Dendritic cells (DC) can be generated by culture of adherent peripheral blood (PB) cells in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-4 (IL-4). There is controversy as to whether these DC arise from proliferating precursors or simply from differentiation of monocytes. DC were generated from myeloid-enriched PB non-T cells or sorted monocytes. DC generated from either population functioned as potent antigen-presenting cells. Uptake of [3H]thymidine was observed in DC cultured from myeloid-enriched non-T cells. Addition of lipopolysaccharide or tumor necrosis factor-alpha led to maturation of the DC, but did not inhibit proliferation. Ki67+ cells were observed in cytopsins of these DC, and by double staining were mononuclear cell culture was relatively immature, differentiation could be induced by inclusion of TNF-alpha or CD40-ligand, or monocyte-conditioned medium late in the culture period. A number of studies concluded that CD14+ monocytes gave rise to DC in the presence of GM-CSF and interleukin-4. Despite this consensus, there has been contention as to whether monocyte-derived DC proliferate in the presence of GM-CSF and IL-4. While it was initially suggested that these DC were derived from proliferating precursors, subsequent investigations failed to detect evidence of proliferation. The current studies examined whether DC derived from monocytes had the potential to proliferate under various conditions, and whether DC differentiation was associated with a reduced proliferative capacity. These studies show that proliferation in these cultures results from small numbers of contaminating progenitor cells, and not proliferation of DC or their monocyte precursors.

MATERIALS AND METHODS

Culturing medium. All cells were cultured in RPMI 1640 (GIBCO, Life Technologies, Mulgrave, VIC, Australia) supplemented with 10% fetal calf serum (FCS; CSL Ltd, Parkville, VIC, Australia), 0.3 mg/mL L-glutamine (Trace Biosciences, Castle Hill, NSW, Australia), 0.12 mg/mL benzylpenicillin ( CSL) and 10 µg/mL gentamicin (Delta West, Pharmacia and Upjohn, Spring Hill, QLD, Australia) at 37°C in 5% CO2-in-air.

Monoclonal antibodies (MoAbs). The MoAbs used in this study are shown in Table 1.

Cell isolation. PB mononuclear cells (PBMC) were prepared as described. Briefly, mononuclear cells were isolated from either heparinized venous blood from healthy volunteers or from buffy coats (Red Cross Blood Transfusion Service, Brisbane, Australia) by density gradient centrifugation over Ficoll-Paque (Pharmacia Biotech, Upsala, Sweden) at 200 g for 45 minutes. PBMC were washed and incubated with neuraminidase-treated sheep erythrocytes. The rosetting fraction (ER+, T cells) was separated from the nonrosetting fraction (ER−, non-T cells) by density gradient centrifugation on Ficoll-Paque; remaining red blood cells (RBCs) were removed by 1 mol/L ammonium chloride lysis, and cells were washed. Myeloid-enriched non-T cells were produced by depletion of T, B, and natural killer (NK) cells from non-T cells, by incubation with MoAb against CD19, CD16, and CD3 (OKT3), followed by goat anti-mouse Ig magnetic beads (Miltenyi Biotech, San Francisco, CA), passage through a separating column in a strong magnetic field (MACS; Miltenyi Biotech), and collection of the unbound fraction. Eighty-five percent to 95% of the negatively selected
populations of monocytes, DC, and B cells were isolated by cell sorting. CD33. 23 fraction were dendritic cell precursors or monocytes that expressed PROLIFERATION IN DENDRITIC CELL CULTURES 1599

monocyte-derived DC as described. 10 Briefly, 5 106 PBMC were cultured with cytokines was determined by [ 3 H]-TdR incorporation. Detection of cyclin-dependent kinase-1 (cdk 1 ) by immunoblotting.

