Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 2588–2592, March 1996
Immunology

$CD14^+$ blood monocytes can differentiate into functionally mature $CD83^+$ dendritic cells

(cytokines/antigen presentation/mixed leukocyte reaction)

LIANG-JI ZHOU AND THOMAS F. TEDDER*

Department of Immunology, Box 3010, Duke University Medical Center, Durham, NC 27710

Communicated by Bernard Amos, Duke University Medical Center, Durham, NC, December 1, 1995 (received for review October 2, 1995)

ABSTRACT **Dendritic cells are potent antigen-presenting cells that initiate primary immune responses. Although dendritic cells derive from bone marrow stem cells, the intermediate stages in their development remain unknown. In this study, plastic-adherent blood monocytes ($CD14^+$, $CD1a^-$) cultured for 7 days with granulocyte–monocyte colony-stimulating factor, interleukin 4, and tumor necrosis factor α were shown to differentiate into $CD1a^+$ $CD83^+$ dendritic cells. These cells displayed all phenotypic and morphologic characteristics of mature dendritic cells and were the most potent stimulatory cells in allogeneic mixed leukocyte reactions. The identification of specific culture conditions that generate large numbers of dendritic cells from purified monocytes uncovers an important step in dendritic cell maturation that will allow the further characterization of their role in autoimmune diseases, graft rejection, and human immunodeficiency virus infection.**

Dendritic cells are the most potent antigen-presenting cells in the immune system and are critically involved in the initiation of primary immune responses, autoimmune diseases, graft rejection, human immunodeficiency virus infection, and the generation of T-cell-dependent antibodies (1). Mature dendritic cells are also the principal stimulatory cells of primary mixed leukocyte reactions (2, 3). However, the study of dendritic cells has proven difficult because they represent only a small subpopulation of bone-marrow-derived leukocytes that includes interdigitating reticulum cells in lymphoid organs, blood dendritic cells, Langerhans cells in the epidermis of the skin, and dermal dendritic cells (1). In addition, dendritic cells have been primarily isolated and studied based on their unique morphology and lack of surface antigens when compared to T cells, B cells, monocytes, and natural killer cells (4–12).

Although dendritic cells can be generated from stem cells and blood progenitor cells (13–22), the intermediate stages of this developmental pathway remain unclear. $CD1a^+$ cells with dendritic cell morphology and function, as well as monocytes, can be generated from bone marrow or blood progenitor cells after culture with combinations of granulocyte–monocyte colony-stimulating factor (GM-CSF), and either tumor necrosis factor α (TNF-α) or interleukin 4 (IL-4) (13–22). Blood monocyte subpopulations or adherent cells with dendritic cell morphology, size, cell surface phenotype, or function have also been identified by using various cell surface markers and methods of purification or culture (4, 23–29). Recently, the identification of CD83 as a selective marker of dendritic lineage cells has aided in their characterization (30–34). Three populations of cells in blood that have or can develop a dendritic cell morphology have been identified: one population is $CD83^+$, one population gains the expression of CD83 after culture, and one population does not express CD83 even after culture or activation and appears to derive from myelomonocytic cells (31, 32). Given the close relationship between dendritic and myelomonocytic lineage cells, the developmental relationship between $CD14^+$ blood monocytes and $CD83^+$ dendritic cells was examined after cytokine-induced differentiation of monocytes.

METHODS

Cell Isolation and Cultures. Mononuclear cells were isolated by Ficoll/Paque (Pharmacia) density gradient centrifugation of heparinized blood obtained from healthy adult donors according to protocols approved by the Human Use Committee of Duke University. Monocytes were isolated by plastic adherence as described (31). Monocytes were recovered by incubation in ice-cold saline for 20 min at 4°C. Resuspended monocytes (5×10^6 cells per ml) were layered onto hypertonic 14.5% (vol/vol) metrizamide (Sigma) and centrifuged at $650 \times g$ for 15 min at room temperature to sediment contaminating lymphocytes. Cells (>98% $CD14^+$) were harvested from the interface of the metrizamide gradient, resuspended (1×10^6 cells per ml) in RPMI 1640 medium (GIBCO/BRL) containing 10% (vol/vol) fetal calf serum, 10 mM glutamine, and penicillin/streptomycin. Blood $CD83^+$ dendritic cells were isolated as described (31).

