Interleukin-3 Cooperates With Tumor Necrosis Factor $\alpha$ for the Development of Human Dendriti/Langerhans Cells From Cord Blood CD34+ Hematopoietic Progenitor Cells

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We have previously shown that tumor necrosis factor (TNF$\alpha$) strongly potentiates the granulocyte-macrophage colony-stimulating factor (GM-CSF)/interleukin (IL)-3-dependent proliferation of CD34+ hematopoietic progenitor cells (HPC) through the recruitment of early progenitors with high proliferative potential. Furthermore, the combination of GM-CSF and TNF$\alpha$ allows the generation of large numbers of dendritic/Langerhans cells (D-Lc). Herein, we analyzed whether IL-3, when combined to TNF$\alpha$ would, as does GM-CSF, allow the generation of CD1a+ D-Lc. Accordingly, cultures of cord blood CD34+ HPC with IL-3 + TNF$\alpha$ yielded 20% to 60% CD14+ cells and 11% to 17% CD1a+ cells, while IL-3 alone did not generate significant numbers of CD1a+ cells. Although the percentage of CD1a+ cells detected in IL-3 + TNF$\alpha$ was lower than that observed in GM-CSF + TNF$\alpha$ (42% to 78%), the strong growth induced by IL-3 + TNF$\alpha$ generated as many CD1a+ cells as did GM-CSF + TNF$\alpha$. The CD14+ and CD1a+ cells generated with IL-3 + TNF$\alpha$ are similar to CD14+ and CD1a+ cells generated in GM-CSF alone and GM-CSF + TNF$\alpha$, respectively. CD1a+ cells differed from CD14+ cells by (1) dendritic morphology, (2) higher expression of CD1a, CD1c, CD4, CD40, adhesion molecules (CD11c, CD54, CD58), major histocompatibility complex (MHC) class II molecules and CD28 ligands (CD80 and CD86), (3) lack of Fc receptor Fc$\gamma$RI (CD64) and complement receptor CR1 (CD35) expression, and (4) stronger induction of allogeneic T-cell proliferation. Thus, in combination with TNF$\alpha$, IL-3 is as potent as GM-CSF for the generation of CD1a+ D-Lc from cord blood CD34+ HPC. The dendritic cell inducing ability of IL-3 may explain why mice with inactivated GM-CSF gene display dendritic cells.

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Materials and Methods

Hematopoietic factors. rhGM-CSF (specific activity: 2.106 U/mg, Schering-Plough Research Institute, Kenilworth, NJ) was used at a saturating concentration of 100 ng/mL (200 U/mL). rhIL-3 (specific activity: 5 × 104 U/mg, kindly provided by Dr. S. Tindall, Schering-Plough Research Institute, Kenilworth, NJ) was used at a saturating concentration of 10 ng/mL (200 U/mL). rhTNF$\alpha$ (specific activity: 2 × 105 U/mg Genzyme, Boston, MA) was used at an optimal concentration of 2.5 ng/mL (50 U/mL).

Collection and purification of cord blood CD34+ HPC. Umbilical cord blood samples were obtained according to institutional guidelines. Cells bearing the CD34 antigen were isolated from non-adherent mononuclear fractions through positive selection by indirect immune "panning" using anti-CD34 monoclonal antibody (MoAb) (Immuno-133.3, Immunotech, Marseille, France). A second purification step was performed using a cocktail of MoAbs and immunomagnetic beads (Dynabeads M450, Dynal, Oslo, Norway), as described.

Thus, in all experiments, the isolated cells were 95% to 99% CD34+ as judged by staining with anti-CD34 MoAb.