Mixed lymphocyte reaction (MLR). Harvested APC were washed and resuspended at 5 106 cells/mL in medium containing 0.08 mg/mL mitomycin C (Sigma) to inhibit cell proliferation. Cells were incubated at 37°C in the dark for 20 minutes, then washed three times in Hanks’ balanced salt solution (HBSS), Various numbers (102 to 104 per well) of APC were incubated in triplicate in round-bottom 96-well tissue culture plates (Costar) with 105 allogeneic T cells for 5 days at 37°C. DNA synthesis was measured by incorporation of [ 3 H]-thymidine ([ 3 H]-TdR 1 µCi/well; Amersham, Buckinghamshire, UK) added during the final 18 hours of the culture period. Cells were harvested onto glass fiber filters by an automated cell harvester (LKB Wallac, Turku, Finland), and incorporation of [ 3 H]-TdR was determined by liquid scintillation spectroscopy. Results are expressed as the mean cpm ± SD.

In vitro differentiation of DC. Cells cultured in the presence of GM-CSF and IL-4 were stimulated in vitro by the addition of 100 U/mL TNF-α (National Institute for Biological Standards and Control, Hertfordshire, UK) or 1 µg/mL lipopolysaccharide (LPS) (Sigma) for the final 24 hours of the culture period.

Immunophenotyping of cells by flow cytometry. Freshly isolated or cultured cells were stained with either FITC- or biotinylated-MoAb or unconjugated primary MoAb (Table 1) on ice for 30 minutes, then washed. Incubation with unlabeled MoAb was followed with biotinylated-rabbit anti-mouse IgG (DAKO), and finally streptavidin-conjugated-FITC (SA-FITC; DAKO) for 30 minutes. Biotinylated-MoAb were followed by SA-FITC as described. Cells were analyzed using an EPICS Elite ESP flow cytometer (Coulter) and data analyzed using Winlist software (Verity Software House, Topsham, ME).

CD34+ progenitor cell determination by flow cytometry. Enumeration of progenitor cells in freshly prepared or cultured myeloid-enriched non-T cells was performed by flow cytometry according to ISHAGE guidelines. 25 Briefly, cells were labeled with CD45-FITC and CD34-PE, and analyzed by sequential gating using side scatter and fluorescence properties of at least 50,000 events.

Cell proliferation assay. In some experiments, proliferation of APC cultured with cytokines was determined by [ 3 H]-TdR incorporation. Cytokine-containing medium, then plated at varying cell numbers (104 to 106) per well in medium without cytokines. Control cells were treated with mitomycin C before culture. All wells were pulsed immediately with [ 3 H]-TdR, cultured for 18 hours, then harvested as described. Cell proliferation is expressed as mean cpm ± SEM for triplicate wells.

CD34+ progenitor cells were identified by the expression of CD34 and CD133 antigens and the absence of CD19 and CD56.

Selection of cells by cell sorting. For some experiments, pure populations of monocytes, DC, and B cells were isolated by cell sorting. For B cells, T and NK cell-depleted ER+ cells were incubated with CD19-phycocerythrin (PE) for 30 minutes on ice, then washed. For DC and monocytes, T, and NK cell-depleted non-T cells were incubated with CD14-fluorescein isothiocyanate (FITC) and CD33-PE. Cells were sorted using an EPICS Elite ESP flow cytometer (Coulter Electronics, Hialeah, FL) by gating on either CD19 or CD34 and monocytes, T, B, and NK cell-depleted non-T cells were incubated with CD45-FITC and CD34-PE.

In some experiments, a gate was set to include all cells. These mock-sort cells were used as a control for the sorting procedure.

Culture of PBMC and monocytes. PBMC were cultured to produce monocyte-derived DC as described. 10 Briefly, 5 106 PBMC were cultured in 1.5 mL medium per well of a 24-well plate (Costar Corp, Cambridge, MA). After 2 hours the cells were gently agitated and the nonadherent cells removed. In preliminary experiments, adherent PBMC were cultured in the presence of 800 U/mL GM-CSF and various doses of IL-4. Optimum APC function was observed if the cells were cultured in the presence of either 400 or 500 U/mL IL-4 (data not shown). The adherent fraction was therefore cultured in fresh medium containing 400 U/mL IL-4 (Sigma) and 800 U/mL GM-CSF (Schering-Plough Pty Ltd, Baulkam Hills, NSW, Australia) for 2 to 7 days. Myeloid-enriched non-T cells or sorted monocytes were cultured similarly in 1.5 mL medium containing IL-4 and GM-CSF. Approximately 2 106 cells were cultured per well in 24-well tissue culture plates for 2 to 14 days. After culture, the wells were washed with several changes of phosphate-buffered saline (PBS) to harvest the loosely adherent cells.

