Check for updates

Proliferation in Monocyte-Derived Dendritic Cell Cultures Is Caused by Progenitor Cells Capable of Myeloid Differentiation

By Lois L. Cavanagh, Russell J. Saal, Karen L. Grimmett, and Ranjeny Thomas

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 [^{3}H]-thymidine was observed in DC cultured from myeloid-enriched non-T cells. Addition of lipopolysaccharide or tumor necrosis factor-α led to maturation of the DC, but did not inhibit proliferation. Ki67$^+$ cells were observed in cytospins of these DC, and by double staining were CD3$^-$CD19$^-$CD11c$^-$CD40$^-$ and myeloperoxidase$^+$, suggesting that they were myeloid progenitor cells. Analysis of the starting population by flow cytometry demonstrated small numbers of CD34$^+$CD33$^-$CD14$^-$ progenitor cells, and numerous granulocyte-macrophage colony-forming units were generated in standard assays. Thus, production of DC in vitro from adherent PB cells also enriches for progenitor cells that are capable of proliferation after exposure to GM-CSF. Of clinical importance, the yield of DC derived in the presence of GM-CSF and IL-4 cannot be expanded beyond the number of starting monocytes.

© ***1998 by The American Society of Hematology.***

Downloaded from http://ashpublications.net/blood/article-pdf/92/5/1598/1647175/1598.pdf by guest on 18 May 2024

DENDRITIC CELLS (DC) are derived from CD34$^+$ progenitors in the bone marrow (BM).[1] Human peripheral blood (PB) DC are closely related to monocytes. Their common precursor can be identified by hematopoietic progenitor colony-forming assay, and is derived from the common granulocyte-macrophage colony-forming unit (CFU-GM).[2,3] DC circulate in the blood in very small numbers, and migrate into various tissues as a part of steady-state traffic. After contact with antigen, DC migrate to draining lymph nodes, where they undergo functional maturation to become specialized antigen-presenting cells (APC). DC are capable of activating naive T cells in the initiation of immune responses,[4] and therefore are desirable APC for clinical adoptive immunotherapy.

Investigations into the ontogeny of DC led to the observation that purified CD34$^+$ stem cells derived from either BM or blood could be differentiated in vitro into functional APC by the addition of the cytokines tumor necrosis factor-α (TNF-α) and granulocyte-macrophage colony-stimulating factor (GM-CSF) to the culture medium.[2,3,5] The yield of DC generated from CD34$^+$ precursors could be augmented with c-*kit* ligand,[6,7] or more effectively by a complex cocktail of cytokines, including c-*kit* ligand, transforming growth factor-β1, and flt3-ligand.[8,9] However, alternative techniques have been developed for the generation of functional DC by the culture of either adherent blood or BM cells in the presence of GM-CSF and IL-4.[10-12]

Although the phenotype of DC derived from adherent mononuclear cell culture was relatively immature, differentiation could be induced by inclusion of TNF-α or CD40-ligand, or monocyte-conditioned medium late in the culture period.[13-15] A number of studies concluded that CD14$^+$ monocytes gave rise to DC in the presence of GM-CSF and interleukin-4 (IL-4).[16-21] 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,[10,13] subsequent investigations failed to detect evidence of proliferation.[21] 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

Culture 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% CO_2-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.[22] 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, Uppsala, Sweden) at 170*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

From the Centre for Immunology and Cancer Research, the Department of Medicine, University of Queensland; and the Department of Haematology, Princess Alexandra Hospital, Queensland, Australia.

Submitted December 1, 1997; accepted May 4, 1998.

Supported by grants from the Queensland Cancer Fund, the National Health and Medical Research Council of Australia. R.T. is supported by the Arthritis Foundation of Queensland.

Address reprint requests to Ranjeny Thomas, MD, University of Queensland, Department of Medicine, Princess Alexandra Hospital, Woolloongabba QLD 4102, Australia.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" *in accordance with 18 U.S.C. section 1734 solely to indicate this fact.*

© 1998 by The American Society of Hematology.

0006-4971/98/9205-0036$3.00/0

Table 1. MoAbs Used in This Study

Antibody	Clone	Source
CD1a	NA1/34	DAKO (Australia), Botany, NSW
CD3	OKT3	American Type Culture Collection (ATCC), Rockville, MD
CD3	CD3	DAKO
CD3-ECD	HIT3A	Coulter Immunotech, Brookvale, NSW, Australia
CD11b	OKM1	ATCC
CD11c	3.9	Ancell Corp, Bayport, MN
CD14	63D3	ATCC
CD14-FITC	Leu-M3	Becton Dickinson (BD), Lane Cove, NSW, Australia
CD14-PE-Cy5	RMO52	Coulter Immunotech
CD16	Leu-11b	BD
CD19	Leu-12	BD
CD19-PE	SJ25-C1	Sigma Chemical Co, Castle Hill, NSW, Australia
CD20	L26	DAKO
CD33-PE	Leu-M5	BD
CD34-FITC/PE	8G12	BD
CD40	G28.5	Gift from P. Lipsky*
CD45-FITC	2D1	BD
CD56	Leu-19	BD
CD80	BB1	Ancell
CD86	IT2.2	Pharmingen, San Diego, CA
cdk_1	cdc2 p34	Santa Cruz Biotechnology, Santa Cruz, CA
CMRF-44	CMRF-44	Gift from D. Hart†
HLA-DR	L243	ATCC
HLA-DR-FITC	HK14	Sigma
HLA-DQ	BU46	The Binding Site, San Diego, CA
Ki67	MIB-1	Coulter Immunotech
p55	55K2	Gift from E. Langhoff‡

*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]

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-phycoerythrin (PE) for 30 minutes on ice, then washed. For DC and monocytes, T, B, 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 $CD19^+$ cells (B cells), or $CD33^+CD14^{bright}$ cells (monocytes) or $CD33^+CD14^{dim}$ cells (DC) as previously described.[24] In some experiments a gate was set to include all cells. These mock-sorted 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, 5×10^6 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 2×10^6 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 CD14, CD16, CD19, or HLA-DR as described above. Greater than 98% of the recovered cells expressed CD3 by flow cytometric analysis.