Monocytes were cultured in six-well tissue culture plates (1×10^6 cells per ml) in medium containing cytokines [GM-CSF (800 units/ml)/IL-4 (500 units/ml)/TNF-α (100 units/ml) (from Genzyme)]. The cultures were fed with fresh medium and cytokines every 3 days and cell differentiation was monitored by light microscopy. In some experiments, $CD83^+$ cells were isolated from cultures of monocytes incubated with GM-CSF/IL-4/TNF-α for 7 days by fluorescence-based cell sorting as described (31). The cell preparations were >99% $CD83^+$ when the isolated cells were reexamined for fluorescence staining.

Immunofluorescence Analysis. Monocyte differentiation was assessed by two-color immunofluorescence staining with flow cytometry analysis as described (31). Antibodies used included fluorescein isothiocyanate-conjugated HB15a (CD83, IgG_{2b}) (31), phycoerythrin-conjugated CD14 (Coulter), phycoerythrin-conjugated CD1a (Coulter), and antibodies from the Fifth International Leukocyte Differentiation Antigen Workshop. Isotype-matched control antibodies (Coulter) were used to determine the levels of background staining. Ten thousand cells were analyzed by flow cytometry using an Elite flow cytometer (Coulter).

Electron Microscopy. Cells were processed for transmission electron microscopy as described (31).

Mixed Leukocyte Reactions. Monocyte-derived $CD83^+$ and $CD83^-$ cells were isolated by cell sorting. Responder T cells used for allogeneic mixed leukocyte reaction assays were isolated by sheep erythrocyte rosette formation of

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. §1734 solely to indicate this fact.

Abbreviations: GM-CSF, granulocyte–monocyte colony-stimulating factor; TNF, tumor necrosis factor; IL-4, interleukin 4.

*To whom reprint requests should be addressed.

mononuclear cells obtained from healthy donors. Assays were carried out as described (12). Briefly, stimulator cells were irradiated (5000 rads; 1 rad = 0.01 Gy) and added in graded doses to 1.5×10^5 allogeneic T cells in 96 well U-bottom tissue culture plates (0.2 ml of medium per well). Cell proliferation during the last 12 h of 5-day cultures was quantified by incubation of cells with 1 μCi (37 kBq) of [*methyl*-^{3}H]thymidine (NEN-DuPont, Boston, MA). The cells were harvested onto filters and radioactivity was measured in a scintillation counter.

RESULTS

Cytokine-Induced Monocyte Differentiation. Plastic-adherent CD14$^+$ CD1a$^-$ monocytes were purified and cultured with different combinations of cytokines (Fig. 1). Monocytes cultured with GM-CSF/IL-4 generated small distinctive clusters of cells similar to those described for cultured blood dendritic cell precursors and CD34$^+$ cells from bone marrow (19, 21, 22, 29). However, these cells remained CD14$^+$ and did not express CD83 at any time point (Fig. 1*A*), although they did become CD1a$^+$ (Fig. 1*B*). Monocytes cultured with GM-CSF/TNF-α primarily adhered to the tissue culture plates and formed only small loose cell clusters similar to those described for cultured CD34$^+$ stem cells (20). Monocytes cultured with either GM-CSF/IL-4 or GM-CSF/TNF-α became large macrophage-like cells after 12 days of culture but remained CD14$^+$ and CD83$^-$. In contrast, monocytes cultured with GM-CSF/IL-4/TNF-α generated small clusters of cells on day 2 that were semiadherent to the tissue culture plates (Fig. 2*A*). The cells were tightly clustered together by day 5 (Fig. 2*B*) with some cells located between clusters (Fig. 2*C*). A large percentage (40–80%) of the monocytes differentiated into CD83$^+$ CD14$^-$ cells, but this usually required 10–12 days of culture (Fig. 1*A*). In contrast, if TNF-α was added to GM-CSF/IL-4 cultures at day 5, the cells became firmly adherent to the tissue culture plates and 78–95% of the cells became CD83$^+$ within 2–3 days (Fig. 1*B*). After acquisition of CD83 expression by cultured monocytes, there was a decrease in CD1a expression (Fig. 1*C*), which is typical of CD83$^+$ blood dendritic cells (31).

In all three of the above culture systems, cell viability remained >95% as determined by trypan blue exclusion until day 14–15, when the cells appeared to increase in size and the cell clusters began to deteriorate. At day 20 or 21, the cells cultured with GM-CSF/IL-4/TNF-α became large round macrophage-like cells. Interestingly, monocytes or CD83$^+$ blood dendritic cells did not expand in cell number or incorporate significant amounts of radiolabeled thymidine when cultured in the presence of cytokines. These results suggest that TNF-α induces the differentiation of blood monocytes cultured with GM-CSF/IL-4 into dendritic-like cells within 7–9 days, with a yield of CD83$^+$ cells similar to the number of monocytes cultured.