Preparation of T cells. T cells were isolated by collecting ER+ cells that passed through a nylon wool column, followed by magnetic immunodepletion of cells expressing CD4+, CD16+, CD19+, or HLA-DR as described above. Greater than 98% of the recovered cells expressed CD3 by flow cytometric analysis.

Table 1. MoAbs Used in This Study

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clone</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD1a</td>
<td>NA1/34</td>
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<td>ATCC</td>
</tr>
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<td>CD11c</td>
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<td>cdki</td>
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<tr>
<td>p55</td>
<td>SK2</td>
<td>Gift from E. Langhoff</td>
</tr>
</tbody>
</table>

*P. E. Lipsky, UT Southwestern Medical Center, Dallas, TX. †D.N.J. Hart, Christchurch Hospital, Christchurch, New Zealand. ‡E. Langhoff, Penn State College of Medicine, Hershey, PA.

fraction were dendritic cell precursors or monocytes that expressed CD33. 23
The presence of GM-CSF and IL-4. To compare the functional either freshly isolated or cultured PBMC give rise to DC in the previously been shown that CD14^{bright} monocytes developed potent APC function after prolonged incubation in the presence of GM-CSF and IL-4. It has been observed that CD14^{+} monocytes sorted from either freshly isolated or cultured PBMC give rise to DC in the presence of GM-CSF and IL-4.2,18,20 To compare the functional outcome of DC generated from sorted monocytes, myeloid-enriched non-T cells, or PBMC, these cell populations were cultured for 4 days in the presence of GM-CSF and IL-4, and their APC function assessed in the allogeneic MLR. As shown in Fig 1A, all preparations of cells gave rise to APC of comparable function. Furthermore, as shown in Fig 1B, only CD14^{bright} monocytes cultured in GM-CSF and IL-4 exhibited APC function comparable to unfractionated or mock sorted PBMC. Neither the CD14^{dim} fraction nor CD19^{+} B cells were effective APC after prolonged incubation in the presence of GM-CSF and IL-4. The data show that monocytes give rise to APC after incubation in GM-CSF and IL-4, and that monocytes do not require the presence of other mononuclear cells to differentiate into functional APC in the presence of GM-CSF and IL-4. Because there were no functional differences observed between DC derived from any of these starting populations, myeloid-enriched non-T cells (B and NK-depleted ER^−) were used for the remaining experiments.

The previous experiments indicated that the presence of lymphocytes in the starting population did not influence the

**RESULTS**

**CD14^{bright} monocytes are responsible for the potent allostimulatory function of PBMC cultured in GM-CSF and IL-4.** Adherent PBMC develop potent APC function after prolonged incubation in the presence of GM-CSF and IL-4. It has been previously shown that CD14^{+} monocytes sorted from either freshly isolated or cultured PBMC give rise to DC in the presence of GM-CSF and IL-4. To compare the functional outcome of DC generated from sorted monocytes, myeloid-enriched non-T cells, or PBMC, these cell populations were cultured for 4 days in the presence of GM-CSF and IL-4, and their APC function assessed in the allogeneic MLR. As shown in Fig 1A, all preparations of cells gave rise to APC of comparable function. Furthermore, as shown in Fig 1B, only CD14^{bright} monocytes cultured in GM-CSF and IL-4 exhibited APC function comparable to unfractionated or mock sorted PBMC. Neither the CD14^{dim} fraction nor CD19^{+} B cells were effective APC after prolonged incubation in the presence of GM-CSF and IL-4. The data show that monocytes give rise to APC after incubation in GM-CSF and IL-4, and that monocytes do not require the presence of other mononuclear cells to differentiate into functional APC in the presence of GM-CSF and IL-4. Because there were no functional differences observed between DC derived from any of these starting populations, myeloid-enriched non-T cells (B and NK-depleted ER^−) were used for the remaining experiments.