Mixed lymphocyte reaction (MLR). Harvested APC were washed and resuspended at 5×10^6 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 (10^2 to 10^4 per well) of APC were incubated in triplicate in round-bottom 96-well tissue culture plates (Costar) with 10^5 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 filtermats 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. Cultured cells were washed three times with PBS to remove residual cytokine-containing medium, then plated at varying cell numbers (10^4 to 10^5) 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.

Detection of cyclin-dependent kinase-1 (cdk_1) by immunoblotting. Cytoplasmic lysates were prepared from cells after varying times in culture with the various cytokine combinations. Cells were resuspended at 2×10^5 cells/5 µL of lysis buffer (0.1% Triton-X-100 in Tris buffer), incubated on ice for 20 minutes with occasional vortexing, then spun at 1,500 rpm for 10 minutes at 4°C. The supernatant was collected and either stored at −20°C for later use, or mixed with an equivalent volume of reduced Laemmli loading buffer (Bio-Rad Laboratories Pty Ltd, Regents Park, NSW, Australia) and boiled for 2 to 3 minutes. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed using a Bio-Rad Protean II minigel apparatus.[26] Proteins were first run through a 5% acrylamide stacking gel, then separated in a 12% acrylamide separating gel. Transfer of proteins from SDS-polyacrylamide gels onto nitrocellulose membrane (Hybond-C; Amer-

Downloaded from http://ashpublications.net/blood/article-pdf/92/5/1598/1647175/1598.pdf by guest on 18 May 2024

sham) was performed using a Bio-Rad mini trans-blot apparatus. The nitrocellulose filters (blots) were transferred to blocking solution (5% skim milk powder in Tris buffered saline, TBS, containing 0.05% tween 20). The blots were blocked either for 2 hours or overnight at 4°C, followed by incubation in anti-cdk$_1$ or an irrelevant control MoAb for 1 hour at room temperature. The blots were washed three times in TBS-tween over 15 minutes, then incubated in sheep anti-mouse Ig conjugated to horseradish peroxidase (HRP; Silenus Laboratories, Hawthorn, VIC, Australia) for 1 hour. After washing, the chemiluminescent ECL reaction (Amersham) was performed for 1 minute, followed by exposure to X-OMAT AR scientific imaging film (Eastman Kodak Co, Rochester, NY).

Immunohistochemistry. 10^5 freshly isolated or cultured cells were cytospun onto Vectabond-coated slides (Vector Laboratories Inc, Burlingame, CA). Cytospins were air dried, then fixed in absolute acetone for 2 minutes at room temperature, and stained or stored frozen until required. For single stains, cytospins were incubated in blocking solution (5% FCS/5% normal sheep serum) for 20 minutes, then incubated with primary MoAb, or irrelevant control antibody (mouse IgG1 or rabbit Ig negative controls; DAKO) for 60 minutes. Slides were washed in TBS between each step. Endogenous peroxidase was blocked by incubation in 0.5% H_2O_2. This was followed by incubation with either biotinylated rabbit anti-mouse Ig (DAKO) or biotinylated swine anti-rabbit Ig (DAKO) for 30 minutes, then horseradish peroxidase (HRP)-streptavidin complex (DAKO) for 30 minutes. The HRP product was developed with liquid DAB$^+$ substrate-chromogen system (DAKO). For double staining, the above method was followed, except that after addition of the HRP complex, slides were again blocked, followed by the second primary MoAb or irrelevant control antibody for 60 minutes, and then incubated in appropriate biotinylated secondary antibody for 30 minutes. Slides were incubated with streptavidin ABC-alkaline phosphatase complex (DAKO) for 30 minutes, then the peroxidase substrate. Finally, Vector red substrate (Vector Laboratories) was used to develop the alkaline phosphatase reaction. Cytospins were counterstained with Mayer's hematoxylin (Sigma) to demonstrate cell nuclei.

For myeloperoxidase staining, cytospins were prepared as described, air dried, then fixed in 10% formal-ethanol solution. Slides were incubated with filtered Kaplow's reagent for 30 seconds, washed in tap water, and counterstained in 20% Giemsa. Slides were finally washed in buffered distilled water.[27]

CFU-GM assay. Freshly isolated myeloid-enriched non-T cells, or DC generated in culture for 11 days, were plated on semi-solid methylcellulose medium containing IL-3, stem cell factor, and GM-CSF (Methocult GF H4534; Stem Cell Technologies Inc, Vancouver, BC, Canada) at a concentration of 3×10^5/plate, and incubated for 14 days. CFU-GM colonies (>50 cells) were counted by visual examination of the plates, according to the method described by the Terry Fox Laboratory Media Preparation Service.[28] The mean colony count per 10^5 cells was calculated.

