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Katsuya Miyatani* Kiyoshi Takahashi† Hiroyuki Yanai‡

Tadashi Yoshino** Tadaatsu Akagi††

*Okayama University,

†Okayama University,

‡Okayama Univeristy,

**Okayama University,

††Okayama University,

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Katsuya Miyatani, Kiyoshi Takahashi, Hiroyuki Yanai, Tadashi Yoshino, and Tadaatsu Akagi

Abstract

Previously, we reported that interleukin-2 (IL-2)-stimulated helper T cells produced an unknown soluble factor which induced dendritic cell-like differentiation in primary cultures of monocytic leukemia cells and we referred to this factor as dendritic cell differentiation factor (DCDF). In this study, we attempted to purify and characterize DCDF and investigated its biological effect on normal human monocytes. Gel filtration chromatography indicated that the molecular weight of DCDF is approximately 30-35 kDa. Chromatofocusing indicated that the isoelectric point of DCDF is approximately 5.0. DCDF, partially purified by subsequent gel filtration, chromatofocusing, and hydrophobic chromatography, significantly enhanced the HLA-DR expression of normal human monocytes and a human monocytic leukemia cell line, THP-1. This biological activity was not neutralized by any known antibodies to human cytokines. DCDF significantly amplified the T-cell stimulatory activity of monocytes in the allogeneic mixed leukocyte reaction (MLR). Moreover, DCDF significantly enhanced IL-1 beta and IL-6 production by monocytes in a dose-dependent manner. These results suggest that DCDF is a novel human cytokine which stimulates the accessory cell function of monocytes.

KEYWORDS: dendritic cell, differentiation, protein purification, cytokine

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Partial Purification and Characterization of Dendritic Cell Differentiation Factor

KATSUYA MIYATANI, KIYOSHI TAKAHASHI*, HIROYUKI YANAI, TADASHI YOSHINO AND TADAATSU AKAGI

Second Department of Pathology, Okayama University Medical School, Okayama 700, Japan

Previously, we reported that interleukin-2 (IL-2)-stimulated helper T cells produced an unknown soluble factor which induced dendritic cell-like differentiation in primary cultures of monocytic leukemia cells and we referred to this factor as dendritic cell differentiation factor (DCDF). In this study, we attempted to purify and characterize DCDF and investigated its biological effect on normal human monocytes. Gel filtration chromatography indicated that the molecular weight of DCDF is approximately 30-35 kDa. Chromatofocusing indicated that the isoelectric point of DCDF is approximately 5.0. DCDF, partially purified by subsequent gel filtration, chromatofocusing, and hydrophobic chromatography, significantly enhanced the HLA-DR expression of normal human monocytes and a human monocytic leukemia cell line, THP-1. This biological activity was not neutralized by any known antibodies to human cytokines. DCDF significantly amplified the T-cell stimulatory activity of monocytes in the allogeneic mixed leukocyte reaction (MLR). Moreover, DCDF significantly enhanced IL-1β and IL-6 production by monocytes in a dose-dependent manner. These results suggest that DCDF is a novel human cytokine which stimulates the accessory cell function of monocytes.

Key words: dendritic cell, differentiation, protein purification, cytokine

Dendritic cells (DC) are immunocytes which have characteristic dendritic morphology and a potent accessory cell function for T cells. Interdigitating reticulum cells (IDC) of the T-lymphoid tissues and Langerhans cells (LC) of the suprabasal layer of the epidermis are main classes of DC (1). Because of the marked similarities in morphology, phenotype and function, tissue-specific DC are generally thought to be derived from the same lineage (2). Although DC have been well-documented to be bone marrow origin (3, 4) and generally thought to belong to the mononuclear phagocyte system, recent immunological and cytological studies have revealed that the DC are quite different from phagocytic macrophages in terms of typical dendritic morphology, and further that they have a potent accessory cell function and lack phagocytic activity (5-7). While these differences imply that DC are independent of the mononuclear phagocyte system (5), many reports suggest a close cytogenic relationship between DC and the monocytic lineage (8, 9). In fact, several unusual cases of monocytic leukemia have been reported in which the leukemia cells in the T-lymphoid tissues or skin showed features of DC (10). The authors consider that, in such cases, the microenvironment of the skin or T-lymphoid tissues induced the leukemic cells to differentiate into DC (8-10). However, little is known about such microenvironmental factors.