The previous experiments indicated that the presence of lymphocytes in the starting population did not influence the
function of the DC generated by culture of adherent PBMC in the presence of GM-CSF and IL-4. To determine whether phenotypic differences occurred, DC were generated in the presence of GM-CSF and IL-4 for 7 days from either adherent PBMC or sorted monocytes. DC were stained with various markers of differentiation and analyzed by flow cytometry. As shown in Fig 2, CD11b was not expressed by either DC population, and CD14 expression was low. CD2 expression was associated with DC generated from PBMC, but not from monocytes, due to contaminating T cells within the cultures. In the case of DC derived from PBMC, these CD3+ T cells were noted to be clustered around DC by confocal microscopy (data not shown). The major histocompatibility complex (MHC) class II molecules HLA-DR and HLA-DQ were expressed by both DC populations. However, DC derived from PBMC also contained MHC class II-lymphocytes. Although CD40 and CD1a were expressed by both DC populations, CD80 and the DC maturation marker CMRF-44 were not. CD86 was expressed by a small percentage of monocyte-derived DC, and a somewhat greater percentage of DC derived from PBMC. The data show that DC derived from monocytes and PBMC exhibit different degrees of purity, and that adherent PBMC-derived DC express higher levels of CD86 than sorted monocyte-derived DC.

Evidence of proliferation within cultures of monocyte-derived DC. The next experiments were performed to determine whether monocyte-derived DC are generated by differentiation from nonproliferating monocytes, or whether a process of proliferation with subsequent differentiation is involved. Myeloid-enriched non-T cells were cultured for 4 days with or without cytokines, then washed, and incubated in medium containing [3H]-TdR for 18 hours. Control cells were treated with mitomycin C to inhibit cell proliferation. As shown in Fig 3, proliferation was detectable only in cultures treated with GM-CSF and IL-4. The low levels of [3H]-TdR uptake suggest either that the cell turnover was low, or that only a subpopulation of cells was indeed proliferating.

To determine whether DC progenitor cells stopped proliferating as they differentiated, myeloid-enriched non-T cells were cultured for 6 days in medium supplemented with GM-CSF in the presence or absence of IL-4. In some wells either LPS (1 µg/mL) or TNF-α (100 U/mL) was added for the final 24 hours of culture. As shown in Fig 4, cells cultured with GM-CSF in the presence or absence of IL-4 incorporated [3H]-TdR, and this capacity was not inhibited by either LPS or TNF-α. Donor-to-donor variability was observed in these assays, as illustrated in the three representative experiments illustrated in Figs 3 and 4. The [3H]-TdR incorporation was particularly high in some cultures in the presence of GM-CSF alone. The data show low-level proliferation of cells in cultures supplemented by GM-CSF and IL-4. Of importance, LPS and TNF-α, which induce DC differentiation, do not inhibit proliferation of those cells. In data not shown, [3H]-TdR uptake was demonstrated after 2, 4, 6, and 9 days in culture, and addition of LPS at early and late time points did not alter the proliferative capacity of the cells.

