Generation of Interferon α–producing Predendritic Cell (Pre-DC)2 from Human CD34+ Hematopoietic Stem Cells

By Bianca Blom, Stephen Ho, Svetlana Antonenko, and Yong-Jun Liu

From the Department of Immunobiology, DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, California 94304

Abstract
Upon viral stimulation, the natural interferon (IFN)-α/β–producing cells (IPCs; also known as pre-dendritic cells (DCs [2]) in human blood and peripheral lymphoid tissues rapidly produce huge amounts of IFN-α/β. After performing this innate antiviral immune response, IPCs can differentiate into DCs and strongly stimulate T cell–mediated adaptive immune responses. Using four-color immunofluorescence flow cytometry, we have mapped the developmental pathway of pre-DC2/IPCs from CD34+ hematopoietic stem cells in human fetal liver, bone marrow, and cord blood. At least four developmental stages were identified, including CD34++CD45RA- early progenitor cells, CD34++CD45RA+ late progenitor cells, CD34+CD45RA++CD4+ interleukin (IL)-3Rα++ pre-DC2, and CD34+CD45RA+++CD4+IL-3Rα+++ pre-DC2/IPCs. Pro-DC2s have already acquired the capacity to produce large amounts of IFN-α/β upon viral stimulation and to differentiate into DCs in culture with IL-3 and CD40 ligand. CD34++CD45RA- early progenitor cells did not have the capacity to produce large amounts of IFN-α/β in response to viral stimulation; however, they can be induced to undergo proliferation and differentiation into IPCs/pre-DC2 in culture with FLT3 ligand.

Key words: dendritic cells • interferon α/β–producing cells • stem cells • FLT3 ligand • interferon-producing cell

Introduction
Dendritic cells (DCs)1 represent heterogeneous populations of hematopoietic-derived cells that display potent ability to induce primary T cell activation, polarization, and in certain circumstances, tolerance (1–4). The distinct capacity of DCs to induce immunity versus tolerance or Th1 versus Th2 responses depends on their maturation stage (2, 5), signals that induce or inhibit DC maturation (2, 5, 6), as well as the lineage origin of DCs (7–12). A lymphoid DC developmental pathway was suggested by the finding that mouse thymic lymphoid precursors can give rise to both T cells and CD8+CD11b- DCs (13, 14). In addition, a well-established myeloid DC pathway giving rise to CD8-CD11b+ DCs has been defined (15–17). Recent studies suggest that CD8+CD11b- lymphoid DCs and CD8-CD11b+ myeloid DCs may have different functions in T cell activation/tolerance or Th1/Th2 differentiation (7, 9, 18–24).

In humans, two distinct populations of DC precursors exist in the blood. Monocytes (pre-DC1), which belong to the myeloid lineage, differentiate into immature DC1 after 5 d of culture in GM-CSF and IL-4 (25, 26). Upon CD40 ligand activation, immature myeloid DC1 undergo maturation and produce large amounts of IL-12 (27, 28). The mature DC1 induced by CD40 ligand are able to polarize naive CD4+ T cells into Th1 cells (29). The second type of DC precursor cells, pre-DC2 (previously known as plasma-cytoid T/monocytes) are characterized by a unique surface phenotype (CD4+IL-3Rα++CD45RA+HLA-DR+ lineage marker-negative and CD11c+), and at the ultrastructural level resemble Ig-secreting plasma cells (30, 31). Several lines of evidence suggest that pre-DC2s are of lymphoid origin: (a) pre-DC2 lack expression of the myeloid antigens CD11c, CD13, CD33, and mannose receptor (30, 32), (b) pre-DC2 isolated from the thymus express the lymphoid markers CD2, CD5, and CD7 (32), (c) pre-DC2 have little phagocytic activity (30), (d) pre-DC2 do not differentiate into macrophages after culture with GM-CSF.
progenitor cells, and (b) to identify the stimuli that can induce CD34\(^+\) hematopoietic progenitor cells to differentiate into pre-DC2/IPCs.

In this paper, we describe the identification of CD34-expressing precursors of pre-DC2/IPCs from human fetal tissues and cord blood. In addition, we report on the generation of pre-DC2/IPCs from early hematopoietic stem cells in vitro cultures.