Previously, we reported a unique case with acute monocytic leukemia in which the neoplastic cells in the lymphoid tissues had features of DC, while leukemic cells in the peripheral blood were essentially monocytic and lacked such features (11). We established an interleukin-2 (IL-2)-dependent, nonneoplastic helper T-cell line from the peripheral blood of the patient and found that the culture supernatant contained a soluble factor which induced IDC-like differentiation in the monocytic leukemia cells (11). Because this factor was immunologically different from the all human cytokines, we referred to it as dendritic cell differentiation factor (DCDF). In this study, we partially purified and characterized DCDF. We also studied the biological effects of DCDF on normal human blood monocytes.

* To whom correspondence should be addressed.

Materials and Methods

Cells. The DCDF-producing IL-2-dependent T-cell line, KAZ-3N, was established from the peripheral blood of the patient with acute monocytic leukemia mentioned above (11). A human monocytic leukemia cell line, THP-1, was obtained from the Japanese Cancer Research Resources Bank (Tokyo, Japan). The non-hematopoietic cell lines, HeLa and HGC-27 (human gastric carcinoma), were also used.

Monocytes were collected from the peripheral blood by plastic adherence. Peripheral blood mononuclear cells (PBM) were obtained from the peripheral blood of healthy individuals by Ficoll-Hypaque density gradient centrifugation. The PBM were suspended at a concentration of 4×10^5/ml in RPMI-1640 medium containing 10% low endotoxin (<0.1 ng/ml) fetal calf serum (FCS), and then plated in culture bottles (75 cm^2; Falcon, Becton Dickinson Labware, Lincoln Park, NJ, USA), which had been treated with heat-inactivated human AB serum for 24 h at 4°C to avoid nonspecific adherence of lymphocytes. After incubation for 60 min, nonadherent cells were removed by gentle washing with RPMI-1640 medium, and adherent cells were incubated in 1/15 M phosphate-buffered saline (PBS) containing 0.2% EDTA-Na_2. After incubation for 30 min at 4°C, the adherent cells detached spontaneously. Cells were washed and suspended in RPMI-1640 medium containing 10% FCS. The purity of the monocytes was about 98%, as determined by the nonspecific esterase method.

Cell culture. Cells were cultured in RPMI-1640 medium containing 10% heat-inactivated low endotoxin FCS supplemented with penicillin G (100 U/ml), kanamycin (60 μg/ml) and streptomycin (250 μg/ml). Cultures were maintained at 37°C in 5% CO_2 in humidified air.