Purification of peripheral blood dendritic cells and monocytes. Dendritic cells, isolated from peripheral blood following the protocol of Freundenthal and Steinman, were used as a standard population of dendritic cells. Briefly, peripheral blood mononuclear cells were depleted of T cells by rosetting with sheep erythrocytes followed by Ficoll-Hypaque sedimentation, and cultured at 3 × 105 cells per mL at 37°C for 36 hours. After culture, monocytes were removed by two serial 30 to 40 minute adherence steps at 37°C. To recover the adherent monocytes, the dishes were washed three times and
cultured with fresh medium for 3 to 4 hours, at which point the monocytes were easily dislodged. The monocyte and T-cell depleted fraction (5 × 10⁶ cells per mL) was layered onto hypertonic 14.5% metrizamide columns and sedimented at 650g for 10 minutes. The dendritic cell enriched interface was returned to isotonicity after two successive washes with 40 and 25 mmol/L NaCl complete medium solutions. The enriched population representing 0.3% to 0.9% of the starting mononuclear cells was composed of 45% to 75% dendritic cells as judged by morphology and high MHC class II expression and lack of CD14, CD19, and CD16 expression. The major other cells were CD14+ monocytes.

**Purification of adult peripheral blood CD4+ T cells.** Mononuclear cells were isolated from adult peripheral blood and depleted of adherent cells by overnight adherence to plastic, in complete medium (see below), at 1 × 10⁶ cells/mL. CD4+ T lymphocytes were then purified by immunomagnetic depletion using a cocktail of MoAbs IOM2 (CD14), ION16 (CD16), ION2 (HLA-DR) (Immunotech, Marseille, France), NKH1 (CD56), OKT8 (CD8) (Ortho Diagnostic System, Raritan, NJ), 4G7 (CD19) and MoAb 89 (CD40). After two rounds of bead depletion, the purity of CD4+ T cells was routinely higher than 95%.

**Cell cultures in liquid medium of CD34+ HPC.** Cultures were established in the presence of GM-CSF or IL-3 with or without TNFα in endotoxin free medium consisting of RPMI 1640 (GIBCO, Grand Island, NY) supplemented with 10% (vol/vol) heat-inactivated fetal bovine serum (FBS; Flow Laboratories, Irvine, UK), 10 mmol/L HEPES, 2 mmol/L L-glutamine, 5 × 10⁻⁵ mol/L 2-mercaptoethanol, penicillin (100 U/mL), and streptomycin (100 µg/mL; referred to as complete medium). CD34+ cells were seeded for expansion in 24-well culture-plates (Lunbro; Flow Laboratories, McLean, VA) at 2 × 10⁴ cells/mL. Optimal conditions were maintained by splitting these cultures every 4 to 5 days with medium containing fresh factors (cell concentration: 1 to 3 × 10⁶ cells/mL). Cells were routinely collected after 12 days of culture, eventually adherent cells were recovered using a 5-mmol/L EDTA solution.

**Cytocentrifuged cell-surface phenotyping.** Indirect immunofluorescence was performed according to standard techniques, using a panel of murine MoAbs shown by phcoerythrin (PE)-conjugated sheep F(ab')₂; antimonose IgG (Ortho). Double-color fluorescence was performed by sequential incubation of the cells with unconjugated MoAbs, PE-conjugated antimonose IgG, normal mouse serum, and OKT6 (CD1a) (Ortho) or Leu-M3 (CD14) (Becton Dickinson, Sunnyvale, CA) MoAbs directly labelled with fluorescein isothiocyanate (FITC). Negative controls were performed with unrelated murine MoAbs. Fluorescence analysis was determined with a FACScan flow-cytometer (laser setting: power, 15 mW, excitation wavelength 488 nm, Becton Dickinson), and 10,000 to 50,000 events were collected for double staining.

**Fluorescence-activated cell sorter (FACS).** For certain experiments, cells generated from CD34+ HPC after 12 days of culture in the presence of GM-CSF, GM-CSF + TNFα, or IL-3 + TNFα, were collected and labelled with FITC-conjugated OKT6 (CD1a) (Ortho) and PE-conjugated Leu-M3 (CD14) (Becton Dickinson). Cells were separated according to CD1a and CD14 expression into CD1a+ CD14+ and CD1a- CD14+ fractions using a FACSStatplus (laser setting: power, 250 mW, excitation wavelength 488 nm, Becton Dickinson). The procedure of staining and sorting was performed in the presence of 5 mmol/L EDTA to avoid cell aggregation. Reanalysis of the sorted populations showed a purity higher than 98%.