**Materials and Methods**

*Flow Cytometric Analysis and Cell Sorting of Human Cord Blood and Fetal Tissues.* Fetal tissue (16–22 wk of gestation) and cord blood were obtained from Advanced Bioscience Resources Inc. Mononuclear cells (MNCs) were isolated from these samples by Ficoll density gradient centrifugation (1.077 g/ml Lymphoprep; Amersham Pharmacia Biotech). MNCs were washed three times in PBS (BioWhittaker), and resuspended in PBS containing 2% (vol/vol) human serum (HS; Gemini Bioproducts) and 2 mM EDTA (PBS/HS/EDTA). Magnetic bead depletion was performed to remove lineage-positive cells. In brief, MNCs were incubated with a mixture of antibodies against CD3 (OKT-3 ascites), CD8 (OKT-8 ascites), CD14 (RPA-M1 ascites), CD16 (3G8; Immunotech), CD19 (4G7 ascites), CD56 (My31 ascites), CD66B (80H3; all from Immunotech), and glycopherin A (10F7MN ascites). After two washes, the cells were incubated with goat anti–mouse IgG coupled to magnetic beads (Dynabeads\(^\text{M-450}\), goat anti–mouse IgG; Dynal) and isolated according to the manufacturer’s instructions. The enriched cells were stained either to perform a four-color flow cytometric analysis or to purify different subsets by cell sorting (FACS\(^\text{®}\); Becton Dickinson). Cells were incubated with a cocktail of the following FITC-conjugated antibodies: CD3 (Leu-4), CD14 (Leu-M3), CD15 (Leu-M1), CD16 (Leu-11a), CD20 (Leu-16), CD57 (Leu-7; all from Becton Dickinson), and CD11c (3.9; Caltag Laboratories). To analyze expression of different antigens on the lineage FITC-negative cells, the cells were stained with CD34-allophycocyanin (APC; HPCA-2; Becton Dickinson) and CD45RA-TRICOLOR (MEM 56; Caltag), and in addition, different PE-conjugated antibodies: anti–HLA-DR (Becton Dickinson), CD4 (Leu-3a; Becton Dickinson), and IL-3Ra (9F5l; BD Pharmingen). For cell sorting, the enriched cells were stained with CD34-APC (HPCA-2), CD45RA-PE (Leu-18), and CD4-biotin (Leu-3a), all from Becton Dickinson. Expression of CD4 was revealed after a second step staining using the streptavidin-alexa594 (Molecular Probes) or avidin-Texas red (BD Pharmingen) conjugate.

*DC2 Culture Conditions.* For generation of DC2, sorted cells were cultured for 5 d in the presence of IL-3 (10 ng/ml; R&D Systems) and CD40 ligand–transfected L cells (10,000/well, irradiated at 7,000 rads) in 25 \(\mu\)l Yssel’s medium (48) containing 2% HS in microwell plates (Robbins Scientific Corporation).

*Proliferation Assay.* Sorted cells (15,000/well) were cultured in duplicate for the indicated durations in the presence of IL-3 (10 ng/ml; R&D Systems), GM-CSF (800 U/ml; a gift from Schering-Plough, Kenilworth, NJ), FLT3 ligand (100 ng/ml; a gift from S. Menon, DNAX Research Institute of Molecular and Cellular Biology), and SCF (10 ng/ml; R&D Systems) in 200 \(\mu\)l Yssel’s medium (48) containing 2% HS in 96-well round-bottomed culture plates (Falcon\(^\text{®}\); Becton Dickinson). [\(\text{H}\)]Thymidine (1 \(\mu\)Ci/well; Amersham Pharmacia Biotech) was added during the last 8 h of the culture. Proliferation data were calculated in counts per minute.
**IFN-α Production.** Cells (10^6/ml) were cultured in 25 μl Yssel's medium (48) containing 2% HS in microwell plates (Robbins Scientific). HSV-1, KO-S strain, attenuated by γ irradiation (a gift from R. Chase, Schering-Plough, Kenilworth, NJ) was added at 10 PFU/cell. After 24 h, supernatants were collected and frozen at −20°C before analysis by an IFN-α-specific sandwich ELISA (Biosource International). Appropriate dilutions of Yssel's medium (48) containing 2% HS in microwell plates (Robbins Scientific) were used for the detection of IFN-α.