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 previously been 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.[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

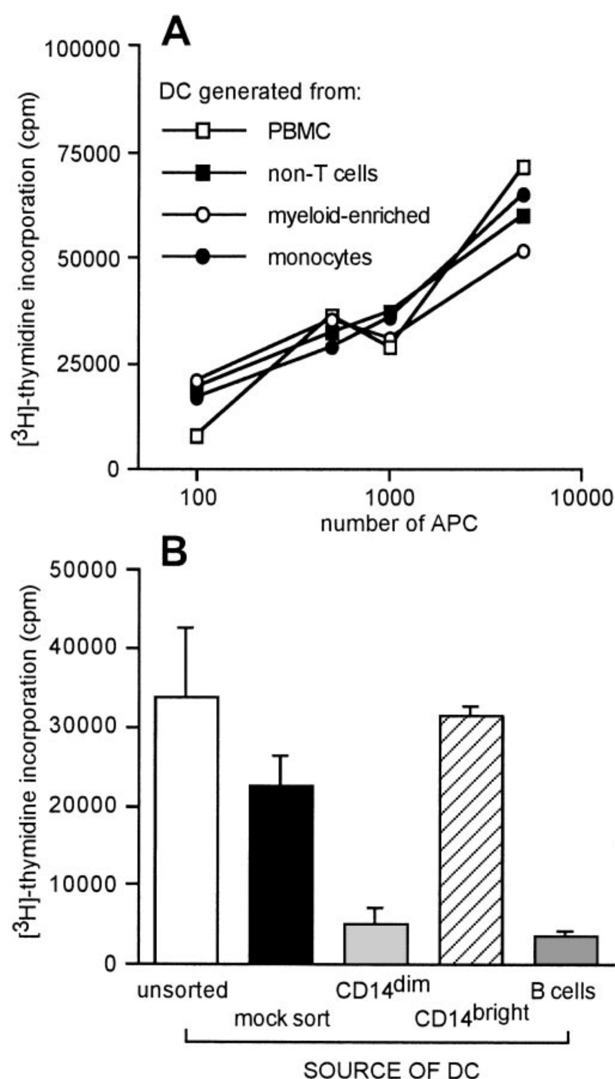

Fig 1. Monocyte-derived DC have potent APC function. DC prepared from adherent PBMC, myeloid-enriched non-T cells, or sorted monocytes (A), or from various sorted PBMC subpopulations (B), were cultured with 800 U/mL GM-CSF and 400 U/mL IL-4 for 4 days. Graded numbers (A) or 10^4 (B) mitomycin-treated APC were incubated with 10^5 allogeneic T cells for 5 days. Results are expressed as mean cpm of triplicate samples.

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

Downloaded from http://ashpublications.net/blood/article-pdf/92/5/1598/1647175/1598.pdf by guest on 18 May 2024

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. CD3 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 histocompatability 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 [^{3}H]-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 [^{3}H]-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 [^{3}H]-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 [^{3}H]-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, [^{3}H]-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

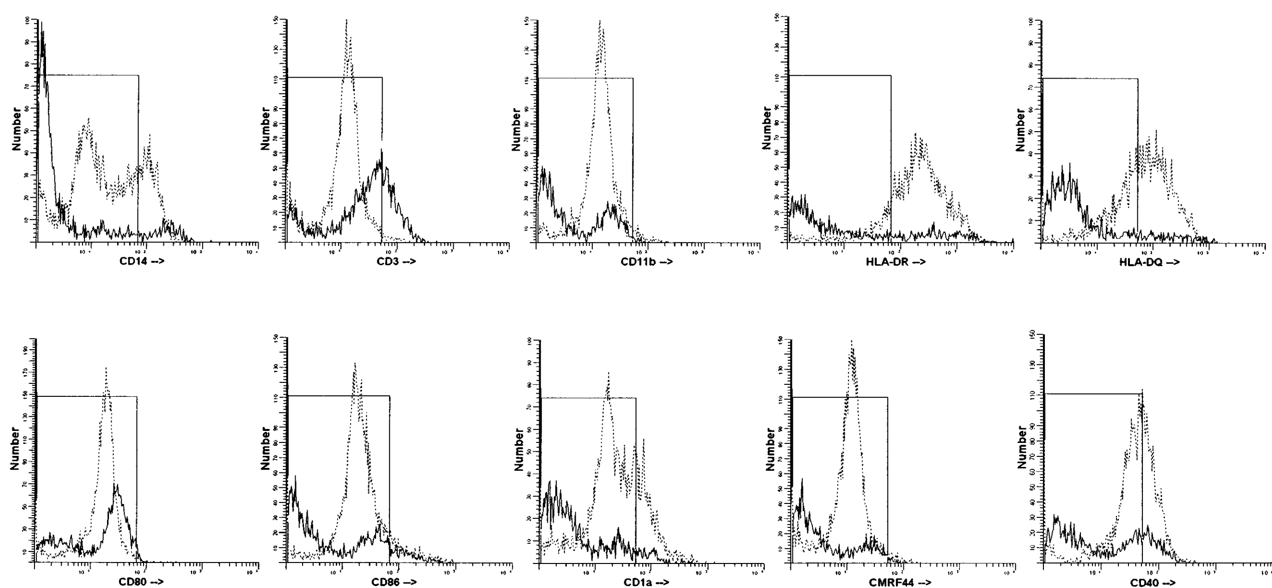

Fig 2. Phenotype of DC generated after culture of adherent PBMC or sorted monocytes. Cells were cultured in the presence of GM-CSF and IL-4 for 7 days before analysis by flow cytometry. In each case staining with an irrelevant control antibody is represented by the box to the left of the histogram. Adherent PBMC are represented by the solid line, and monocytes by the dashed line. Dead cells were excluded by gating on forward and side scatter properties, and the remaining viable cells were analyzed. In the case of DC generated from adherent PBMC, contaminating lymphocytes were included. Histograms represent data acquired from 10,000 events. Data are representative of four separate experiments.