To confirm that the addition of LPS induced DC differentiation regardless of the time of addition, myeloid-enriched non-T cells were analyzed by flow cytometry after either 2 or 7 days in culture. The phenotype of each cell population was compared with that of freshly isolated myeloid-enriched non-T cells (Table 2). Cells were cultured either in medium, or in the presence of GM-CSF, GM-CSF and IL-4, or GM-CSF and IL-4 with LPS added for the final 24 hours of incubation. Nearly all
freshly isolated myeloid-enriched non-T cells expressed high levels of CD14, and the majority of cells expressed HLA-DR but not HLA-DQ, CD86, or CD1a. In culture medium alone, or with additional GM-CSF, 45% to 65% of monocytes retained surface expression of CD14 at 7 days. CD14 expression was somewhat downregulated by the addition of IL-4, and LPS had little additional effect. While CD1a, CD86 and MHC class II molecules were expressed late in the cultures, LPS addition particularly after 2 days upregulated their expression. The most marked effect of LPS after 7 days was the upregulation of CD86 expression. The data indicate that LPS enhances DC differentiation whether added early or later to cultures containing GM-CSF and IL-4. Taken together with the previous experiments, it is clear that in the presence of GM-CSF, IL-4, and LPS, proliferation and DC differentiation occur simultaneously.

To confirm that cells were capable of proliferation in cultures of monocyte-derived DC, the next experiments examined the activity of cyclin-dependent kinase 1 (cdk1)—an intracellular protein expressed by cycling cells—and the expression of Ki67. Expression of cdk1 was determined by immunoblotting. Detergent-solubilized cytoplasmic lysates from 10^5 myeloid-enriched non-T cells that were either freshly isolated or that had been cultured for 4 days with additional cytokines were prepared. Cdk1 was detected in all samples (Fig 5). However, by comparison with equivalent cell numbers of the cycling cell line, BJAB, the levels of cdk1 protein in freshly isolated or cultured myeloid cells were relatively low. These data show that an essential cyclin-dependent kinase is expressed at low levels by freshly isolated and by cultured myeloid-enriched non-T cells. Thus, although cdk1 expression is observed in cultured cells, stimulation of proliferation by GM-CSF and IL-4 is unlikely to be at the level of cdk1 induction.

The low levels of [3H]-TdR uptake and of cdk1 might indicate either a low cell turnover, or a small percentage of cells with a high level of proliferation. To determine which cells were proliferating, expression of Ki67, a nuclear antigen, that is absent in G_0, was examined by immunohistochemical staining of cytospins. Figure 6A represents Ki67 staining of cells cultured in the presence of GM-CSF and IL-4 for varying lengths of time. Very occasional nuclear Ki67^+ cells (brown, 0.78%) were observed among freshly isolated cells. Although the frequency of Ki67^+ cells increased during culture, only a small percentage of cells was cycling at any time sampled. Similar numbers of cycling cells were found in cultures that contained LPS. Taken together with the previous data, these results indicate that proliferation was independent of DC.
differentiation. In accordance with the \[ ^3H \]-TdR incorporation, culture with GM-CSF was associated with an increased proportion of Ki67$^+$ cells (data not shown). From these data, it is likely that few, if any, DC or DC precursors were proliferating.

To investigate the phenotype of the Ki67$^+$ cells, a variety of lineage and DC differentiation markers was examined in combination with Ki67. In each case, the Ki67 staining is represented in brown, and the other marker in red (Fig 6B). Although occasional CD3$^+$ T cells and CD20$^+$ B cells were identified, no lymphocyte colocalized Ki67. DC differentiation markers CD40, CD1a, CD11c, and p55 were expressed by large numbers of cells and their expression was upregulated by the addition of LPS. However, Ki67 did not colocalize with any of these markers. From these data it is evident that the proliferating cells were neither residual T or B cells, nor DC.