Pre-DC2/IPC Generation from Stem Cells. Sorted cells (25,000–50,000/well) were cultured in 200 μl Yssel's medium containing 2% HS in 96-well round-bottomed culture plates. Cytokines were added at the following concentrations: FLT3 ligand (100 ng/ml; provided by S. Menon, DNAX Research Institute of Molecular and Cellular Biology), GM-CSF (800 U/ml; a gift from Schering-Plough), SCF (10 ng/ml; R&D Systems), IL-3 (10 ng/ml; R&D Systems), IL-7 (10 ng/ml; R&D Systems), and G-CSF (5 ng/ml; R&D Systems). Cell cultures were refreshed every 5 d by demi-depletion and split if necessary.

Results

Identification of Pro-DC2, the CD34-expressing Immediate Precursors of Pre-DC2/IPCs. Pre-DC2/IPCs have been identified in tonsil, adult peripheral blood (29, 30), and postnatal thymus (32). To define the developmental pathway of pre-DC2/IPCs from CD34^+ hematopoietic progenitors, lineage-positive cells (including T, B, and NK cells, and monocytes, granulocytes, and erythrocytes) from fetal liver, bone marrow, cord blood, and adult blood were depleted using magnetic beads. The remaining lineage-negative cells were analyzed by four-color flow cytometry analysis after staining with: (a) FITC-conjugated antibodies against lineage markers (CD3, CD11c, CD14, CD15, CD16, CD20, and CD57), in order to exclude the remaining lineage-positive cells; (b) anti-CD34 antibody conjugated to APC, to follow the populations of hematopoietic progenitor cells; (c) anti-CD45RA antibody conjugated to Tricolor as a positive marker to identify pre-DC2/IPCs and to distinguish CD34^+CD45RA^- early progenitors from CD34^+CD45RA^+ late progenitors; and (d) various PE-conjugated antibodies known to detect antigens expressed on pre-DC2/IPCs, such as CD4 and IL-3Ra. After gating on FITC-negative cells, four populations of cells (A, B, C, and D) were identified (Fig. 1). CD34^+CD45RA^- cells (population A) are enriched for early multipotent progenitor cells (49), whereas CD34^+CD45RA^+ cells (population B) are enriched for myeloid/lymphoid progenitors, which have lost the potential to develop into the erythroid lineage (42). These two populations of CD34^+ progenitor cells expressed lower levels of CD4 and IL-3Ra than expressed by pre-DC2/IPCs derived from adult peripheral blood (Fig. 2) or tonsils (data not shown). A substantial proportion of CD34^+CD45RA^+ cells in population C and CD34^-CD45RA^- cells in population D expressed a moderate level of CD4 and a high level of IL-3Ra, similar to the expression level detected on pre-DC2/IPCs from adult peripheral blood (Fig. 2) or tonsils (data not shown). The CD4^- and IL-3Ra^- fraction of cells in populations C and D expressed NKRP1A (data not shown), and most likely represent cells of the NK lineage (50). Thus, according to these phenotypical analyses, the CD34^-CD45RA^+CD4^-IL-3Ra^- cells in population D may represent pre-DC2/IPCs. Furthermore, because of the low CD34 expression, the CD34^+CD45RA^-CD4^+IL-3Ra^- cells in population C may represent the immediate progenitors of pre-DC2/IPCs. Accordingly, the CD34^-CD45RA^-CD4^-IL-3Ra^- cells in population C will be referred to as pro-DC2 (for progenitor of pre-DC2). Both pre-DC2 in fraction D and pro-DC2 in fraction C display a similar size profile (intermediate size between lymphocytes and monocytes) revealed by forward scatter (FSC)/side scatter (SSC) (Fig. 2) and a plasmacytoid morphology by Giemsa staining (not shown). The presence of pro-DC2 could be detected in cord blood, fetal bone marrow, fetal liver, and at very low numbers in peripheral blood, but not in fetal thymus (Fig. 1). All tissues analyzed contained pre-DC2 in population D (Fig. 1 and data not shown).