Downloaded from http://ashpublications.net/blood/article-pdf/92/5/1598/1647175/1598.pdf by guest on 18 May 2024

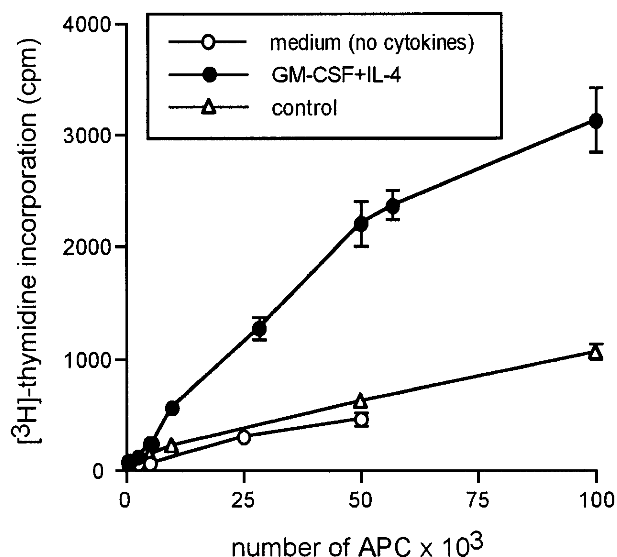

Fig 3. Proliferation of cultured myeloid-enriched cells. Myeloid-enriched non-T cells were cultured in the presence or absence of GM-CSF and IL-4, washed, and reincubated for 18 hours with [^{3}H]-TdR. Control cells were cultured with GM-CSF and IL-4 that had been treated with mitomycin C before assay. Cell proliferation is expressed as the mean [^{3}H]-TdR incorporation of triplicate wells ± SD.

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 (cdk_1)—an intracellular protein expressed by cycling cells—and the expression of Ki67. Expression of cdk_1 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. Cdk_1 was detected in all samples (Fig 5). However, by comparison with equivalent cell numbers of the cycling cell line, BJAB, the levels of cdk_1 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 cdk_1 expression is observed in cultured cells, stimulation of proliferation by GM-CSF and IL-4 is unlikely to be at the level of cdk_1 induction.

The low levels of [^{3}H]-TdR uptake and of cdk_1 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_o, 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

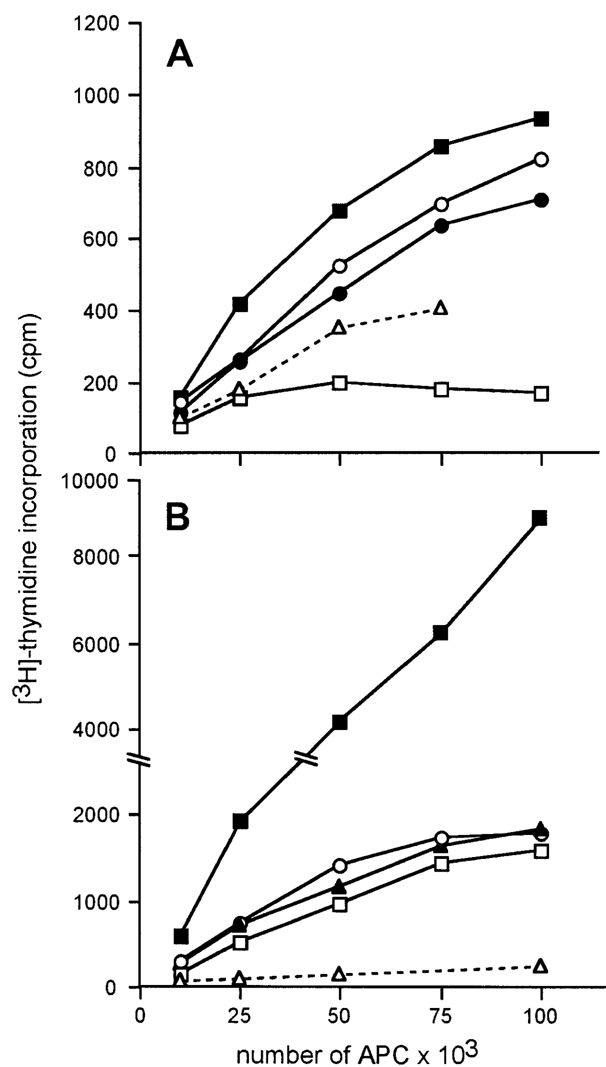

Fig 4. Effect of LPS and various cytokines on the proliferative response in DC cultures. Myeloid-enriched non-T cells were cultured in the presence of various cytokine combinations, then washed and reincubated for 18 hours with [^{3}H]-TdR. Where added, LPS or TNFα were included during the final 24 hours of culture. Control cells were cultured with GM-CSF and IL-4, and treated with mitomycin C before assay. Proliferation is represented as the mean [^{3}H]-TdR incorporation of triplicate wells. The data presented are representative of three separate experiments. (□), Medium; (■), GM-CSF; (○), GM-CSF + IL-4; (●), GM-CSF/IL-4 + LPS; (▲), GM-CSF/IL-4 + TNF; (△), control.