The nuclear morphology of Ki67$^+$ cells in the cytospins was reminiscent of early myeloid progenitors. Moreover, cells with this appearance were myeloperoxidase$^+$ (data not shown). Because myeloperoxidase is an early marker of the myeloid lineage, the presence of CD34$^+$ stem cells in the myeloid-enriched cell fraction was investigated by flow cytometry. Whereas CD34$^+$ cells constituted 0.13% to 0.15% of PBMC, 0.27% to 0.75% of the myeloid-enriched cells were CD34$^+$, representing a twofold to fivefold enrichment. By three-color flow cytometry the myeloid-enriched population contained approximately 0.72% (range, 0.32% to 1.15%) CD34$^+$ cells, of which none expressed either CD33 or CD14. The data indicate that progenitor cells are present in the myeloid-enriched non-T fraction, and that their frequency varies between donors. CFU-GM assays were performed to determine their myeloid colony-forming potential. Freshly isolated myeloid-enriched non-T cells gave rise to many CFU-GM colonies, whereas myeloid-enriched cells that had been cultured previously for 11 days in the presence of GM-CSF and IL-4 did not produce colonies in this assay; rather, only single viable cells were observed across the plates. To confirm that CD34$^+$ cells were proliferating in these cultures, CD34$^+$ cells were separated by immunomagnetic beads from myeloid-enriched non-T cells. The CD34-enriched fraction, the CD34-depleted, and the starting populations were each cultured in the presence of GM-CSF and IL-4. After 6 days, unfractionated cells contained 1.7%, CD34-depleted fraction contained 0.6%, and the CD34-enriched cells contained 5.5% Ki67$^+$ cells.

To establish that proliferating progenitor cells did not influence...
ence the yield of monocyte-derived DC, either myeloid-enriched non-T cells, or these cells pretreated with mitomycin C, were cultured with GM-CSF and IL-4 for 7 days, then analyzed by flow cytometry, or in the allogeneic MLR. As shown in Table 3, the yield of DC was unaffected by mitomycin C treatment. The data indicate that the DC yield in cultures of myeloid-enriched cells ranges from 50% to 70% of the starting population, and is not dependent on proliferating progenitors. Furthermore, the APC function of DC derived from the mitomycin-pretreated cells was equivalent to that of mock-treated cells (Fig 7).

DISCUSSION

In the presence of GM-CSF and IL-4, PB blood monocytes differentiate in vitro into cells with the phenotype and function of DC. The initial identification of proliferating DC precursors in adherent blood cultures supplemented with GM-CSF and IL-4 suggested the possibilities that PB monocytes may be induced to proliferate under these conditions, and that large yields of DC might be generated by expansion from such precursors. Therefore, the current studies were performed to determine the nature of the proliferating cells in such cultures. Initially, the function and phenotype of DC generated from
adherent PBMC, myeloid-enriched non-T cells and sorted monocytes were compared directly. Whereas DC generated from either pure monocytes or adherent starting populations were similar in function, phenotypic differences were detected in that the DC generated from adherent PBMC expressed higher levels of CD86 than DC derived from sorted monocytes. In view of the clusters of CD3^+ T cells noted around DC derived from PBMC, it is likely that these T cells contributed to DC activation by CD40 ligation.13,29-31 In previous reports, monocyte-derived DC have varied widely in the expression of CD86. At least one factor in this variability is likely to relate to the presence or absence of T cells in the starting population.

The studies described here show that CD34^+CD33^- progenitor cells, capable of giving rise to CFU-GM under appropriate conditions, were responsible for the low levels of proliferation in cultures of monocyte-derived DC. Ki67 expression and [^3]H-TdR incorporation was evident over several days of incubation. Although there was some variability between donors, the addition of GM-CSF was generally required for the detection of a proliferative response. There was no enhancement of this response with IL-4. Rather, IL-4 had a greater effect on the phenotype of the monocytes in culture and the development of DC, as has been previously described.16,32-34 In contrast, DC generated from CD34^+ precursors from BM or blood require the addition of GM-CSF and TNF-α. TNF-α, in particular, is likely to be required for the initial phase of DC development.35-37 Subsequently, exclusion of TNF-α and addition of IL-4 leads to optimal development of DC.33,34 The inability of 11-day-cultured cells to generate CFU-GM, despite ongoing proliferation, indicates that myeloid progenitors differentiated in vitro. This notion is supported by the increasing numbers of cells expressing myeloperoxidase during culture. In addition, IL-4 has been shown to strongly inhibit the development of CFU-GM, but only weakly inhibits granulocyte development.38 It is unlikely that these proliferating precursors would develop into DC in the absence of other cytokines, such as TNF-α, which must be included in CD34^+ cell cultures for prolonged periods to generate DC.2,3,5 The proliferative response observed in cultures of myeloid-enriched cells is consistent with previous reports of proliferating cells in cultures of adherent PBMC.10 In contrast, other studies that used pure, or semi-purified monocyte populations, found no evidence of proliferation.14,19,21 These data are all consistent with the idea that myeloid progenitors proliferate in response to GM-CSF in these cultures. In the current studies, the yield of DC generated from either monocytes or myeloid-enriched non-T cells was not altered by blockade of mitosis by mitomycin C. These data indicate that nonproliferating precursors give rise to DC in these cultures. This has important implications for the generation of monocyte-derived DC using GM-CSF and IL-4 alone, for clinical trials. First, the maximum theoretical yield of DC can be predicted from the monocyte count of each individual donor. Second, in the absence of other cytokines that might induce differentiation of the myeloid progenitor cells, the DC generated cannot be expanded beyond the starting monocyte input.