Monocyte-Derived CD83$^+$ Cells Exhibit a Dendritic Morphology. Monocyte-derived CD83$^+$ cells exhibited the characteristic cellular projections and motility of dendritic cells (Fig. 2*C*) and continually extended, retracted, and reoriented their cellular processes and veils. These CD83$^+$ cells were morphologically homogenous (Fig. 2*D*) with large irregular-shaped nuclei, numerous mitochondria and vesicles, relatively few cytoplasmic granules, and obvious abundant cellular projections (Fig. 2 *E* and *F*). Their morphology was identical to that of freshly isolated CD83$^+$ blood dendritic cells (Fig. 2*H*) but was distinct from that of monocytes (Fig. 2*G*).

Monocyte-Derived CD83$^+$ Cells Exhibit a Dendritic Cell Phenotype. The phenotypes of monocyte-derived CD83$^+$ cells and CD83$^+$ dendritic cells isolated from blood were similar (31). Monocyte-derived CD83$^+$ cells expressed CD13 and CD33 but lacked most other myeloid markers (Fig. 3 and Table 1). Cultured CD83$^+$ cells expressed CD1c, CD13, CD29, CD31, CD40, CD45RO, CD86, and high levels of major histocompatibility complex class I and class II molecules, as do blood CD83$^+$ dendritic cells. However, in contrast with blood CD83$^+$ dendritic cells, monocyte-derived CD83$^+$ cells expressed CD1a, CD1b, CD4, and CD80 but were negative or mostly negative for CD2, CD5, CD15s, and CD25. Small subpopulations of CD83$^+$ cells that lacked CD4, yet expressed CD2 and CD5 were observed in some cultures (Fig. 3), suggesting further differentiation of these cells.

Stimulating Activity of CD83$^+$ Cells in Mixed Leukocyte Reactions. Monocytes cultured with GM-CSF/IL-4/TNF-α induced significantly ($P < 0.02$, paired Student's *t* test) more proliferation of allogeneic T cells than monocytes cultured

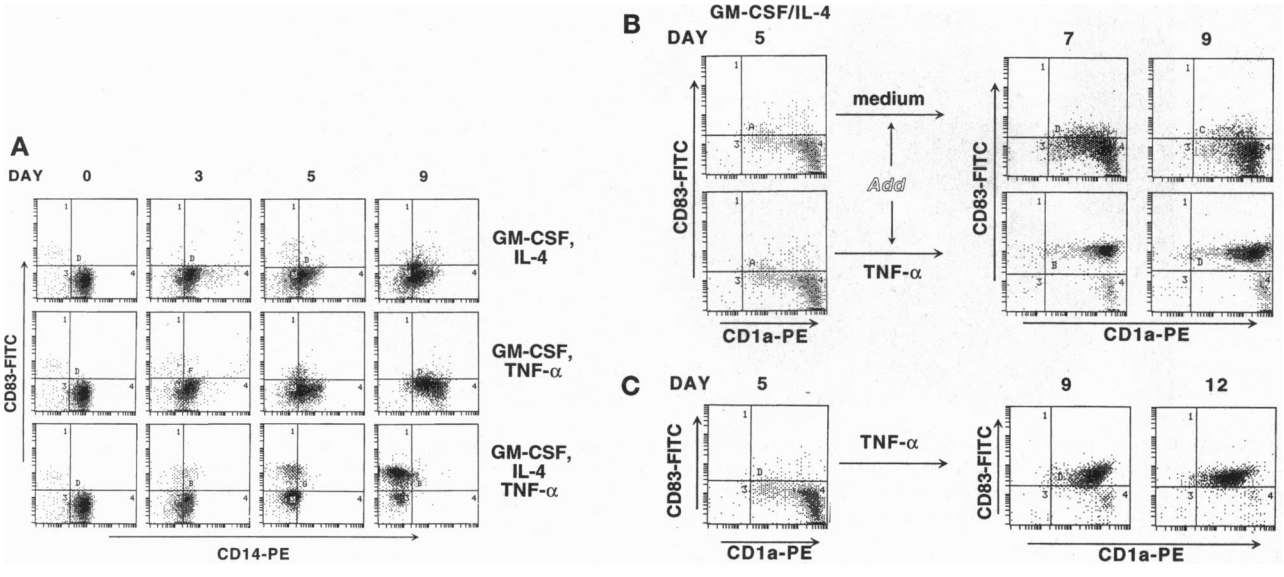

FIG. 1. Monocytes cultured with GM-CSF/IL-4/TNF-α differentiate into CD83$^+$ CD1a$^+$ cells. CD14$^+$ blood monocytes were cultured with the indicated combinations of cytokines (*A*) or with GM-CSF/IL-4 (*B* and *C*) for 5 days before the cultures were split and TNF-α was added to the indicated cultures. The cell surface phenotype of cultured cells was examined in at least 10 experiments by two-color immunofluorescence analysis over the subsequent 12 days. CD14-PE, phycoerythrin-conjugated CD14; CD83-FITC, fluorescein isothiocyanate-conjugated CD83.