**T-cell proliferation assay.** After 12 days of culture, CD34+ HPC derived cells were collected and, after irradiation (30 Gy), used as stimulator cells for restaging allogeneic adult peripheral blood CD4+ T cells (2.5 × 10⁴ per well). From 10 to 10³ stimulator cells were added to the T cells in 96-well round-bottomed microtissue culture plates (Nunc, Roskilde, Denmark). Cultures were performed in RPMI 1640 medium supplemented with 10% heat inactivated human AB serum, glutamine, and antibiotics as above. After 5 days incubation, cells were pulsed with 1 µCi of ³H-TdR (specific activity 25 Ci/mmol) per well, for the last 8 hours, harvested, and counted. Tests were performed in triplicate and results were expressed as mean counts per minute (cpm). Standard deviations were always below 10%. The levels of thymidine uptake by stimulator cells alone were always below 100 cpm.

**RESULTS**

When combined with TNFα, IL-3, as well as GM-CSF, can induce CD34+ HPC to differentiate into CD1a+ D-Lc. We have recently shown that TNFα directs the GM-CSF-dependent development of CD34+ HPC toward the DC lineage characterized by the expression of the CD1a antigen. As TNFα was also found to synergize with IL-3 to induce the growth/maturity of cord blood CD34+ HPC, we wondered whether IL-3 would, as GM-CSF, allow the generation of CD1a+ D-Lc. Although among DC, CD1a expression is restricted to Langerhans cells, CD1a remains the best antigen available allowing the identification of DC derived from CD34 HPC. As previously described, mainly CD15+ granulocytic cells (range, 31% to 72%, n = 6) were generated in the presence of IL-3 alone, while CD14+ cells (33%, range, 22% to 63%, n = 6) represented the major population after culture in the presence of IL-3 + TNFα (Fig 1). In addition, the subset of cells expressing the CD1a antigen, barely detectable in presence of IL-3 alone (≤ 4%) represented 14% (range, 11% to 17%, n = 6) in the presence of IL-3 + TNFα. As a comparison, GM-CSF alone allowed strong development of CD14+ cells (47%, range, 35% to 61%, n = 10), which is not observed in GM-CSF + TNFα (15%, range, 11% to 25%, n = 10). Conversely, the major development of CD1a+ cells (47%, range, 42% to 78%, n = 10) seen in GM-CSF + TNFα was not detected in GM-CSF alone (8%, range, 7% to 15%, n = 10). Furthermore, 73% (range, 67% to 73%, n = 10) and 86% (range, 70% to 90%, n = 10) of the cells expressing the CD1a antigen in IL-3 + TNFα and GM-CSF + TNFα, respectively, did not bear the CD14 antigen.

In terms of morphology, as observed following May–Grünwald–Giemsa staining, after 12 days of culture, no D-Lc could be detected within cells generated in IL-3 alone, while in the presence of IL-3 + TNFα 8.0% ± 2.0% of the cells were D-Lc (not shown). In presence of GM-CSF 4.3% ± 2.9% were D-Lc compared with 54.0% ± 8.5% in GM-CSF + TNFα (not shown). In addition, the generation of CD1a+ and CD14+ cells, as measured by flow cytometry from CD34+ HPC cultured in the presence of IL-3 + TNFα was not altered by a MoAb to GM-CSF added at the outset of the culture, which, in the same experimental conditions, blocks the GM-CSF dependent proliferation of CD34+ HPC (not shown).