Partial purification of DCDF. KAZ-3N cells were cultured in complete serum-free medium Cosmedium 001 (Cosmo Bio, Tokyo, Japan) containing 2% serum-free medium TYI-100 (Dainippon Seiyaku, Tokyo, Japan) and recombinant human (rh) IL-2 (75 JRU/ml; Shionogi, Osaka, Japan) at a concentration of 1×10^6 cells/ml. After 72 h in culture, the culture supernatant was collected, and cell debris was removed by centrifugation at 10,000 g for 30 min. Protein in 20 l of culture supernatant was precipitated by cooled 50% acetone (−20°C), dissolved in 10 ml of 20 mM Tris-acetate buffer, pH 9.0. After filter-sterilization, the sample was loaded on a Superdex™ 200 gel filtration column (Pharmacia, Uppsala, Sweden), which had been equilibrated with the same buffer. After flow-through, protein was eluted with the buffer at a flow rate of 8 ml/min (1 min/fraction). The column was calibrated using thyroglobulin, gamma globulin, ovalbumin, myoglobin and cyanocobalamin with Mr of 670, 158, 44, 14, and 1.3 kDa, respectively. Individual fractions were diluted in RPMI-1640 medium containing 10% FCS at dilutions of 1:10, 10^2, 10^3, and 10^4, and were filter-sterilized. THP-1 cells ($n = 10^5$) were incubated in the culture media. The biological activity of DCDF in individual fractions was evaluated based on the maximum dilution which enhanced the HLA-DR expression of monocytes, as examined by flow-cytometry. The positive fractions were collected and loaded on a Mono-P HR 5/5 isoelectric focusing column (Pharmacia), which had been equilibrated with 20 mM Tris-acetate buffer, pH 9.0. After flow-through, protein was eluted with the elution buffer: polybuffer 96 (30%) + polybuffer 74 (70%) (Pharmacia) adjusted to pH 4.5 with acetate, and diluted with distilled water at 1:10. Running conditions were a flow rate of 0.5 ml/min, 1 min/fraction. Individual fractions were bioassayed as described above. The positive fractions were collected and 1.7 M $(NH_4)_2SO_4$ was added to the sample. The sample was then loaded on a phenylsuperose column (Pharmacia), which had been equilibrated with 20 mM Tris-HCl buffer, pH 7.4, containing 1.7 M $(NH_4)_2SO_4$. After flow-through, a linear gradient of $(NH_4)_2SO_4$ in the starting buffer was run from 1.7 to 0.01 M for 30 min. Running conditions were a flow rate of 0.5 ml/min, 1 min/fraction. Ammonium sulfate in individual fractions were removed by sephadex G50 (Pharmacia) chromatograhy and the DCDF biological activity of individual fractions were assayed as described above. The positive fractions were collected and analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) on 20-10% polyacrylamide gradient gels under either reducing or non-reducing conditions. The gels were then stained with Coomassie brilliant blue R-250. The protein concentration of the final sample was calculated by optical density at 280 nm.

Enzyme-linked immunosorbent assay (ELISA). The human cytokine levels in the culture supernatant from KAZ-3N cells, including IL-1α, IL-1β, IL-6, interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), and GM-CSF, were assayed by ELISA using Genzyme ELISA kits (Genzyme, Boston MA,

USA) according to the manufacturer's instructions.

DCDF treatment. The biological effects of DCDF on various normal and neoplastic cells were examined. THP-1, HeLa, HGC-27 cells and human peripheral blood monocytes ($n=10^6$/well) were cultured in RPMI-1640 medium containing 10% FCS and partially purified DCDF at concentrations of 50 ng protein/ml for 72 h, and then examined.

Flow cytometry. For flow cytometry, we used a fluorescein isothiocyanate (FITC)-conjugated monoclonal antibody (mab) to human HLA-DR (Becton Dickinson). Control or DCDF-treated cells were incubated with FITC-conjugated mab to human HLA-DR diluted at a dilution of 1:20 for 30 min at 4°C, washed, and then examined by flow cytometry using an Epics 750 flow cytometer (Coulter Electrics, Hialeah, FL, USA) as described previously (11, 12).

Effects of DCDF on cytokine production of monocytes. Monocytes ($n=10^6$) were cultured in RPMI-1640 medium containing 10% FCS and partially purified DCDF at concentrations of 50, 25, 12.5, and 6.25 ng protein/ml. After incubation for 72 h, the levels of IL-1α, IL-1β, IL-6, and TNF-α were investigated using ELISA, as described above.