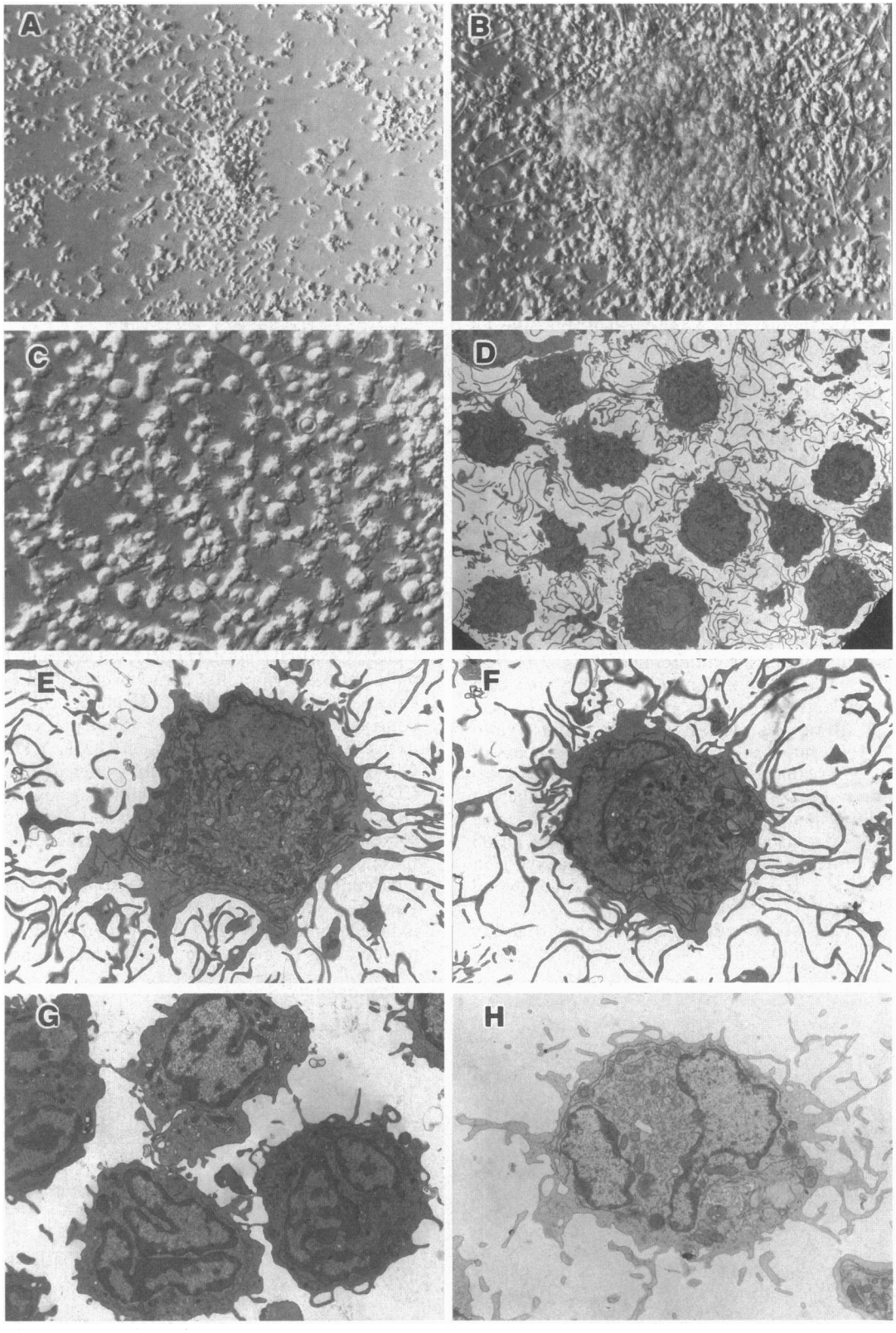

FIG. 2. Monocytes cultured with GM-CSF/IL-4/TNF-α develop a dendritic-cell morphology. $CD14^+$ monocytes were cultured with cytokines for 2 (*A*) or 5 (*B* and *C*) days and examined by phase-contrast microscopy. (*A* and *B*, ×180; *C*, ×360.) (*D–H*) Representative transmission electron micrographs of monocyte-derived $CD83^+$ cells isolated by cell sorting from 7-day GM-CSF/IL-4/TNF-α cultures (*D–F*), blood $CD83^+$ dendritic cells (*G*), and $CD14^+$ monocytes before culture (*H*). (*D*, ×1670; *E–H*, ×5200.)