CD1a+ cells differ from CD14+ cells in morphology and phenotype. We further compared the morphology and phenotype of CD1a+ cells generated in IL-3 + TNFα with those generated in GM-CSF + TNFα. GM-CSF + TNFα cultures contain low numbers of CD14+ cells with low intensity of
CD14 expression, and a large proportion of those cells can also express CD1a. The low frequency of these cells did not permit their full characterization. Rather, the CD14<sup>+</sup> cells, which represent a major population in GM-CSF cultures, are mostly CD1a<sup>-</sup> and could be fully characterized. CD14<sup>+</sup>CD1a<sup>-</sup> from IL-3 + TNFα or GM-CSF cultures and CD14<sup>-</sup>CD1a<sup>-</sup> cells from IL-3 + TNFα or GM-CSF + TNFα cultures, were FACS-sorted and analyzed for morphology. As shown in Fig 2, CD14<sup>+</sup>CD1a<sup>-</sup> cells generated either in IL-3 + TNFα or GM-CSF alone appeared as small regularly shaped monocytes (Figs 2A and B), while CD14<sup>-</sup>CD1a<sup>-</sup> cells generated either in IL-3 + TNFα or GM-CSF + TNFα represented an homogeneous population of large cells displaying a characteristic dendritic morphology (Figs 2C and D).

The phenotype, determined by two-color analysis, of the CD14<sup>+</sup> and of the CD1a<sup>-</sup> generated in GM-CSF alone and GM-CSF + TNFα, respectively, was compared with that of CD14<sup>-</sup> and CD1a<sup>-</sup> cells generated in IL-3 + TNFα. Cells cultured for 12 days with either GM-CSF, GM-CSF + TNFα, or IL-3 + TNFα were initially stained using a panel of unlabelled MoAbs shown with PE coupled antimouse Ig, then, after saturation in mouse serum, labelled with FITC coupled CD14 or CD1a. Figure 3 shows the flow cytometry histograms of antibody staining gated on the CD1a<sup>-</sup> cells (in white) and on the CD14<sup>+</sup> cells (in black). Differences in phenotype between CD14<sup>+</sup> and CD1a<sup>-</sup> cells generated in IL-3 + TNFα were similar to those observed between CD14<sup>+</sup> and CD1a<sup>-</sup> cells generated in GM-CSF alone and GM-CSF + TNFα, respectively. Between 10% to 30% of CD1a<sup>-</sup> cells expressed CD14 and less than 10% of CD14<sup>-</sup> expressed CD1a. CD1b was weakly expressed on both subsets, while CD1c was mainly expressed on CD1a<sup>-</sup> cells. While the FCγ receptor RII (CD32) was highly expressed on both cell types, the FCγ RI (CD24) was only expressed on CD14<sup>-</sup> cells. While the complement receptor CR2 (CD21) was negative on both cell types, the CR3 (CD11b) and the CR1 (CD35) were only expressed on
CD14+ cells. Concerning adhesion molecules, while the α chain of the LFA1 (CD11a) was expressed at a similar density on both populations, the β chain (CD18) was expressed at higher levels on CD14+ cells. In contrast, the level of expression of the ligand for leukocyte function-associated 1 antigen (LFA1), intracellular adhesion molecule (ICAM1) (CD54) was much higher on CD14+ cells. LFA3 (CD58) and CD11c were also expressed at higher levels on CD14+ cells. CD4 and CD40 were expressed on both subsets, but the level of expression was higher on CD14+ cells. Finally, HLA class II, CD80, and CD86 molecules were more strongly expressed on CD14+ cells generated in GM-CSF + TNFα than on CD14+ cells generated in IL-3 + TNFα. However, whatever the culture conditions, the levels of HLA class II molecules were 10 to 50-fold higher, that of CD80 (B7/BB-1) 5 to 15-fold higher, and that of CD86 (B70/B7-2) 10 to 20-fold higher on CD14+ cells than on CD14+ cells. Thus, the CD14+ cells generated in the presence of IL-3 + TNFα are similar in morphology and phenotype to those obtained in the presence of GM-CSF + TNFα.