Pro-DC2 and Pre-DC2 Produce Large Amounts of IFN-α after Viral Stimulation. A key function feature of pre-DC2/IPCs is the rapid production of huge amounts of IFN-α/β in response to viral stimulation. (37, 38). To determine if CD34^- pro-DC2s in population C have ac-
quired this function, pro-DC2 were stimulated with irradiated HSV-1 (10 PFU/cell) for 24 h, in parallel with pre-DC2 and CD34\(^+\) progenitor cell populations A and B. CD34\(^+\)CD45RA\(^-\) (population A) isolated from either cord blood or fetal liver only produced low levels of IFN-\(\alpha\) (population A, \(<17–111 \text{ pg/ml, } n = 11; \text{ Table I}\) ). Similar low IFN-\(\alpha\) levels were found for virally stimulated cord blood CD34\(^+\) CD45RA\(^+\) (population B) cells (population B, 17–48 pg/ml, \(n = 4\) ). However, the range of IFN-\(\alpha\) production from fetal liver–derived CD34\(^+\) CD45RA\(^+\) cells was more variable (population B, \(<17–4,257 \text{ pg/ml, } n = 11\) ). Pro-DC2 in population C and pre-DC2 in population D isolated from either cord blood or fetal liver produced huge amounts of IFN-\(\alpha\) (pro-DC2, 172–90,464 pg/ml, \(n = 10\); pre-DC2, 1,024–15,830 pg/ml, \(n = 8; \text{ Table I}\) ). No IFN-\(\alpha\) was detectable from the CD4\(^+\) cells in population D after purification and viral stimulation, suggesting that these cells are different from the pre-DC2 (data not shown).

These data indicate that pre-DC2s from fetal liver and cord blood are similar to pre-DC2/IPCs isolated from adult blood and tonsils regarding their surface phenotype and function in antiviral innate immunity. Furthermore, although pro-DC2 express the CD34 antigen, these cells already acquired the functional capacity to produce a large amount of IFN-\(\alpha\) in response to viral stimulation, and therefore pro-DC2 may represent the earliest IPCs during hematopoiesis. In addition, the ability to rapidly produce vast amounts of type 1 IFN is acquired during hematopoietic development, as CD34\(^+\) early hematopoietic progenitors only produced a low amount of IFN-\(\alpha\) in response to viral stimulation.

**Pro-DC2s Differentiate into Mature DCs upon IL-3 and CD40 Ligand Stimulation.** Adult blood and tonsil-derived pre-DC2 depend on IL-3 for their survival (30). Culture of pre-DC2 with IL-3 and CD40 ligand–transfected L cells results in their differentiation into mature DCs (DC2 [29, 30]). To determine whether pro-DC2 have the potential to
differentiate into mature DCs, the cells were cultured for 5 d with IL-3 and CD40 ligand in parallel with pre-DC2s. After 5 d of culture, both pro-DC2 and pre-DC2 acquired mature DC morphology (data not shown). Moreover, flow cytometry analysis revealed that both cultured pro-DC2 and pre-DC2 expressed mature DC markers, such as high HLA-DR, CD80, CD86, CD40, and CD83 (Fig. 3). These mature DC2 strongly induced the proliferation of allogeneic naive CD4 T cells (data not shown). These results suggest that pro-DC2 and pre-DC2 have an equal potential to develop into mature DCs.

Pro-DC2 Have a Limited Proliferative Capacity. The proliferation potential of pro-DC2 in response to hematopoietic cytokines was analyzed, in parallel with that of pre-DC2, CD34+CD45RA− (population A), and CD34+CD45RA+ (population B) from fetal liver. Cells of each population were cultured for a total of 5 d in a cytokine cocktail consisting of GM-CSF, IL-3, SCF, and FLT3 ligand. [3H]Thymidine was added to the cultures 8 h before incorporation was analyzed. Pro-DC2 proliferated moderately better than pre-DC2 (Fig. 4 A), but 50–100 times less than CD34+CD45RA− (population A) and CD34+CD45RA+ (population B; Fig. 4 B). In addition, both pro-DC2 and pre-DC2 failed to increase cell numbers in these cultures (data not shown). These data suggest that although pro-DC2 still express the CD34 antigen and proliferate moderately better in response to cytokines than pre-DC2, the pro-DC2 may have lost the expansion potential of hematopoietic progenitor cells.