with GM-CSF/IL-4 (Fig. 4*A*). Furthermore, the $CD83^+$ cells from the GM-CSF/IL-4/TNF-α cultures initiated significantly higher ($P < 0.002$) stimulatory effects when compared with $CD83^-$ cells, even at a 1:300 (stimulator/responder) ratio (Fig. 4*B*). The potent T-cell stimulatory activity of monocyte-derived $CD83^+$ cells was comparable with that of $CD83^+$ blood dendritic cells (31), confirming the functional maturity of monocyte-derived $CD83^+$ cells.

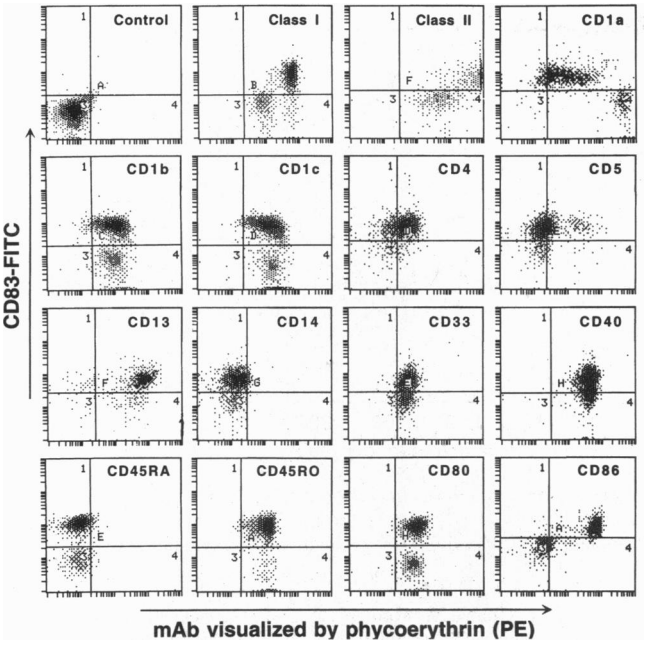

FIG. 3. Cell surface phenotype of monocyte-derived CD83$^+$ cells. Each histogram is representative of results obtained from 2 to 10 cell preparations and the figure is a compilation of results from multiple experiments. CD14$^+$ monocytes were cultured in GM-CSF/IL-4 for 5 days with TNF-α added during the final 2 days of the 7-day cultures. In all cases, similar flow cytometer settings were used to ensure uniformity, and the gates that delineated the histograms obtained from control cell populations stained with control antibodies are shown. The fluorescence intensity of CD83$^+$ cell expression of class II antigens exceeded the four-decade logarithmic scale and, therefore, was accumulated on the far right of the histogram. mAb, Monoclonal antibody; FITC, fluorescein isothiocyanate.

Table 1. Surface antigen expression by cell populations

Antigen	Antibody	Blood monocytes	Differentiated monocytes CD83$^+$	Differentiated monocytes CD83$^-$	CD83$^+$ blood DC
Class I	W6.32	++	+++	++	+++
Class II	I3	+++	+++++	+++	++++
CD1a	T6	−	++	++++	−
CD1b	4A7.6.5	−	++	++	−
CD1c	10C3	−	++	++	++
CD2	T11	−	+/−, few ++	+/−	++
CD3	T3	−	−	+/−	−
CD4	T4	+/−	+, few −	+	−
CD5	T1	+	+/−, few ++	+/−	+++
CD13	MY7	++	+++	+++	++
CD14	MY4	++	−	−	−
CD15s	csLex	−	−	−	+++
CD19	B4	−	−	−	−
CD20	B1	−	−	−	−
CD25	IL-2R	−	−, few +	−	+
CD29	4B4	++	++	++	++
CD31	1F11	++	+	+	+/−
CD33	MY9	++	+, weak	+	++
CD40	HB14	+	++	++	+
CD45	KC56	++	+	+	+
CD45RA	2H4	−	−	−	−
CD45RO	UCHL-1	−	++	++	++
CD54	HAE-4a	−	+/−	+, weak	+
CD80	B7-g, BB1	−	+	+	+
CD86	FUN-1	+/−	+++	−	+++
α2,6-SA	HB6	−	−	−	++
CMRF-44		−	−	−	+

DC, dendritic cells; SA, sialic acid. The methods and levels of surface antigen staining are as in Fig. 3. −, No staining above background levels; +/−, variable weak reactivity; + to +++++, increasingly positive staining based on a relative linear scale.