**CD14+ D-Lc generated with IL-3 + TNFα are a strong inducer of alloreactive T-cell proliferation.** As D-Lc are potent stimulators of allogeneic T-cell proliferation, we assayed CD34+ HPC cultured for 12 days in these different conditions for their capacity to induce proliferation of resting allogeneic CD4+ T cells (Fig 4). As shown in Fig 4A, bulk progeny generated in the presence of GM-CSF + TNFα was a much stronger potentiator of allogeneic CD4+ T-cell proliferation than cells arising from HPC cultured in GM-CSF alone. Similarly, although less potent than GM-CSF + TNFα progeny, bulk progeny generated in IL-3 + TNFα was a fairly strong inducer of allogeneic T-cell proliferation compared with that generated in IL-3 alone. H TdR incorporation of $2.5 \times 10^5$ CD4+ T cells is increased 50-fold by $1.5 \times 10^7$ cells (0.68 to 2.4 × 10³) generated in IL-3 + TNFα, $6.5 \times 10^5$ cells (1 to 8 × 10³) generated in GM-CSF, and 100 cells (40 to 250) generated in GM-CSF + TNFα (means and ranges from five experiments). Bulk progeny generated in the presence of GM-CSF + TNFα were compared with a 45% to 75% enriched population of DC isolated from adult peripheral blood (PBL DC) using the protocol of Freudenthal and Steinman. As shown in Fig 4B, GM-CSF + TNFα cells were either as good as or even better than PB-DC in supporting CD4 T-cell proliferation. Furthermore, among
cells generated in IL-3 + TNFα, CD14+ CD1a+ cells appeared to be 50 to 100 times more efficient than CD14+ CD1a+ cells, and 200 to 1,000 times than CD14+ CD1a+ cells in inducing proliferation of resting alloreactive CD4+ T cells (Fig 5A). H TdR incorporation of $2.5 \times 10^6$ CD4+ T cells was increased 50-fold by 37 CD14- CD1a+ cells (range, 12 to 60) or by $2 \times 10^5$ CD14+ CD1a+ cells (range, $1 \times 10^5$ to $5 \times 10^5$). Similarly, as few as 30 CD14+ CD1a+ D-Lc, FACS-sorted from GM-CSF + TNFα cultures induced a strong proliferation of allogeneic CD4+ T cells, while CD14+ CD1a+ cells, FACS-sorted from GM-CSF cultures, were fairly poor antigen presenting cells (Fig 5B) as monocytes purified from peripheral blood (Fig 4B).

Thus, when combined with either IL-3 or GM-CSF, TNFα is able to induce the development of functional CD1a+ D-Lc.

When combined with TNFα, IL-3 yields as many viable CD1a+ D-Lc as does GM-CSF. As IL-3 is a better inducer of CD34+ HPC growth,25 (Fig 6A) than is GM-CSF, the overall yield of CD1a+ D-Lc was followed in cultures performed with or without TNFα. As shown in Fig 6B, CD1a+ cells could be detected from day 6 to day 20 in IL-3 + TNFα cultures of CD34+ HPC. As previously mentioned (Fig 1), whereas less than 4% of CD1a+ cells were observed in the presence of IL-3, 7% to 15% CD1a+ cells were detected with GM-CSF. A higher proportion of CD1a+ cells was detected with IL-3 + TNFα (11% to 17%) and particularly with GM-CSF + TNFα (47% to 78%) (ranges from 10 experiments after 12 days of culture) (Fig 6B). In terms of overall cell yield (Fig 6A), IL-3 + TNFα is twofold to threefold more potent than either IL-3 alone or GM-CSF + TNFα, conditions that are themselves threefold to fourfold more efficient than GM-CSF alone. Thus, as shown in Fig 6C, the curves showing the cumulative numbers of CD1a+ cells obtained with IL-3 alone or with IL-3 + TNFα are nearly superposable to those of GM-CSF and GM-CSF + TNFα, respectively. In fact, starting cultures with $10^5$ CD34+ HPC, in the presence of IL-3 or GM-CSF alone allowed, after 12 days of culture, the generation of $1 \times 10^7$ to $3 \times 10^7$ CD1a+ cells, while those performed with IL-3 + TNFα or GM-CSF + TNFα yielded $2 \times 3 \times 10^7$ CD1a+ cells (ranges from 10 experiments) (Fig 6C).
**DISCUSSION**

