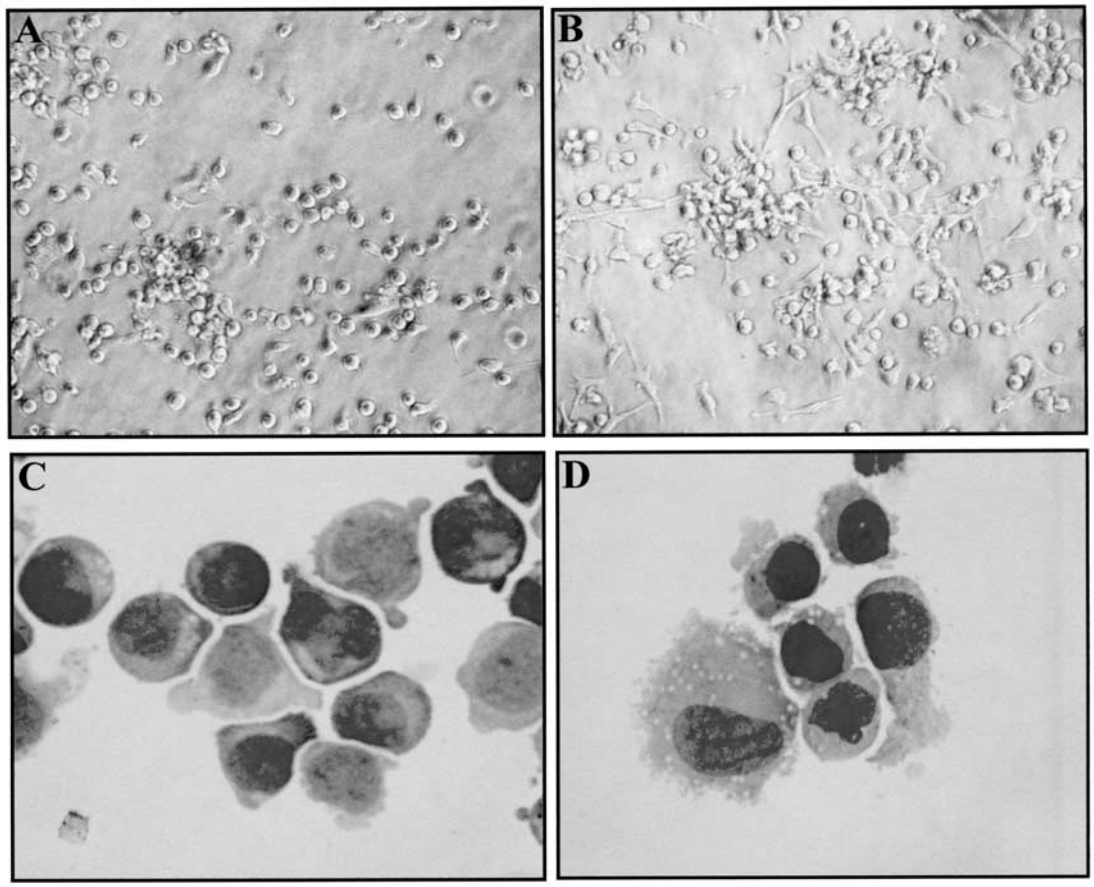

Figure 6. Morphology of STF3 primary cultures induced to differentiate into plasmacytoid dendritic cells. $CD34^+$ cells were cultured for 7 days with SCF, FL, thrombopoietin, and IL-3 (STF3) and incubated with SCF, FL, IL-3, and TNF-α for 3 days **(A)** or 6 days **(B)**. Original magnification × 20. May-Grünwald Giemsa staining of STF3-derived cells exposed to TNF-α for 3 **(C)** and 6 **(D)** days. Original magnification × 50. Abbreviations: FL, FLT3-ligand; IL, interleukin; SCF, stem cell factor; TNF-α, tumor necrosis factor alpha.