DISCUSSION

These results demonstrate that functionally mature dendritic cells can derive directly from blood monocytes under the influence of a specific cascade of cytokines. Monocyte differentiation into CD83$^+$ dendritic cells can be divided into several stages. First, an intermediate maturation stage is induced by GM-CSF/IL-4 where the cells were CD14$^+$ (Fig. 1 *A* and *B*) and expressed CD1a at high levels as described (29, 35). During the second stage, TNF-α induced the majority of cells to differentiate into CD83$^+$, CD14$^-$, and CD4$^+$ cells with a dendritic morphology. Continued culture of monocyte-derived CD83$^+$ cells led to decreased CD1a expression (Fig. 1*C*) and acquisition of a dermal dendritic cell phenotype (Fig. 3 and Table 1). The CD83$^+$ dendritic cells were the most potent of all stimulator cells in mixed leukocyte reactions (Fig. 4). Three populations of dermal dendritic cells have been previously identified as CD14$^+$/CD1a$^-$, CD14$^-$/CD1a$^+$, and CD14$^-$/CD1a$^-$, each with increasing antigen-presenting capacity (36). This linear differentiation scheme is also consistent with most CD83$^+$ cells in skin being CD1a$^+$ (30), while blood CD83$^+$ cells lack CD1a (31). Acquisition of CD45RO expression by monocyte-derived CD83 cells (Table 1) also correlates with dendritic-cell maturation (37, 38). Therefore, monocyte differentiation into dermal dendritic cells could be induced during local inflammatory reactions within tissues where all three cytokines are produced.

Earlier studies have reported that combinations of GM-CSF and either TNF-α or IL-4 can generate dendritic-like cells from blood leukocytes or their precursors (4, 13–29). Dendritic cells were identified in those studies based on a dendritic-cell morphology, size, surface phenotype, or function. This study extends those findings by demonstrating that pure populations of mature CD14$^+$ monocytes from blood can be induced to directly differentiate into functionally mature dendritic cells. In addition, when monocytes were cultured with appropriate combinations of GM-CSF, IL-4, and TNF-α, they expressed CD83 (Fig. 1), a marker for mature dendritic cells. Relative to our studies, Sallusto and Lanzacecchia (29) have shown that adherent blood mononuclear cells cultured with GM-CSF and IL-4 can generate dendritic cell lines with the phenotype and antigen-processing capacity of immature dendritic cells. These dendritic-cell lines express little or no CD14 and result from cell growth (29), which differs from the monocyte-derived dendritic cells described in our study (Fig. 1). Nonetheless, incubation of their dendritic cell lines with TNF-α or soluble CD40-ligand for 24 h upregulated their capacity to stimulate naive allogeneic T cells and downregulated their capacity to process/present soluble antigen. Therefore, it may be advantageous to provide monocyte-derived dendritic cells (cultured with GM-CSF/IL-4 only) with soluble antigens before inducing their further maturation into potent CD83$^+$ stimulator cells with TNF-α.

The ability to generate CD83$^+$ dendritic cells directly from blood monocytes provides important clues as to the differentiation pathways of this cell lineage and will facilitate the further functional and molecular characterization of this rare cell type. Specifically, the finding that dendritic cells expressed CD4 during their intermediate stages of differentiation from monocytes provides new insight into the controversial issue of whether dendritic cells can be infected with the human immunodeficiency virus (32, 39–44). The entry of virus-infected monocytes into cytokine-rich microenvironments may induce their differentiation into dendritic cells that can then transmit a vigorous cytopathic infection to T cells (32, 39, 40, 43). Given