FLT3 Ligand Induces CD34+CD45RA− Early Hematopoietic Progenitor Cells to Differentiate into IFN-α−producing Cells. CD34+CD45RA− cells did not produce IFN-α after viral stimulation, but were found to have the best

Table I. IFN-α−producing Capacity of Purified Pro-DC2, Pre-DC2, and CD34+ Progenitor Subsets from Cord Blood and Fetal Liver

| CD34+CD45RA− | CD34+CD45RA+ | Pro-DC2 | Pre-DC2 |
|----------------|----------------|---------|---------|
| pg/ml          | pg/ml          | pg/ml   | pg/ml   |
| Cord blood     |                |         |         |
| 1              | 32 (±10)       | 36 (±5) | 4,295 (±69) | 3,254 (±55) |
| 2              | 41 (±3)        | 48 (±3) | 172 (±6) | 1,024 (±7) |
| 3              | 43 (±20)       | 43 (±5) | 6,938 (±67) | 13,020 (±322) |
| 4              | −              | −       | 1,284 (±43) | 3,484 (±333) |
| 5              | −              | −       | 10,835 (±780) | 9,241 (±712) |
| 6              | 20 (±1)        | 17 (±5) | −       | −       |
| Fetal liver    |                |         |         |
| 1              | <17            | 1,270 (±150) | 90,464 (±1391) | −       |
| 2              | 76 (±6)        | 1,241 (±22) | 39,577 (±678) | 15,830 (±1,121) |
| 3              | 111 (±9)       | 41 (±37) | 20,308 (±714) | 900 (±48) |
| 4              | 81 (±32)       | 1,496 (±41) | 20,308 (±714) | 900 (±48) |
| 5              | 32 (±3)        | 4,257 (±95) | 90,010 (±856) | −       |
| 6              | 23 (±3)        | 46 (±9) | −       | −       |
| 7              | <17            | <17     | −       | −       |

CD34+ progenitor subsets, pro-DC2, and pre-DC2 were purified by flow cytometric cell sorting and stimulated with HSV-1 for 24 h. The amount of IFN (pg/ml) produced in the supernatants was measured by ELISA. The detection limit of the ELISA was 17 pg/ml. Numbers in parentheses represent standard deviations. −, not tested.

Figure 3. Flow cytometric analysis of pro-DC2 and pre-DC2 after 5 d of culture with IL-3 and CD40 ligand L cells. Harvested cells were stained using PE-conjugated antibodies against HLA-DR, IL-3Rα, CD80, CD86, CD40, and CD83. Open histograms represent expression after staining with an isotype control antibody. The shaded histograms represent expression of the indicated antigen.
The amount of IFN-α 20,000 cells were stimulated with HSV-1 for 24 h and the ligand, GM-CSF, or G-CSF. After 11 and 25 d of culture, one of the following cytokines: IL-3, IL-7, SCF, FLT3 induced to differentiate into pre-DC2/IPCs in vitro with SCF produced moderate levels of IFN-α by ELISA. At day 11, cells cultured with FLT3 ligand or CD34 not shown). None of the other cytokines had induced the ment was 24-fold in FLT3 ligand and 6-fold in SCF (data not shown). The concentration of IFN-α in the supernatants was analyzed by ELISA. At day 11, cells cultured with FLT3 ligand or SCF produced moderate levels of IFN-α (Fig. 5). Cellular expansion in this experiment increased six- to sevenfold after 4 wk of culture (data not shown).

FLT3 Ligand Induces CD34++CD45RA− Early Progenitor Cells to Differentiate into CD4+HLA-DR−IL-3Ra++ CD45RA−CD11c− Pre-DC2. Next, we used four-color flow cytometry to determine whether the production of the vast amounts of IFN-α by FLT3 ligand–cultured cells was due to the generation of pre-DC2/IPCs. In normal human adult blood, the frequency of CD4+HLA-DR−IL-3Ra++CD45RA−CD11c− pre-DC2s is between 0.3 and 0.8% of total peripheral blood MNCs (30). The percentage of CD4+HLA-DR−IL-3Ra++CD45RA−CD11c− cells in freshly isolated CD34++CD45RA− early progenitor cells from fetal liver or in 5 d FLT3 ligand–cultured cells was <0.05% (Fig. 1 B and Fig. 6, B and C). A progressive increase (from 1.5 to 6%) in the percentage of HLA-DR−IL-3Ra++ cells was observed from days 11 to 20 of culture (Fig. 6, B and C). Between days 25 and 30, up to 10% of the cultured cells were HLA-DR−IL-3Ra++ cells (Fig. 6, B and C). Detailed flow cytometric analysis of CD34++CD45RA− cells at day 30 of FLT3 ligand culture revealed that the HLA-DR−IL-3Ra++ cells expressed CD4, CD45RA, and low levels of CD11c (Fig. 7 A), a typical phenotype of pre-DC2/IPCs. Most interestingly, a clear correlation between the ability of cultured cells to produce large amounts of IFN-α in response to viral stimulation and the appearance of CD4+HLA-DR−IL-3Ra++...
CD45RA⁺CD11c⁻ cells in culture with FLT3 ligand was evidenced (Fig. 6, A and C).