Downloaded from http://ashpublications.net/blood/article-pdf/92/5/1598/1647175/1598.pdf by guest on 18 May 2024

Table 2. LPS-Induced Differentiation of 2- and 7-Day–Cultured Monocyte-Derived DC

					Cells Cultured in:		
Marker	Day of Culture	Control MFI	Freshly Isolated % Positive (Δ MFI)	Medium % Positive (Δ MFI)	GM-CSF % Positive (Δ MFI)	GM-CSF/IL-4 % Positive (Δ MFI)	GM-CSF/IL-4 + LPS % Positive (Δ MFI)
CD14	0	30.7	89.2 (92)				
	2	49.6		71.7 (77)	51.0 (45)	33.1 (23)	52.5 (37)
	7	77.6		64.6 (46)	47.9 (21)	45.4 (17)	30.8 (9)
HLA-DR	0	30.7	78.8 (55)				
	2	49.6		62.0 (50)	89.8 (75)	57.1 (48)	94.1 (70)
	7	77.6		41.2 (6)	31.4 (0)	80.5 (53)	86.2 (53)
HLA-DQ	0	29.4	3.9 (7)				
	2	28.9		55.9 (43)	48.3 (39)	53.2 (46)	93.4 (78)
	7	78.8		84.2 (23)	84.6 (27)	89.6 (32)	96.6 (47)
CD1a	0	29.4	6.7 (10)				
	2	28.9		17.0 (2)	33.6 (29)	51.4 (36)	65.1 (53)
	7	78.8		79.7 (5)	77.9 (4)	97.2 (30)	95.8 (28)
CD86	0	25.7	2.0 (16)				
	2	66.5		12.3 (0)	11.0 (0)	10.3 (0)	45.3 (33)
	7	71.1		41.9 (4)	55.2 (15)	46.6 (8)	94.3 (47)

Myeloid-enriched non-T cells were either freshly isolated (day 0) or cultured for either 2 or 7 days in either medium, medium supplemented with GM-CSF alone, GM-CSF and IL-4, or GM-CSF and IL-4 with LPS added for the last 24 hours of culture. Cells were stained with various MoAbs or control MoAb, and the % positive and MFI were analyzed. Data are representative of 4 separate experiments.

Abbreviation: Δ MFI, mean fluorescence intensity of sample minus control MFI.

differentiation. In accordance with the [^{3}H]-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.

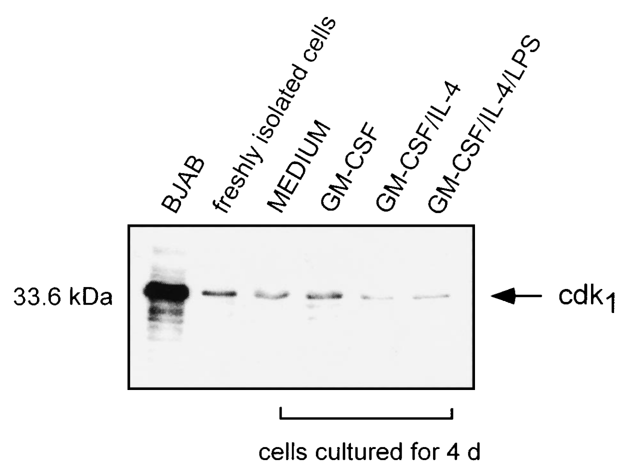

Fig 5. Cdk$_1$ is expressed by freshly isolated and cultured myeloid-enriched non-T cells. Myeloid-enriched non-T cells were cultured in the presence of various cytokines with or without LPS for the final 24 hours. Lysates from 10^5 cells were immunoblotted as described in Materials and Methods using anti-cdk$_1$ MoAb. The cycling BJAB cell line was used as a positive control. Data presented are representative of four separate experiments.

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% $\pm$ 0.18% (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 influ-

Downloaded from http://ashpublications.net/blood/article-pdf/92/5/1598/1647175/1598.pdf by guest on 18 May 2024