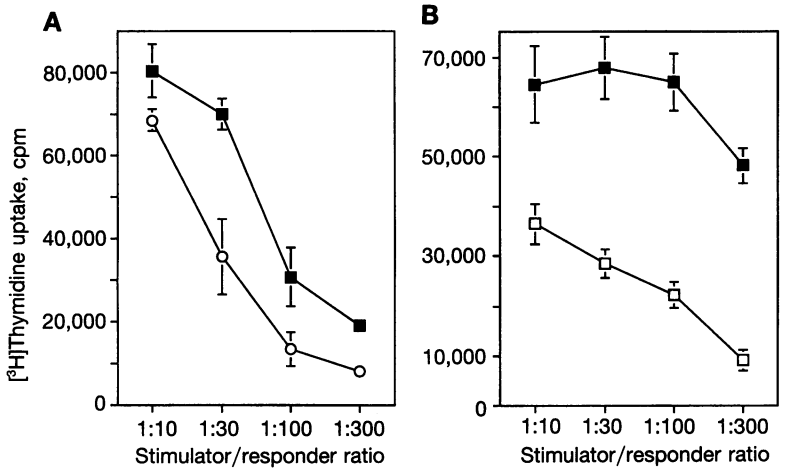

FIG. 4. Monocyte-derived CD83$^+$ cells are potent stimulator cells in an allogeneic mixed leukocyte reaction. (*A*) CD14$^+$ monocytes cultured for 7 days with GM-CSF/IL-4 (○) or GM-CSF/IL-4/TNF-α (■) were used as stimulator cells. (*B*) CD83$^+$ (■) or CD83$^-$ (□) cells obtained from the GM-CSF/IL-4/TNF-α cultures were used as stimulator cells. Values are the mean ± SD obtained for triplicate cultures and are representative of those obtained in two experiments.

the capacity of dendritic cells to elicit strong antigen-specific helper and cytotoxic T-cell responses, the ability to generate large numbers of homogeneous preparations of CD83$^+$ cells from plastic-adherent monocytes will also facilitate the manipulation of this cell lineage for vaccine development, organ grafting, and *ex vivo* therapy for a broad range of human diseases.

We thank Mr. Nathan Green, Ms. Susan Hester, and Dr. David Rothstein for help with these studies and reagents. This work was supported by National Institutes of Health Grants AI-26872, CA-54464, and HL-50985. T.F.T. is a Stohlman Scholar of the Leukemia Society of America.