Effects of DCDF on the accessory cell function of monocytes. We evaluated the accessory cell function of monocytes by means of a primary autologous and allogeneic MLR assay. The capacity of the primary population of leukocytes to act as MLR stimulators requires an accessory function in addition to the expression of MHC-encoded transplantation antigens (13). Therefore, stimulatory activity in the primary MLR was used as a measure of the accessory cell function that is required to activate antigen-specific, resting T cells. Normal autologous and allogeneic T cells, which were isolated by sheep erythrocyte rosetting from normal PBM, were used as responder cells in the primary autologous and allogeneic MLR assay. Fresh PBM were used as stimulators. Monocytes (autologous: $n=10^4$, allogeneic: $n=10^3$) were incubated in 200 ml culture medium containing partially purified DCDF at a concentration of 50, 5, or 0.5 ng protein/ml, or in control medium in the individual wells of round-bottomed 96-well microtiter plates (Corning) for 72 h. Thereafter, the cells were washed twice with RPMI-1640 by centrifugation at 400 g, and the medium was replaced with 100 ml RPMI-1640 containing 10% heat-inactivated human AB serum. Then, 10^5 autologous or allogeneic T cells suspended in 100 μl RPMI-1640 medium containing 10% heat-inactivated human AB serum were added to each well and incubated. Four days later, the cultures were incubated with 1 μCi/well of [^{3}H] thymidine for 16 h and harvested to measure the [^{3}H] thymidine incorporation into DNA. Responses were reported as the mean cpm of triplicate autoradiography.

Immunoinhibition studies. Rabbit neutralizing antibodies against human IL-1α, -1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, GM-CSF, M-CSF, G-CSF, IFN-α, IFN-β, IFN-γ, or TNF-α were diluted in medium containing partially purified DCDF (50 ng/ml) or control medium at a concentration of 1:20 and incubated for 2 h at 37°C. This concentration is within the range of neutralizing activity indicated by the manufacturers of these antibodies. After incubation, THP-1 cells were cultured with each sample for 72 h. DCDF activity of the samples was evaluated by the positive conversion of HLA-DR antigen of THP-1 cells.

Results

Contents of Cytokines in the Conditioned Medium

ELISA assay indicated that the culture supernatant from IL-2-supplemented KAZ-3N cultures contained a large amount of GM-CSF (18,000 pg/ml), and trace amounts of cytokines including IL-1β (142 pg/ml), TNF-α (11 pg/ml), and IL-6 (80 pg/ml), but not IL-1α, IL-3, IL-4 or IFN-γ.

Characterization of DCDF

DCDF significantly enhanced HLA-DR expression in a human monocytic leukemia cell line, THP-1. Neutralized antibodies to human cytokines including IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, GM-CSF, M-CSF, G-CSF, TNF-α, IFN-α, IFN-β, and IFN-γ had no effect on the biological activity of DCDF, indicating that DCDF is immunologically distinctive from these known human cytokines. Gel filtration chromatography indicated that the molecular weight of DCDF is approximately 30–35 kDa (Fig. 1a). Chromatofocusing indicated that the isoelectric point of DCDF is approximately 5.0 (Fig. 1b). The results of hydrophobic chromatography indicated that DCDF is a hydrophobic protein (Fig. 1c). No cytokines were detected by ELISA in this final sample. The numerous faint bands in SDS-PAGE gels stained with Coomassie brilliant blue R-250, indicated the presence of many polypeptides, but