ODN) mimic bacterial DNA, and are able to induce IFN-I secretion by acting on toll-like-receptor 9 (TLR9) [34]. Plasmacytoid DCs, unlike myeloid DCs, express high levels of TLR9 [35] and have been identified as a primary target for CpG ODN [33]; thus, we determined IFN-α production after stimulation with ODN. As can be observed in Figure 8B, STF3-derived primary cultures maturated with TNF-α were able to secrete IFN-α when stimulated with ODN, demonstrating the generation of true plasmacytoid DCs.

Discussion

In this study we describe the generation of DCs from $CD34^+$ cells in a two-step procedure under serum-free conditions. The first step was the expansion of hematopoietic progenitor cells under the action of FL, SCF, TPO, and IL-6 or IL-3, and the second step was the induction of the differentiation and maturation of DCs in the presence of GM-CSF, IL-4, and TNF-α. This procedure has allowed us to study the effect of these early-acting cytokines on the generation of DC precursors, omitting the effects of serum, commonly used in the generation and expansion of DCs from $CD34^+$ cells. As previously described, two different myeloid DC precursors emerge independently from the $CD34^+$ cells culture, $CD14^-CD1a^+$ and $CD14^+CD1a^-$, considered precursors of LCs and dermal DCs, respectively. We have demonstrated that the relative appearance of these DC precursors can be modulated by interleukins. Thus, IL-6 had an inhibitory effect on the generation of $CD1a^+$ cells, favoring the expansion of $CD14^+$ cells. Moreover, taking into account that CD1a was expressed in 50% of $CD14^+$ cells appearing in STF3 cultures and only in 13% of $CD14^+$ cells derived from STF6 cultures, we can speculate that IL-6 also had an inhibitory effect on the maturation of this CD14-derived intermediate precursor. These results are in accordance with the inhibitory effect on the colony-forming unit DC growth of $CD34^+$ cells [7] and on DC maturation of $CD14^+CD1a^-$ cells [36] induced by IL-6. Myeloid DCs express CD11c, and here we report that approximately half of the $CD1a^+$ cell population presented this molecule on its surface versus 70%–80% of $CD14^+$ cell population, thus demonstrating a different acquisition time of CD11c by these two DC precursors. Taking into account that $CD34^+$