1. Steinman, R. M. (1991) *Annu. Rev. Immunol.* **9,** 271–296.
2. Steinman, R. M., Gutchinov, B., Witmer, M. D. & Nussenzweig, M. C. (1983) *J. Exp. Med.* **157,** 613–627.
3. Kuntz Crow, M. & Kunkel, H. G. (1982) *Clin. Exp. Immunol.* **49,** 338–346.
4. Knight, S. C., Farrant, J., Bryant, A., Edwards, A. J., Burman, S., Lever, A., Clarke, J. & Webster, A. D. B. (1986) *Immunology* **57,** 595–603.
5. Young, J. W. & Steinman, R. M. (1988) *Cell. Immunol.* **111,** 167–182.
6. Van Voorhis, W. C., Hair, S. L., Steinman, R. M. & Kaplan, G. (1982) *J. Exp. Med.* **155,** 1172–1187.
7. Xu, H., Friendrichs, U., Gieseler, R. K. H., Ruppert, J., Ocklind, G. & Peters, J. H. (1992) *Scand. J. Immunol.* **36,** 689–696.
8. Wood, G. S. & Freudenthal, R. S. (1992) *Am. J. Pathol.* **141,** 789–795.
9. Egner, W., Prickett, T. C. R. & Hart, D. N. J. (1992) *Trans. Proc.* **24,** 2318.
10. Thomas, R., Davis, L. S. & Lipsky, P. E. (1993) *J. Immunol.* **150,** 821–834.
11. Egner, W., Andreesen, R. & Hart, D. N. J. (1993) *Transplantation* **56,** 945–950.
12. Freudenthal, P. S. & Steinman, R. M. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 7689–7702.
13. Reid, C. D., Fryer, P. R., Clifford, C., Kirk, A., Tikerpae, J. & Knight, S. C. (1990) *Blood* **76,** 1139–1149.
14. Reid, C. D. L., Stackpoole, A., Meager, A. & Tikerpae, J. (1992) *J. Immunol.* **149,** 2681–2688.
15. Santiago-Schwarz, F., Belilos, E., Diamond, B. & Carsons, S. E. (1992) *J. Leukocyte Biol.* **52,** 274–281.
16. Inaba, K., Inaba, M., Deguchi, M., Hagi, K., Yasumizu, R., Ikehara, S., Muramatsu, S. & Steinman, R. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 3038–3042.
17. Santiago-Schwarz, F., Divaris, N., Kay, C. & Carsons, S. E. (1993) *Blood* **82,** 3019–3028.
18. Szabolcs, P., Moore, M. A. S. & Young, J. W. (1995) *J. Immunol.* **154,** 5851–5861.
19. Inaba, K., Steinman, R. M., Pack, M. W., Aya, H., Inaba, M., Sudo, T., Wolpe, S. & Schuler, G. (1992) *J. Exp. Med.* **175,** 1157–1167.
20. Caux, C., Dezutter-Dambuyant, C., Schmitt, D. & Banchereau, J. (1992) *Nature (London)* **360,** 258–261.
21. Inaba, K., Inaba, M., Romani, N., Aya, H., Degichi, M., Ikehara, S., Muramatsu, S. & Steinman, R. (1992) *J. Exp. Med.* **176,** 1693–1702.
22. Romani, N., Gruner, S., Brang, D., Kampgen, E., Lenz, A., Trockenbacher, B., Konwalinka, G., Fritsch, P. O., Steinman, R. M. & Schuler, G. (1994) *J. Exp. Med.* **180,** 83–93.
23. Grage-Griebenow, E., Lorenzen, D., Fetting, R., Flad, H. D. & Ernst, M. (1993) *Eur. J. Immunol.* **23,** 3126–3135.
24. Kabel, P. J., De Haan-Meulman, M., Voorbij, H. A. M., Kleingeld, M., Knol, E. F. & Drexhage, H. A. (1989) *Immunobiology* **179,** 395–411.
25. Najar, H. M., Bru-Capdeville, A. C., Gieseler, R. K. H. & Peters, J. H. (1990) *Eur. J. Immunol.* **51,** 339–346.
26. Peters, J. H., Ruppert, J., Gieseler, R. K., Najar, H. M. & Xu, H. (1991) *Pathobiology* **59,** 122–126.
27. Ruppert, J., Friedrichs, D., Xu, H. & Peters, J. H. (1991) *Immunobiology* **182,** 449–464.
28. Levine, T. & Chain, B. M. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 8342–8346.
29. Sallusto, F. & Lanzavecchia, A. (1994) *J. Exp. Med.* **179,** 1109–1118.
30. Zhou, L.-J., Schwarting, R., Smith, H. M. & Tedder, T. F. (1992) *J. Immunol.* **149,** 735–742.
31. Zhou, L.-J. & Tedder, T. F. (1995) *J. Immunol.* **154,** 3821–3835.
32. Weissman, D., Li, Y., Ananworanich, J., Zhou, L.-J., Adelsberger, J., Tedder, T. F., Baseler, M. & Fauci, A. S. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 826–830.
33. Engel, P., Wagner, N. & Tedder, T. F. (1995) in *CD83 Workshop Report*, eds. Schlossman, S. F., Boumsell, L., Gilks, W., Harlan, J. M., Kishimoto, T., Morimoto, C., Ritz, J., Shaw, S., Silverstein, R., Springer, T., Tedder, T. F. & Todd, R. F. (Oxford Univ. Press, Oxford), Vol. 1, pp. 693–695.
34. Zhou, L.-J. & Tedder, T. F. (1995) *Blood* **86,** 3295–3301.
35. Kasinrerk, W., Baumruker, T., Majdic, O., Knapp, W. & Stockinger, H. (1993) *J. Immunol.* **150,** 579–584.
36. Nestle, F. O., Zheng, X.-G., Thompson, C. B., Turka, L. A. & Nickoloff, B. J. (1993) *J. Immunol.* **151,** 6535–6545.
37. O'Doherty, U., Steinman, R. M., Peng, M., Cameron, P. U., Gezelter, S., Kopeloff, I., Swiggard, W. J., Pope, M. & Bhardwaj, N. (1993) *J. Exp. Med.* **178,** 1067–1078.
38. O'Doherty, U., Peng, M., Gezelter, S., Swiggard, W. J., Betjes, M., Bhardwaj, N. & Steinman, R. M. (1994) *Immunology* **82,** 487–493.
39. Pope, M., Betjes, M. G. H., Romani, N., Hirmand, H., Cameron, P. U., Hoffman, L., Gezelter, S., Schuler, G. & Steinman, R. M. (1994) *Cell* **78,** 389–398.
40. Cameron, P. U., Freudenthal, P. S., Barker, J. M., Gezelter, S., Inaba, K. & Steinman, R. M. (1992) *Science* **257,** 383–387.
41. Langhoff, E. & Haseltine, W. A. (1992) *J. Invest. Dermatol.* **99,** 89S–94S.
42. Langhoff, E., Kalland, K. H. & Haseltine, W. A. (1993) *J. Clin. Invest.* **91,** 2721–2726.
43. Pinchuk, L. M., Polacino, P. S., Agy, M. B., Klaus, S. J. & Clark, E. A. (1994) *Immunity* **1,** 317–325.
44. Patterson, S., Gross, J., English, N., Stackpoole, A., Bedford, P. & Knight, S. C. (1995) *J. Gen. Virol.* **76,** 1155–1163.