The present study aimed at determining whether IL-3 could be used in place of GM-CSF for the in vitro maturation of CD34+ HPC into dendritic/Langerhans cells. As previously reported, IL-3 poorly supported the development of monocytic cells (<10%), but addition of TNFα resulted in their emergence in a significant proportion (28% to 45%). Cells with a dendritic morphology and expressing the CD1a molecule, barely detectable in the presence of IL-3 alone, represented 11% to 17% of cells generated in the presence of IL-3 + TNFα. The percentage of CD1a+ D-Lc detected in the presence of IL-3 + TNFα was three to five times lower than that observed in GM-CSF + TNFα, but as three to four times more cells were generated in the presence of IL-3 + TNFα, the number of viable CD1a+ cells generated in IL-3 + TNFα was nearly identical to that recovered in GM-CSF + TNFα. Furthermore, blocking antibody to GM-CSF does not alter the yield of either CD1a+ cells or CD14+ cells observed in the presence of IL-3 + TNFα, demonstrating that endogenous GM-CSF production was not involved in these cultures (not shown). The CD14+ and CD1a+ cells generated with IL-3 + TNFα were identical in many aspects to CD14+ and CD1a+ cells generated in GM-CSF alone and in GM-CSF + TNFα, respectively. Sorted CD14+ cells generated from HPC cultured with GM-CSF or IL-3 + TNFα appeared in May-Grünwald-Giemsa staining as regularly shaped monocytes and will thus be referred to as CD14+ monocytes. Sorted CD1a+ cells generated with GM-CSF + TNFα or IL-3 + TNFα presented a morphology typical of dendritic cells. CD1a+ D-Lc from GM-CSF + TNFα or IL-3 + TNFα cultures, expressed higher levels of CD1c, CD4, and CD40 than CD14+ monocytes from IL-3 + TNFα and GM-CSF cultures, as described for epidermal Langerhans cells and dendritic cells. As in skin, some CD1a+ cells (10% to 30%) were found to coexpress...
CD14, CD1a+ cells expressed significant levels of FcγRII (CD32) consistent with the description of its expression on skin Langerhans cells. Moreover, CD1a+ cells lack FcγRI (CD64) and complement receptors as previously described, while CD14+ monocytes expressed the FcγRI, the CR1 (CD35), and the CR3 (CD11b). In addition, CD1a+ cells also expressed higher levels of adhesion molecules such as CD11c, CD54, and CD58 than CD14+ monocytes, as described for fresh DC and Langerhans cells. Finally, CD1a+ D-Lc expressed much higher levels of MHC class II molecules and CD28 ligands CD80 (B7/BB-1) and CD86 (B7/BB-2) antigens than CD14+ monocytes, as observed earlier with fresh DC and Langerhans cells. Importantly, CD14+ monocytes from GM-CSF or IL-3 + TNFα cultures (or from fresh peripheral blood) were 50 to 100 times less efficient than CD1a+ D-Lc from GM-CSF + TNFα or IL-3 + TNFα cultures (or fresh peripheral blood DC) in inducing resting alloreactive CD4+ T-cell proliferation, as previously reported for freshly isolated cells and for normal T cells or T cells from T-cell receptor transgenic mice. These findings demonstrate that IL-3 and GM-CSF, when combined with TNFα, allowed similar development of dendritic/Langerhans cells from CD34+ HPC. They further show that CD14+ and CD1a+ cells derived from CD34+ HPC represent populations of cells comparable in morphology, function, and phenotype to purified monocytes and dendritic cells, respectively.