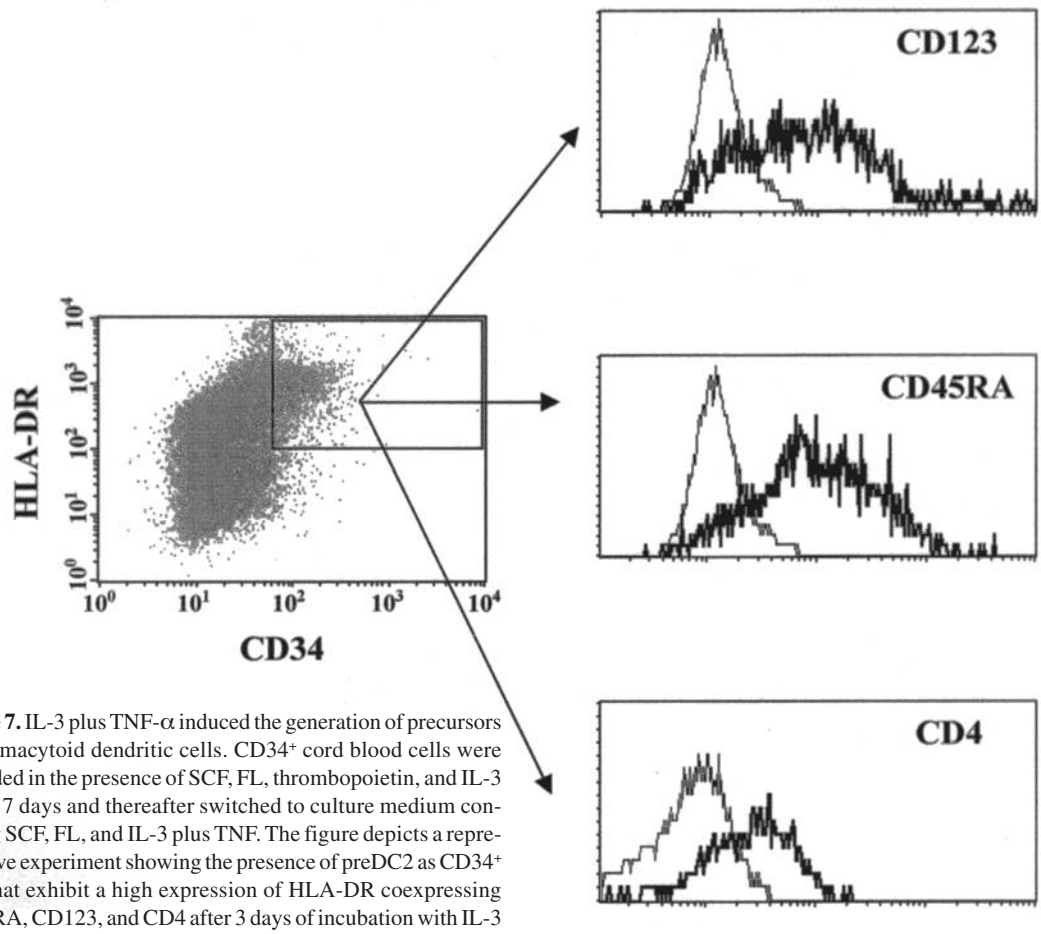

Figure 7. IL-3 plus TNF-α induced the generation of precursors of plasmacytoid dendritic cells. $CD34^+$ cord blood cells were expanded in the presence of SCF, FL, thrombopoietin, and IL-3 during 7 days and thereafter switched to culture medium containing SCF, FL, and IL-3 plus TNF. The figure depicts a representative experiment showing the presence of preDC2 as $CD34^+$ cells that exhibit a high expression of HLA-DR coexpressing CD45RA, CD123, and CD4 after 3 days of incubation with IL-3 plus TNF-α. Abbreviations: FL, FLT3-ligand; IL, interleukin; SCF, stem cell factor; TNF-α, tumor necrosis factor alpha.