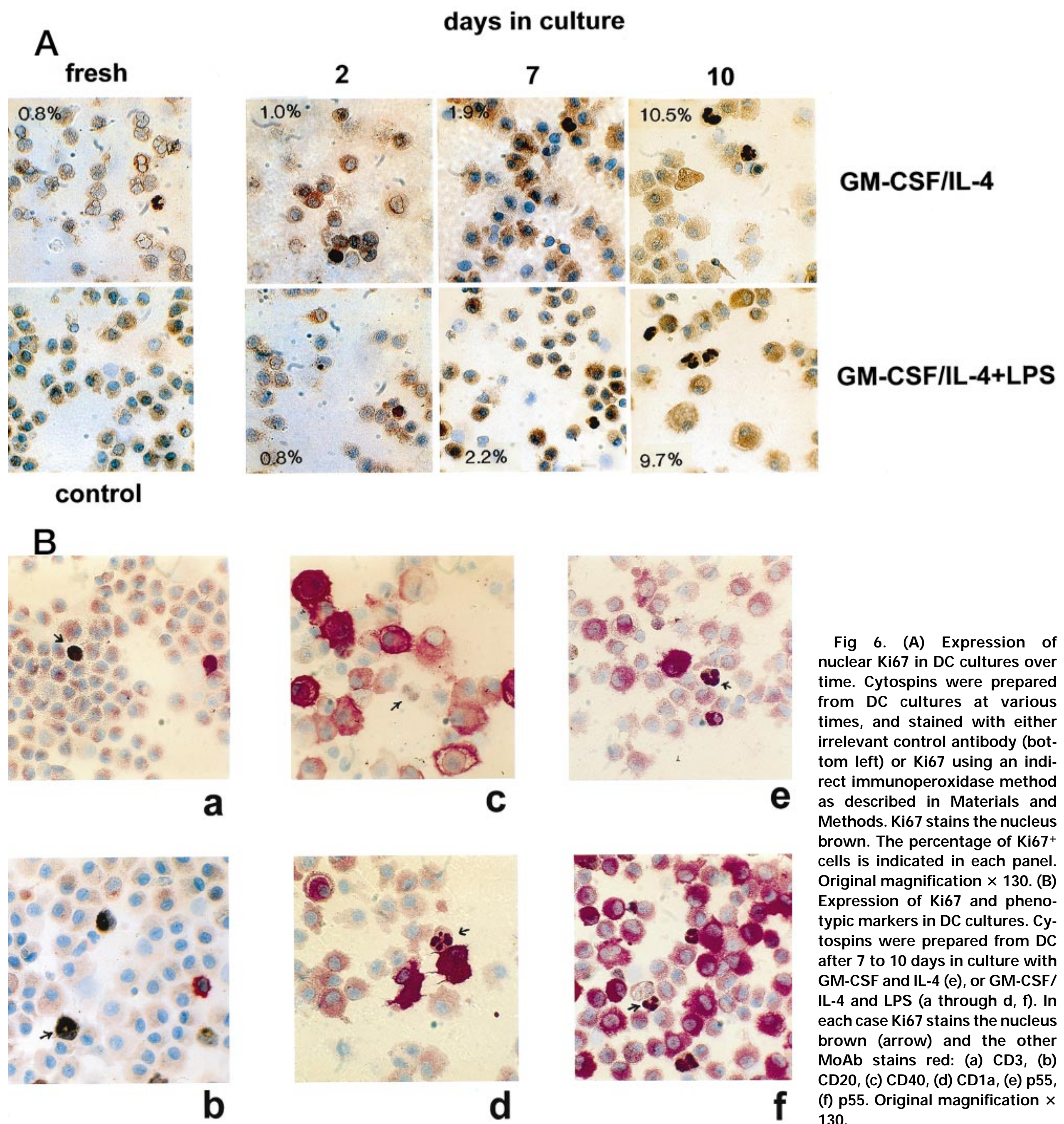

Fig 6. (A) Expression of nuclear Ki67 in DC cultures over time. Cytospins were prepared from DC cultures at various times, and stained with either irrelevant control antibody (bottom left) or Ki67 using an indirect immunoperoxidase method as described in Materials and Methods. Ki67 stains the nucleus brown. The percentage of Ki67^{+} cells is indicated in each panel. Original magnification × 130. (B) Expression of Ki67 and phenotypic markers in DC cultures. Cytospins were prepared from DC after 7 to 10 days in culture with GM-CSF and IL-4 (e), or GM-CSF/IL-4 and LPS (a through d, f). In each case Ki67 stains the nucleus brown (arrow) and the other MoAb stains red: (a) CD3, (b) CD20, (c) CD40, (d) CD1a, (e) p55, (f) p55. Original magnification × 130.

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.[10,18-20] 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.[10] 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

Downloaded from http://ashpublications.net/blood/article-pdf/92/5/1598/1647175/1598.pdf by guest on 18 May 2024

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

	Donor 1		Donor 2		Donor 3	
Mitomycin C Treatment	+	−	+	−	+	−
Total yield (×10⁵/well)	6.5	6.7	10	8	9	9
% Recovery	33	34	50	40	45	45
DC yield (% total cells)	46	54	59	61	70	66
	% Positive Recovered Cells					
HLA-DR	68	58	48	39	78	76
CD14	9	7	5	1	13	3
CD40	55	42	36	51	47	69
CD1a	30	55	53	64	49	70

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]

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

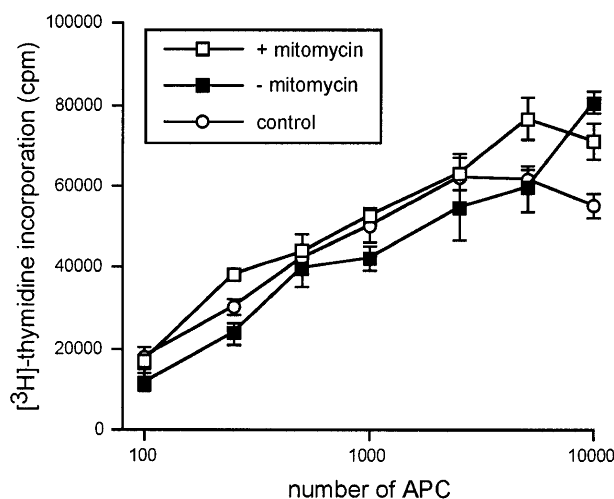

Fig 7. Effect of mitomycin C pretreatment on APC function of monocyte-derived DC. DC prepared from myeloid-enriched non-T cells either treated with mitomycin C (+ mitomycin), mock-treated (− mitomycin), or untreated (control) were cultured for 6 days in the presence of GM-CSF and IL-4. APC were incubated with 10^5 allogeneic T cells for 5 days. Results are expressed as mean cpm (±SEM) of triplicate samples.

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 cells[39,40] 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 committment. Of interest, one of the DC precursor populations could be identified by cell-surface expression of CD14. From this precursor it was

Downloaded from http://ashpublications.net/blood/article-pdf/92/5/1598/1647175/1598.pdf by guest on 18 May 2024

possible to generate potent APC in the presence of GM-CSF and TNF-α, and macrophage-like cells in the presence of M-CSF.[36] As has been shown for circulating monocytes, these $CD14^+$ 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.[21,35,46]

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 differentiation of DC. Observed proliferation depends instead on $CD34^+$ progenitor cells with the potential for myeloid differentiation that are enriched by various monocyte-enrichment procedures, such as adherence. Of clinical relevance, the yield of DC obtained in such cultures can be estimated from the starting number of monocytes evaluated in individual donors.

ACKNOWLEDGMENT

We thank Drs P. Lipsky, D. Hart, E Langhoff, and N. Saunders for the gifts of MoAbs; Dr K. MacDonald and Prof I. Frazer for helpful discussions; and A. Pettit and Dr G. Thomas for excellent technical assistance.