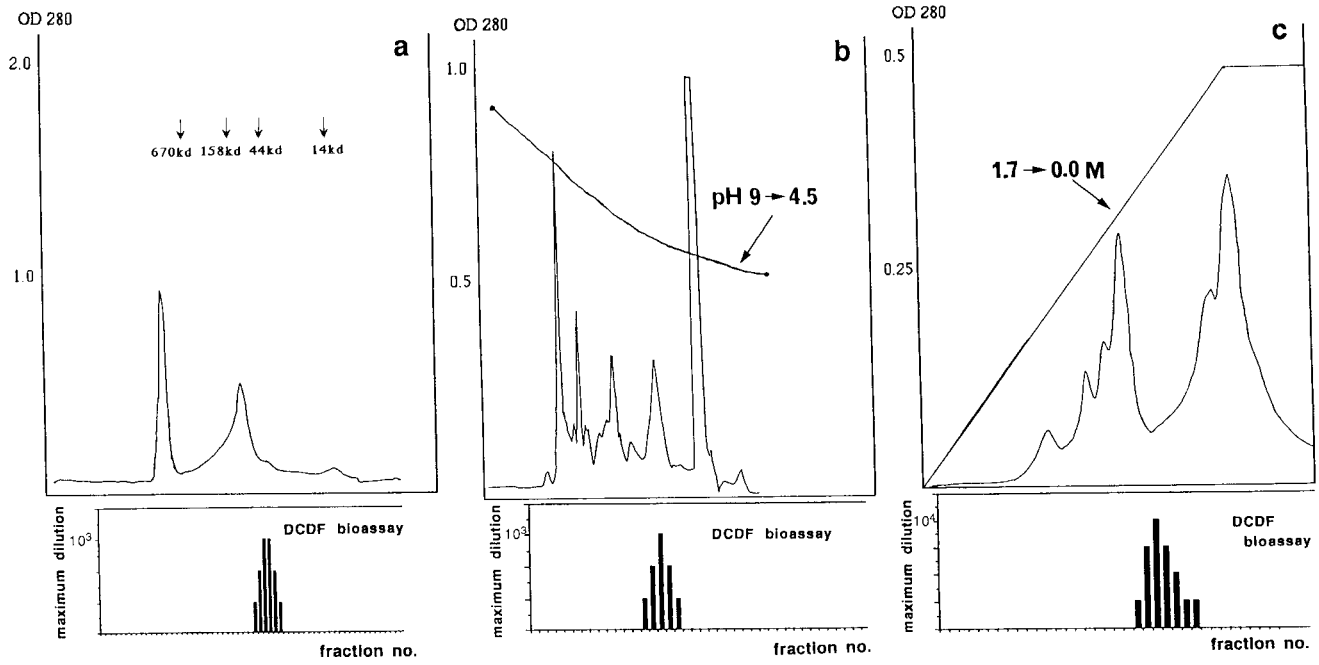

Fig. 1 Biochemical properties of dendritic cell differentiation factor (DCDF).
a. Gel filtration column chromatography. **b**. Mono-P (isoelectric focusing) column chromatography.
c. Phenylsuperose (hydrophobic) column chromatography.
The biological activity of DCDF was evaluated based on the enhancement of HLA-DR expression of monocytes. The degree of its activity was expressed as the maximum dilution which enhances HLA-DR expression of monocyte in cells. It shows a single peak in each chromatography.

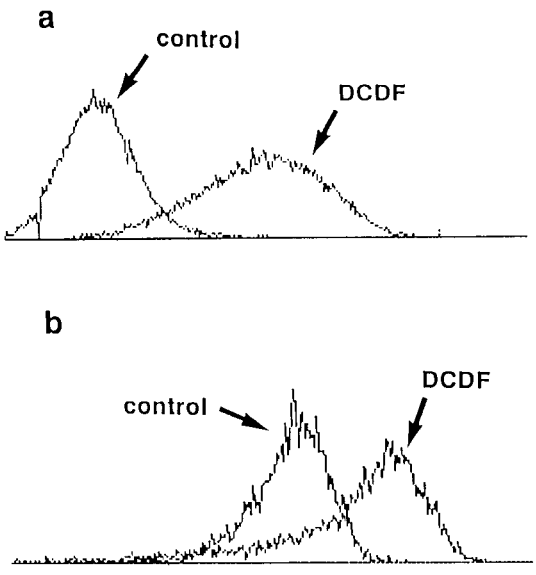

Fig. 2 Flow cytometry. HLA-DR antigen expression by THP-1 cells (**a**) and monocytes (**b**) cultured in the control or DCDF-containing medium. DCDF: See Fig. 1.

we failed to identify which band corresponded to DCDF biological activity (data not shown).

Effects of DCDF on Human Cell Lines

Flow cytometry indicated that DCDF significantly upregulated HLA-DR expression in THP-1 cells (Fig. 2a). DCDF had no significant effect on HLA-DR expression of nonhematopoietic cell lines, HGC-27 and HeLa.

Effects of DCDF on Normal Human Monocytes

Monokine induction. The results of ELISA showed that partially purified DCDF significantly enhanced IL-1β and IL-6 production by monocytes in a dose-dependent manner (Fig. 3), but it had no significant effect on IL-1α and TNF-α production by monocytes (data not shown).