To directly prove that the IL-3Rα⁺⁺HLA-DR⁺ cells generated from CD34⁺⁺CD45RA⁻ early progenitor cells with FLT3 ligand were pre-DC2/IPCs, FLT3 ligand–cultured cells were separated into IL-3Rα⁺⁺HLA-DR⁺ and IL-3Rα⁺⁺⁺HLA-DR⁺ populations by cell sorting. Stimulation of sorted cells with HSV-1 for 24 h revealed that the IL-3Rα⁺⁺⁺HLA-DR⁺ produced the larger amount of IFN-α (50,238 pg/ml), which was seven times more than produced by the IL-3Rα⁺⁺⁺HLA-DR⁺ (7,125 pg/ml; Fig. 7). Notably, the amount of IFN-α produced by these in vitro...
generated cells is comparable to that produced by freshly isolated IPCs from fetal liver (Table I).

These data indicate that pre-DC2/IPCs were generated from CD34<sup>+</sup>CD45RA<sup>+</sup> early hematopoietic progenitor cells in culture with FLT3 ligand.

**Other Hematopoietic Cytokines Do Not Promote the FLT3 Ligand–induced Generation of Pre-DC2/IPCs from CD34<sup>+</sup>CD45RA<sup>-</sup> Early Progenitor Cells.** To determine if other cytokines could enhance the activity of FLT3 ligand to induce the generation of pre-DC2/IPCs, CD34<sup>+</sup>CD45RA<sup>-</sup> early hematopoietic progenitor cells were cultured with FLT3 ligand with each of the following cytokines: GM-CSF, G-CSF, IL-3, IL-7, and SCF. Although G-CSF, and to a lesser extend GM-CSF and SCF, enhanced the cellular expansion of CD34<sup>+</sup>CD45RA<sup>-</sup> cells after 25 d of culture (Fig. 8 A), none of the cytokines tested promoted the generation of pre-DC2/IPCs induced by FLT3 ligand as determined by the capacity of IFN-α/β production or by the pre-DC2/IPC phenotype (CD4<sup>+</sup>HLA-DR<sup>+</sup>IL-3Rα<sup>+</sup>CD11c<sup>+</sup>; Fig. 8, B and C). Moreover, the tested cytokines, in particular IL-3, inhibited the generation of pre-DC2/IPCs induced by FLT3 ligand.

**Discussion**

The evolutionary pressure to fight numerous types of microorganisms has endowed us with the development of not only the sophisticated adaptive immune system, including T and B lymphocytes, but also the divine innate immune system. Whereas neutrophils and macrophages are dedicated to eat and kill various bacteria, the eosinophils, basophils, and mast cells have evolved to kill parasites. The robust production of IFN-α/β of pre-DC2/IPCs in response to viral stimulation suggests that pre-DC2/IPCs represent a new member cell type of the hematopoietic family and an effector cell type of the innate immune system against antiviral infection.