REFERENCES

1. Reid CDL, Fryer PR, Clifford C, Kirk A, Tikerpae J, Knight SC: Identification of hematopoietic progenitors of macrophages and dendritic Langerhans cells (DL-CFU) in human bone marrow and peripheral blood. Blood 76:1139, 1990

2. Santiago-Schwartz F, Belilos E, Diamond B, Carsons SE: TNF in combination with GM-CSF enhances the differentiation of neonatal cord blood stem cells into dendritic cells and macrophages. J Leukoc Biol 52:274, 1992

3. Reid CDL, Stackpoole A, Meager A, Tikerpae J: Interactions of tumor necrosis factor with granulocyte-macrophage colony-stimulating factor and other cytokines in the regulation of dendritic cell growth in vitro from early bipotent $CD34^+$ progenitors in human bone marrow. J Immunol 149:2681, 1992

4. Steinman RM: The dendritic cell system and its role in immunogenicity. Annu Rev Immunol 9:271, 1991

5. Caux C, Dezutter-Dambuyant C, Scmitt D, Banchereau J: GM-CSF and TNF-α cooperate in the generation of dendritic Langerhans cells. Nature 360:258, 1992

6. Young JW, Szabolcs P, Moore MAS: Identification of dendritic cell colony-forming units among normal human $CD34^+$ bone-marrow progenitors that are expanded by c-*kit*-ligand and yield pure dendritic cell colonies in the presence of granulocyte/macrophage colony-stimulating factor and tumor necrosis factor α. J Exp Med 182:1111, 1995

7. Szabolcs P, Moore MAS, Young JW: Expansion of immunostimulatory dendritic cells among the myeloid progeny of human $CD34^+$ bone marrow precursors cultured with c-*kit* ligand, granulocyte-macrophage colony-stimulating factor, and TNF-α. J Immunol 155: 5851, 1995

8. Herbst B, Kohler G, Mackensen A, Veelken H, Kulmburg P, Rosenthal FM, Schaefer HE, Mertelsmann R, Fisch P, Lindemann A: In vitro differentiation of $CD34^+$ hematopoietic progenitor cells toward distinct dendritic cell subsets of the Birbeck granule and MHC-positive Langerhans cell and the interdigitating dendritic cell type. Blood 88:2541, 1996

9. Strobl H, Bello-Fernandez C, Riedl E, Pickl WF, Majdic O, Lyman SD, Knapp W: flt3 ligand in cooperation with transforming growth factor-β1 potentiates in vitro development of Langerhans-type dendritic cells and allows single-cell dendritic cell cluster formation under serum-free conditions. Blood 90:1425, 1997

10. Romani N, Gruner S, Brang D, Kampgen E, Lenz A, Trockenbacher B, Konwalinka G, Fritsch PO, Steinman RM, Schuler G: Proliferating dendritic cell progenitors in human blood. J Exp Med 180:83, 1994

11. Gabrilovich DI, Nadaf S, Corak J, Berzofsky JA, Carbone DP: Dendritic cells in antitumor immune responses. II. Dendritic cells grown from bone marrow precursors, but not mature DC from tumor-bearing mice, are effective antigen carriers in the therapy of established tumors. Cell Immunol 170:111, 1996

12. Zorina T, Mayordomo JI, Watkins S, Lotze MT, DeLeo AB, Ilstad ST: Culture of dendritic cells from murine bone marrow supplemented with GM-CSF and TNF-alpha. J Immunother 16:247, 1996

13. Sallusto F, Lanzavecchia A: Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin-4 and downregulated by tumor necrosis factor-alpha. J Exp Med 179:1109, 1994

14. Bender A, Sapp M, Schuler G, Steinman RM, Bhardwaj N: Improved methods for generation of dendritic cells from nonproliferating progenitors in human blood. J Immunol Methods 196:121, 1996

15. Romani N, Reider D, Heuer M, Ebner S, Kampgen E, Eibl B, Niederwieser D, Schuler G: Generation of mature dendritic cells from human blood. An improved method with special regard to clinical applicability. J Immunol Methods 196:137, 1996

16. Peters JH, Ruppert J, Giesler RKH, Najar HM, Xu H: Differentiation of human monocytes into CD14 negative accessory cell: Do dendritic cells derive from the monocytic lineage? Pathobiology 59:122, 1991

17. Rossi G, Heveker N, Thiele B, Gelderblom H, Steinbach F: Development of a Langerhans cell phenotype from peripheral blood monocytes. Immunol Lett 31:189, 1992

18. Kiertscher SM, Roth MD: Human $CD14^+$ leukocytes acquire the phenotype and function of antigen-presenting dendritic cells when cultured in GM-CSF and IL-4. J Leukoc Biol 59:208, 1996

19. Zhou L-J, Tedder TF: $CD14^+$ blood monocytes can differentiate into functionally mature $CD83^+$ dendritic cells. Proc Natl Acad Sci USA 93:2588, 1996

20. Pickl WF, Majdic O, Kohl P, Stockl J, Riedl E, Scheinecker CS, Bello-Fernandez C, Knapp W: Molecular and functional characteristics of dendritic cells generated from highly purified $CD14^+$ peripheral blood monocytes. J Immunol 157:3850, 1996

21. Chapuis F, Rosenzwajg M, Yagello M, Ekman M, Biberfeld P, Gluckman JC: Differentiation of human dendritic cells from monocytes in vitro. Eur J Immunol 27:431, 1997

22. Thomas R, Davis LS, Lipsky PE: Comparative accessory cell function of human peripheral dendritic cells and monocytes. J Immunol 151:6840, 1993

23. Thomas R, Quinn C: Functional differentiation of dendritic cells in rheumatoid arthritis. Role of CD86 in the synovium. J Immunol 156:3074, 1996