HLA-DR expression. Flow cytometry indicated that DCDF significantly enhanced HLA-DR expression in monocytes (Fig. 2b).

Accessory cell function. The accessory function of monocytes was evaluated by their T-cell

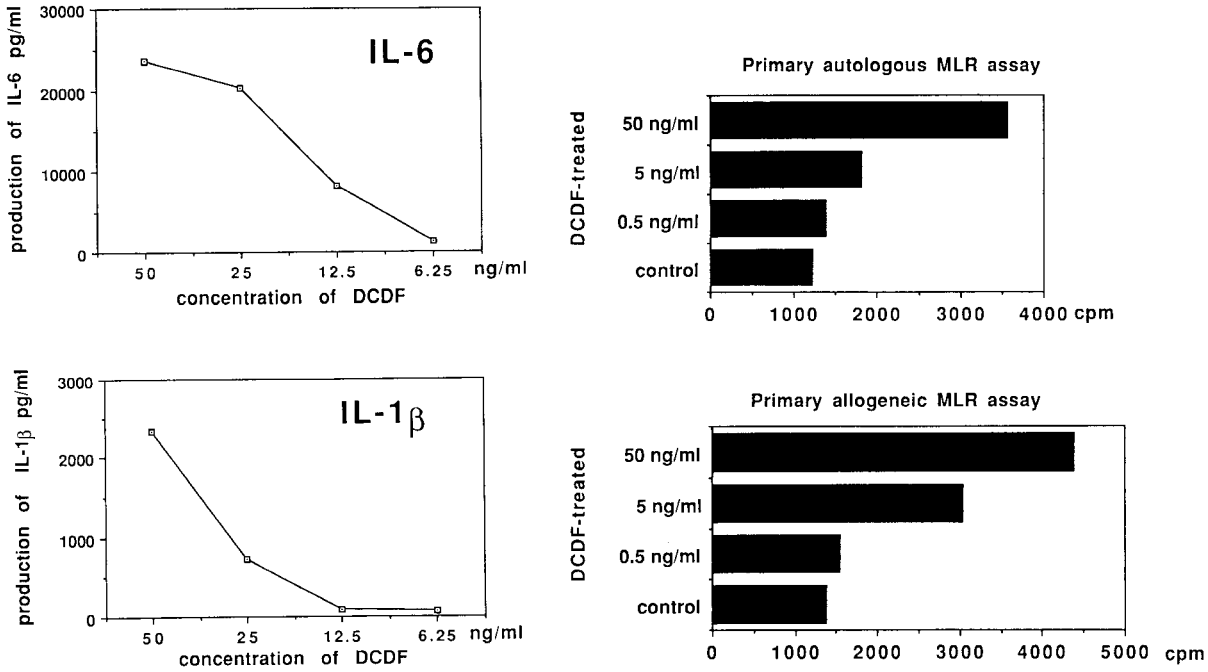

Fig. 3 (Left) Effects of DCDF on the cytokine production by monocytes after 72h in culture (monocytes: 1 x 10^6cell/ml). IL-1β and IL-6 production were significantly enhanced in a dose-dependent manner. DCDF: See Fig.1.

Fig. 4 (Right) Effects of DCDF on the accessory cell function of monocytes evaluated in primary autologous and allogeneic mixed leulkocyte reaction (MLR) (monocyte-autologous: 10^4, allogeneic: 10^3, T cell: 10^5). DCDF enhanced the T cell-mitogenic activity in a dose-dependent manner. DCDF: See Fig. 1.

mitogenicity in primary autologous and allogeneic MLR. As shown in Fig. 4, in both autologous and allogeneic MLR, DCDF augmented the T-cell mitogenic activity in a dose-dependent manner.

Immunoinhibition Assay

None of the neutralizing antibodies to human cytokines examined had any effect on the DCDF biological activities as evaluated by HLA-DR positive conversion of both THP-1 and monocytes (data not shown). This finding indicates that DCDF is immunologically different from all known human cytokines.