Hematopoietic stem cells develop into committed effector cells depending on their microenvironment, which provides them with the necessary stimuli. This development is a multistep process that requires the orchestrated availability of cytokines and chemokines in order for the cells to proliferate, differentiate, and migrate accurately. This study suggests a developmental pathway of pre-DC2/IPCs from CD34<sup>+</sup>CD45RA<sup>-</sup> early progenitors, to CD34<sup>+</sup>CD45RA<sup>+</sup> late progenitors, to CD34<sup>+</sup>CD45RA<sup>+</sup>IL-3Rα<sup>+</sup>CD4<sup>+</sup>HLA-DR<sup>+</sup>CD11c<sup>+</sup> pro-
DC2, and finally to CD34+CD45RA+IL-3Rα+CD4+HLA-DR+CD11c– pre-DC2/IPCs (Fig. 9). This differentiation pathway may provide a basis to study further the function and development of pre-DC2 in normal and disease states. For example, it was recently reported that both G-CSF and FLT3 ligand treatment of healthy donors increased pre-DC2 numbers in the peripheral blood. This study provides a model to study the effects of both cytokines on the different developmental stages of pre-DC2 in vivo. Furthermore, it has been reported that there is a progressive loss of the IFN-α/β-producing capacity of peripheral blood cells in response to viral stimulation in patients with hairy cell leukemia, steroid treatment, idiopathic CD4 lymphopenia, and HIV (for a review, see reference 51). It will be interesting to determine the stage where pre-DC2 development and function is disturbed in these patients in order to anticipate clinical treatment.

This study also demonstrated the generation of pre-DC2/IPCs in vitro from CD34+CD45RA– early hematopoietic progenitor cells with FLT3 ligand. The generated cells not only had the capacity to produce huge amounts of IFN-α/β in response to viral stimulation, but also displayed a typical pre-DC2/IPC phenotype of CD4+IL-3Rα+CD45RA+HLA-DR+CD11c–. Notably, the finding that neither pro-DC2 nor pre-DC2 could survive and proliferate in culture with FLT3 ligand over a 2-wk culture period indicates that the presence of pre-DC2/IPCs was due to their differentiation from CD34+CD45RA– early progenitor cells, and not the selective expansion of contaminating pro-DC2s or pre-DC2s. Injection of FLT3 ligand was previously shown to dramatically increase the numbers of both myeloid and lymphoid DCs in blood and lymphoid tissues of mice (46, 52). A recent study by Pulendran et al. (45) showed that FLT3 ligand treatment also increased the number of both CD11c– myeloid DCs and CD4+IL-3Rα+CD11c– pre-DC2/IPCs in the peripheral blood of human donors. These findings, together with this study, suggest that FLT3 ligand directly induces the differentiation of a proportion of CD34+CD45RA– early progenitor cells into pre-DC2/IPCs. Whether FLT3 ligand promotes the migration of pre-DC2 generated in bone marrow into the blood circulation remains unknown. Two recent studies showed that in G-CSF-treated patients or stem cell donors, the number of pre-DC2 in blood was increased fivefold, whereas the number of CD11c+ DCs was not affected (10, 45). Our current finding that G-CSF was unable to induce CD34+CD45RA– early progenitor cells to differentiate into pre-DC2/IPCs suggests that unlike FLT3 ligand, G-CSF did not support differentiation of early hematopoietic progenitor cells into pre-DC2/IPCs, but may promote the migration of pre-DC2 into peripheral blood.

IFN-α/β has been widely used in treating patients with viral hepatitis (53). Potentially, combination therapy of IFN-α/β and FLT3 ligand or G-CSF can be used to increase the number of pre-DC2/IPCs to treat patients with chronic viral infection. Several studies suggest that in HIV-infected patients, there is a progressive loss of the ability of blood leukocytes to produce IFN-α/β in response to viral infections (54–56). Pre-DC2/IPCs express CD4 and chemokine receptors CXCR4 and CCR5 (Kanzler, H., V. Soumelis, and Y.-J. Liu, unpublished observation), which are the receptors allowing HIV entry (57, 58). We have recently observed that there is a dramatic decrease in pre-DC2/IPC numbers in HIV-infected patients with AIDS, in particular in AIDS patients with complication of infections and of Kaposi sarcoma (Soumelis, V., and Y.-J. Liu, unpublished observation). Although it remains to be established whether pre-DC2/IPCs are infected by HIV viruses, FLT3 ligand together with the therapies preventing HIV infection may be beneficial for patients to ultimately eliminate viruses.

In conclusion, this study has mapped the pathways and identified the cytokine regulation of pre-DC2/IPC development from early hematopoietic stem cells in humans. The pre-DC2/IPC developmental pathway may provide a basis for monitoring pre-DC2/IPC numbers and development in patients with tumors, autoimmune diseases, and infectious diseases. FLT3 ligand and G-CSF may have the potential to be used to stimulate the generation and mobilization of pre-DC2/IPCs in patients who are fighting against cancers and infectious diseases.

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