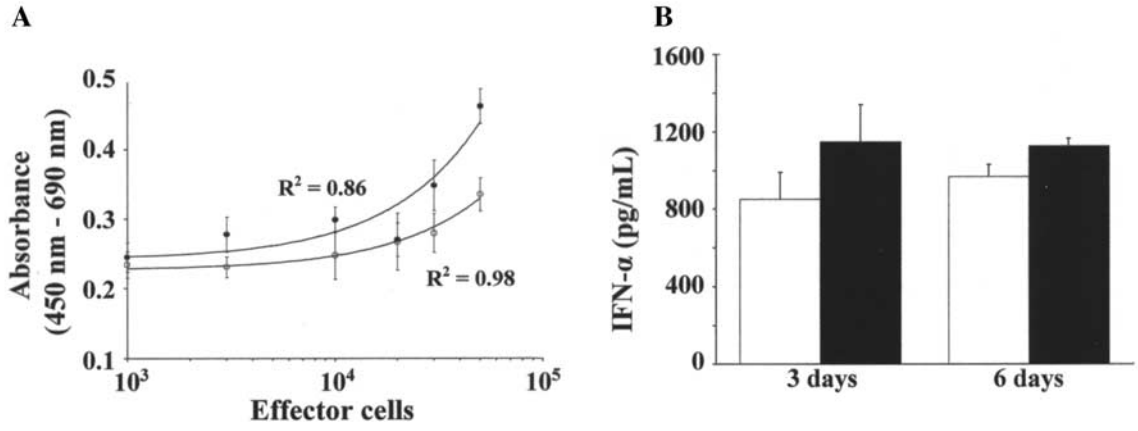

Figure 8. Plasmacytoid dendritic cells derived from STF3 primary cultures are functional dendritic cells. Cells derived from STF3 primary cultures were incubated during 3 or 6 days with interleukin-3 plus TNF-α as described in Materials and Methods. These cells were used as stimulators in graded doses to 1×10^5 allogenic $CD3^+$ T cells from adult peripheral blood in an MLR assay **(A)** or were cultured in the presence of CpG ODN 2006 at 2 μg/ml for 24 or 48 hours for IFN-α production **(B)**. Results are presented as mean ± standard error of duplicate samples corresponding to three independent experiments. MLR activity after 3 days (○) or 6 days (●) of exposure to TNF-α. Open bars and filled bars correspond to IFN-α secretion after 24 or 48 hours of ODN stimulation, respectively. Abbreviations: IFN, interferon; MLR, mixed leukocyte reaction; ODN, oligodeoxynucleotide; TNF-α, tumor necrosis factor alpha.

cells did not express CD11c and that we could not detect either $CD1a^+$ or $CD14^+$ cells after 3 days of culture (data not shown), the possibility that two $CD14^+$ and two $CD1a^+$ cell populations expressing or not expressing CD11c emerged independently from $CD34^+$ cells cannot be ruled out, but it is also possible that DC precursors acquired CD11c expression along the way.

Several receptors expressed by immature DCs belong to the C-type lectin superfamily, including Langerin (CD207), the macrophage mannose receptor (CD206), and CD205 [37]. More recently, a novel C-type lectin, DC-SIGN (CD209), has been identified [38]. It mediates DC trafficking [39] and transient adhesion with T cells to initiate primary immune response [40]. In peripheral blood, DC-SIGN seems to be restricted on a small subset of $CD14^+$ cells [41, 42], and in peripheral tissues its expression is restricted to subsets of immature DC in tissues and on specialized macrophages in the placenta and lung [43]. Immunohistochemical analysis in skin demonstrated that DC-SIGN was only expressed by dermal DCs, whereas LCs only expressed Langerin [44, 45]. It has also been reported that Mos lack DC-SIGN expression, but DC-SIGN expression is overexpressed in immature Mo-derived DCs [39, 40, 45]. Additionally, whereas LCs do not express DC-SIGN, Langerhans-like cells obtained from Mos cultured in the presence of TGF-β did [46]. These data prompted us to use DC-SIGN as a possible marker of differentiation of different myeloid DC subsets obtained in the cultures. We could identify a small cell subset representing only 2%–3% of generated cells in primary cultures that stained DC-SIGN. In addition, 20%–27% of CD1a cells also stained DC-SIGN, indicating that this Ag is also expressed on some DC precursors. As happened with $CD1a^+$ and $CD14^+$ cells obtained from STF6 or STF3 cultures, a lower percentage of DC-$SIGN^+$ derived from IL-6–treated cultures expressed CD11c.

DC precursors derived from both primary cultures were capable of differentiating into immature and mature DCs depending on the absence or presence of TNF-α, in addition to GM-CSF and IL-4. Moreover, by the analysis performed in this study, we could observe that cells derived from primary cultures were able to induce a continuous generation of DC precursors due to the maintenance of FL and SCF in the cultures.

Removal of IL-6 and IL-3 from the cultures allowed the generation of a similar percentage of $CD1a^+$ and $CD14^+$ cells gated on R2, whereas in R1 the proportion of $CD14^+$ cells between them was similar to that observed in primary cultures, but that of $CD1a^+$ cells was reversed. Although a significant increase in the percentage of cells expressing costimulatory molecules was observed in cultures derived from STF6 treatment, there was no difference in the DC yield measured as $CD83^+$ cells between two cytokine combinations used to expand $CD34^+$ cells. These results demonstrate that from one $CD34^+$ cell cultured in the absence of serum, a mean average of four $CD83^+$ cells could be generated, in addition to a wide expansion of DC precursors. Furthermore, we could not detect differences on DC functionality, assessed by internalization of FITC-dextran and MLR between cells derived from both primary cultures.