The basis for the overlapping activities of GM-CSF and IL-3 has been elucidated by the analysis of the receptors that are composed of a β chain common to IL-3, GM-CSF, and IL-5 receptors, and an α chain specific for each factor. In this context, the stimulation of early myelopoiesis by TNFα was associated with the upregulation of the β chain on CD34+ HPC. Thus, the crucial role of TNFα in inducing the development of D-Lc might be the recruitment of D-Lc specific progenitors through the upregulation of the β chain.
IL-3 COOPERATES WITH TNFα IN THE GENERATION OF DENDRITIC CELLS

Fig 6. Combined with either IL-3 or GM-CSF, TNFα allows the generation from CD34+ HPC of similar numbers of CD1a+ cells. Cord blood CD34+ HPC were cultured in the presence of IL-3, IL-3 + TNFα, GM-CSF, and GM-CSF + TNFα. At the time point indicated, independent triplicate aliquots of cells were recovered, enumerated by trypan blue exclusion (A), percentage of CD1a+ cells was determined by indirect immunofluorescence (B), and the number of CD1a+ cells was calculated as a percentage of total cell number (C). Results are representative of three experiments, and SD was always below 10%.

of the IL-3/GM-CSF/IL-5 receptor complex. However, TNFα does not enhance the proliferation of CD34+ HPC induced by IL-5 (not shown), suggesting that IL-5 α chain specific receptor is not expressed on the progenitors targeted by TNFα. Alternatively, as TNFα has been involved in the induction of monocytic differentiation of myeloid cell lines4 and leukemic cells,5 its effect on the D-LC generation might reflect an induction of differentiation toward the dendritic cell lineage. The need of TNFα in the generation of DC in humans has been set forth by different groups.11-13 Other groups have reported that IL-4 when combined with GM-CSF allow the development of CD1a+ DC from human peripheral blood adherent mononuclear cells.44-47 Several studies performed in mice reported the development of DC from peripheral blood or bone marrow progenitors in the presence of GM-CSF alone.47 Thus, a central role of GM-CSF on the generation of dendritic cells is in line with the reported key role of GM-CSF in the development of accessory function of Langerhans cells.23,24 However, mice with a disrupted GM-CSF gene have been reported to have DC and Langerhans cells in normal numbers,49 suggesting that other factors, eg, IL-3, can substitute for GM-CSF. However, IL-3 has not been reported to activate mature Langerhans cells or DC, suggesting a potential loss of the IL-3 receptor α chain during DC maturation.

The redundancy of the activity of IL-3 and GM-CSF, strengthened by our observations on D-Lc development, is not unique and is also observed with IL-4 and IL-1350 or TNFα and TNFβ.51 Whereas GM-CSF is produced by many cell types including fibroblasts, keratinocytes, monocytes, and T lymphocytes, the production of IL-3 is restricted to antigen stimulated T cells, activated mast cells, and keratinocytes.14-15 Therefore, IL-3 is supposed to play a major role in antigen-induced hematopoiesis, while GM-CSF might predominantly be involved in constitutive hematopoiesis. As DC represent key cells involved in antigen specific immunity, their development during an ongoing immune response under the production of IL-3 (or GM-CSF), and TNFα by activated T cells or mastocytes might be crucial.

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REFERENCES


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41. Takeda K, Iwamoto S, Sugimoto H, Takuma T, Kawatani N,


45. Porcelli S, Morita CT, Brenner MB: CD1b restricts the response of human CD4-8-T lymphocytes to a microbial antigen. Nature 360:593, 1992


49. Zurawski G, de Vries JE: Interleukin 13, an interleukin 4-like cytokine that acts on monocytes and B cells, but not on T cells. Immunol Today 15:19, 1994


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