24. Thomas R, Lipsky PE: Human peripheral blood dendritic cell subsets—Isolation and characterization of precursor and mature antigen-presenting cells. J Immunol 153:4016, 1994

25. Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680, 1970

26. Kaplow LS: Simplified myeloperoxidase stain using benzidine dihydrochloride. Blood 26:215, 1965

27. The Terry Fox Laboratory Media Preparation Service: Colony assays of hematopoietic cells using methylcellulose media, in: An Introductory Technical Manual. Stem Cells Inc, Vancouver, Canada, 1992

28. Sutherland DR, Anderson L, Keeney M, Nayar R, Chin-Yee I:

Downloaded from http://ashpublications.net/blood/article-pdf/92/5/1598/1647175/1598.pdf by guest on 18 May 2024

The ISHAGE guidelines for CD34^{+} cell determination by flow cytometry. J Hematother 5:213, 1996

29. Hollenbaugh D, Grosmaire LS, Kullas CD, Chalupny NJ, Barest-Andersen S, Noelle RJ, Stamenkovic I, Ledbetter HA, Aruffo A: A human T cells antigen gp39, a member of the TNF gene family, is the ligand for the CD40 receptor: Expression of a soluble form of gp39 with B cell co-stimulatory activity. EMBO J 11:4313, 1992

30. Graf D, Korthauer U, Mages H, Senger G, Kroczek RA: Cloning of TRAP, a ligand for CD40 on human T cells. Eur J Immunol 22:3191, 1992

31. Caux C, Massacrier C, Vanbervliet B, Dubois B, van Kooten C, Durand I, Banchereau J: Activation of human dendritic cells through CD40 cross-linking. J Exp Med 180:1263, 1994

32. Hart PH, Jones CA, Finlay-Jones JJ: Monocytes cultured in cytokine-defined environments differ from freshly isolated monocytes in their responses to IL-4 and IL-10. J Leukoc Biol 57:909, 1995

33. Lauener RP, Goyert SM, Geha RS, Vercelli D: Interleukin 4 down-regulates the expression of CD14 in normal human monocytes. Eur J Immunol 20:2375, 1990

34. Jansen JH, Wientjens G-JHM, Fibbe WE, Willemze R, Kluin-Nelemans HC: Inhibition of human macrophage colony formation by interleukin 4. J Exp Med 170:577, 1989

35. Caux C, Vanbervliet B, Massacrier C, Dezutter-Dambuyant C, de Saint-Vis B, Jacquet C, Yoneda K, Imamura S, Schmitt D, Banchereau J: CD34^{+} hematopoietic progenitors from human cord blood differentiate along two independent dendritic cell pathways in response to GM-CSF and TNFα. J Exp Med 184:695, 1996

36. Caux C, Massacrier C, Vanbervliet B, Dubois B, Durand I, Cella M, Lanzavecchia A, Banchereau J: CD34^{+} hematopoietic progenitors from human cord blood differentiate along two independent dendritic cell pathways in response to granulocyte-macrophage colony-stimulating factor plus tumor necrosis factor α: II. Functional analysis. Blood 90:1458, 1997

37. Ye Z, Gee AP, Bowers WE, Lamb LS, Turner MW, Henslee-Downey PJ: In vitro expansion and characterisation of dendritic cells derived from human bone marrow CD34^{+} cells. Bone Marrow Transplant 18:997, 1996

38. Snoeck H-W, Lardon F, van Bockstaele DR, Peetermans ME: Effects of interleukin-4 (IL4) on myelopoiesis: studies on highly purified CD34^{+} hematopoietic progenitor cells. Leukemia 7:625, 1993

39. Inaba K, Steinman RM, Witmer-Pack M, Aya H, Inaba M, Sudo T, Wolpe S, Schuler G: Identification of proliferating dendritic cell precursors in mouse blood. J Exp Med 175:1157, 1992

40. Inaba K, Inaba M, Romani N, Aya H, Deguchi M, Ikehara S, Muramatsu S, Steinman RM: Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor. J Exp Med 176:1693, 1992

41. Sonoda Y, Kimura T, Sakabe H, Tanimukai S, Ohmizono Y, Nakagawa S, Yokota S, Lyman SD, Abe T: Human FLT3 ligand acts on myeloid as well as multipotential progenitors derived from purified CD34^{+} blood progenitors expressing different levels of c-kit protein. Eur J Haematol 58:257, 1997

42. Saunders D, Lucas K, Ismaili J, Wu L, Maraskovsky E, Dunn A, Shortman K: Dendritic cell development in culture from thymic precursor cells in the absence of granulocyte/macrophage colony-stimulating factor. J Exp Med 184:2185, 1996

43. Wu L, Li C-L, Shortman K: Thymic dendritic cell precursors: Relationship to the T Lymphocyte lineage and phenotype of the dendritic cell progeny. J Exp Med 184:903, 1996

44. Strunk D, Rappersberger E, C., Strobl H, Kromer E, Elbe A, Maurer D, Stingl G: Generation of human dendritic cells/Langerhans cells from circulating CD34^{+} hematopoietic progenitor cells. Blood 87:1292, 1996

45. Rosenzwajg M, Canque B, Gluckman JC: Human dendritic cell differentiation pathways from CD34^{+} hematopoietic precursor cells. Blood 87:535, 1996

46. Akagawa KS, Takasuka N, Nozaki Y, Komuro I, Azuma M, Ueda M, Naito M, Takahashi K: Generation of CD1^{+}RelB^{+} dendritic cells and tartrate-resistant acid phosphatase-positive osteoclast-like multinucleated giant cells from human monocytes. Blood 88:4029, 1996

Downloaded from http://ashpublications.net/blood/article-pdf/92/5/1598/1647175/1598.pdf by guest on 18 May 2024