When we analyzed for expression of DC-SIGN, we could observe a high proportion of $CD1a^+$ cells generated in culture that did not express DC-SIGN irrespectively of primary culture and the factors used to induce the generation of immature or mature DCs, thus evidencing two different myeloid DC subsets. It has been reported that $CD34^+$ cells cultured in the presence of GM-CSF, TNF-α, TGF-β, SCF, and FL induce LC phenotype [30], characterized by the presence of Langerin and Birbeck granules. In these conditions, Soilleux et al. [47] did not detect DC-SIGN expression by reverse transcription–polymerase chain reaction. However, Langerhans-like cells obtained from Mo by the action of GM-CSF, IL-4, and TGF-β lacked CD14 expression but acquired a high expression of CD1a and DC-SIGN [46]. In addition, the data previously reported suggesting that expression of DC-SIGN and the LC-restricted molecule CD1a are mutually exclusive [40, 47] cause us to propose that $CD1a^+$DC-$SIGN^-$ and $CD1a^+$DC-$SIGN^+$ cell subsets obtained from $CD34^+$ cells correspond to LCs and dermal DCs, respectively.

A considerable body of evidence supports the notion that DCs can originate either from myeloid or lymphoid precursors. Of the cytokines used in this study, the hematopoietic growth factor FL has been shown to increase the numbers of myeloid DCs and plasmacytoid DCs (DC2) in the blood stream when injected into humans [24, 48]. DC2 is present in T-cell areas of the lymph nodes [14] and peripheral blood, and has the capacity to produce high levels of type I IFN in response to viral stimulation [49, 50]. It can be distinguished from $CD11c^+$ myeloid DCs by lacking myeloid Ags, like CD13, CD33, and CD11c. Moreover, it has high levels of IL-3 receptor (IL-3R) but sparse expression of GM-CSF receptor [51]. Recently, culture systems to generate DC2 in vitro from $CD34^+$ cells in coculture either with the murine bone marrow stromal cell line S17 [52] or IL-3 and CD40 ligand-transfected L cells [53] in the presence of serum have been developed. Blom et al. [53] recently identified a precursor of DC2 (pro-DC2) that expressed high levels of IL-3Rα, CD4, and CD34 and that develops from a $CD34^+CD45RA^+$ late progenitor. In our culture conditions, we observed that primary culture cells derived from STF3 treatment contained a considerable proportion of cells lacking CD13 expression that were not present when IL-6 was added. Taking into account the effect induced by FL and considering that DC2 is nonresponsive to GM-CSF [51] but can be induced in the

presence of IL-3, it was not surprising that $CD13^-$ cells could give rise to DC2 upon appropriate stimulus. In fact, when expanded cells were incubated with IL-3 and TNF-α, maintaining FL and SCF to preserve cell viability, a high number of DC2s capable of producing IFN-α after stimulation with CpG ODN was obtained. In addition, the developmental stages from $CD34^+$ hematopoietic stem cells, previously described by Blom et al. [53], were identified.

In conclusion, this study extends previous results on the generation of DCs from human $CD34^+$ cells and identifies the regulatory effect of IL-6 and IL-3 in combination with SCF, FL, and TPO, avoiding the masking effects of serum on the generation of distinct DC precursors, providing a system to study the developmental pathway of different DC subsets. Because DCs play vital roles in the regulation of immune responses and are being used as vaccines, our results indicate that it is possible to generate a massive production of myeloid and lymphoid DCs for clinical purposes.

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Selective Generation of Different Dendritic Cell Precursors from CD34+ Cells by Interleukin-6 and Interleukin-3

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