Based on these results, it is interesting to consider whether DC arising from proliferating cultures of murine blood and BM are indeed derived from stem cells39,40 or whether they differentiate from a relatively more mature cell, particularly in the absence of growth factors other than GM-CSF. In contrast to these mixed cultures, the development of DC from proliferating precursors has been more readily shown in cultures of purified human CD34^+ cells or murine thymic precursors,7,35,37,41-43 In general, however, these cultures require more stringent growth factor supplementation. Recently, human CD34^+ progenitor cells derived from cord blood or BM have been shown to proliferate up to 25-fold in cultures containing GM-CSF and TNF-α.37,44,45 Furthermore, using a two-step culture technique, DC precursors were observed to proliferate at early timepoints. However, two distinct populations of DC precursors were evident after 5 days in culture, and neither showed any proliferative capacity.35 These data suggest a biphasic differentiation of DC from progenitors whereby cells proliferate early, but lose this capacity after lineage commitment. Of interest, one of the DC precursor populations could be identified by cell-surface expression of CD14. From this precursor it was

Table 3. Yield and Phenotype of DC Generated After 7-Day Culture in the Presence of GM-CSF and IL-4

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<thead>
<tr>
<th>Mitomycin C Treatment</th>
<th>Donor 1</th>
<th>Donor 2</th>
<th>Donor 3</th>
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<tr>
<td>Total yield (×10^6/well)</td>
<td>6.5</td>
<td>6.7</td>
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<tr>
<td>% Recovery</td>
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<tr>
<td>DC yield (% total cells)</td>
<td>46</td>
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<td>59</td>
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% Positive Recovered Cells

<table>
<thead>
<tr>
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<th>HLA-DR</th>
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<td>Donor 3</td>
<td>48</td>
<td>5</td>
<td>36</td>
<td>53</td>
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Freshly isolated cells were treated with or without mitomycin C then cultured. The total yield was calculated based on a starting population of 2 × 10^6 cells/well. The DC yield was calculated by flow cytometry, based on forward and side scatter properties.10
possible to generate potent APC in the presence of GM-CSF and TNF-α, and macrophage-like cells in the presence of M-CSF.\textsuperscript{46} As has been shown for circulating monocytes, these CD14\textsuperscript{+} cells were unable to proliferate in response to a range of cytokines including GM-CSF and M-CSF. However, in contrast to monocytes, this macrophage-like cell was not dependent on IL-4 for APC function.\textsuperscript{71,15,46}

Taken together, the previous and current studies indicate that the generation of DC from PB monocytes by culture with GM-CSF and IL-4 does not involve cell proliferation, but rather the generation of DC from PB monocytes. This macrophage-like cell was not dependent on cytokines including GM-CSF and M-CSF. However, in contrast to monocytes, this cell was unable to proliferate in response to a range of cytokines, including GM-CSF and M-CSF. As has been shown for circulating monocytes, these CD14\textsuperscript{+} cells were unable to proliferate in response to a range of cytokines, including GM-CSF and M-CSF. However, in contrast to monocytes, this macrophage-like cell was not dependent on IL-4 for APC function.\textsuperscript{71,15,46}

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