Discussion

In this study, we partially purified and characterized DCDF and clarified its biological effects on human normal monocytes. The fact that the biological activity of DCDF was not neutralized by any of the neutralizing antibodies to known human cytokines indicates that DCDF is immunologically distinct. We failed to identify any protein band by SDS-PAGE which corresponded to DCDF biological activity, and one possible explanation for this could be that DCDF has a high potency and was thus below the levels of detection. Further large scale studies should be performed to completely purify DCDF.

It is noteworthy that DCDF significantly upregulates the expression of HLA-DR antigen of monocytes and enhances their T-cell stimulatory activity in primary MLR, while monocytes usually do not stimulate T cells in primary MLR (5, 6, 14), and to our knowledge there are no reports of cytokines which upregulate this activity. Although IFN-γ significantly upregulates HLA-DR antigen expression in monocytes, it is also ineffective in upregulating T cells in primary MLR (11, 15). Dendritic cells have a potent T-cell stimulatory activity in primary MLR, whereas monocytes and phagocytic macrophages lack this activity, although they express a large amount of HLA-DR antigen (5, 6, 14), which is considered to be

the most important feature of dendritic cells (14). Thus, it is likely that DCDF induces monocytes to functionally differentiate into dendritic cells.

IL-1 secretion by monocytes/macrophages can be stimulated by exposing these cells to lipopolysaccharides (LPS), TNF-α, IL-6 and to IL-1β itself (16). IL-6 secretion by monocytes can also be stimulated with LPS, IL-1, or TNF-α (17, 18). As shown here, DCDF induced monocytes to produce IL-1β and IL-6; Northern blotting analysis indicated that DCDF stimulates the transcription of both genes (unpublished data). Compared with other cytokines, such as GM-CSF and IFN-γ, DCDF is extremely potent in inducing IL-1β and IL-6 production by monocytes (unpublished data). Unlike LPS, DCDF does not significantly induce IL-1α and TNF-α production by monocytes, suggesting that the enhancement of IL-1β and IL-6 production by DCDF is not due to augmentation of the response of monocytes to LPS which is present at trace levels in culture media.

Both IL-1β and IL-6, but not IL-1α and TNF-α, are produced by dendritic cells such as Langerhans cells, and are essential for their accessory cell function (19). The present findings that DCDF stimulates the production of IL-1β and IL-6 by monocytes seem to favor the view that DCDF induces the functional differentiation of monocytes into dendritic cells.

In this study, we did not investigate the morphological and phenotypic effects of DCDF on monocytes. We recently found that DCDF blocks the differentiation of monocytes into macrophages and induces several characteristic phenotypic features of dendritic cells such as CD1 antigen (unpublished data). We consider that DCDF is a novel human cytokine which plays an important role in the accessory cell-mediated immunological reactions and that DCDF may be an important microenvironmental factor in T-lymphoid organs that induces the differentiation of locally migrated monocytes into dendritic cells.

References

1. Ruco LP, Uccini S and Baroni CD: The Langerhans' cells. Allergy (1989) **9**, 27-30.
2. Hoefsmit ECM, Duijvestinjin AM and Kamperdijk AM: Relation between Langerhans cells, veiled cells, and interdigitating cells. Immunobiology (1982) **161**, 255 265.
3. Bowers WE, Berkowitz M, Labadie JH and Klinkert WEF: Differentiation of dendritic cells from rat bone marrow. Transplant Proc (1983) **15**, 1611-1612.
4. Katz SI, Tamaki K and Sachs DH: Epidermal Langerhans cells are derived from cells originating in bone marrow. Nature (1979) **282**, 324 326.
5. Van Voorhis WC, Hair LS, Steinman RM and Kaplan G: Human dendritic cells: Enrichment and characterization from peripheral blood. J Exp Med (1982) **155**, 1172 1187.
6. Braathen LR and Thorsby E: Human epidermal Langerhans cells are more potent than blood monocytes in inducing some antigen-specific T-cell responses. Br J Dermatol (1983) **108**, 139 146.
7. Takahashi K, Yamaguchi H, Ishizeki J, Nakajima T and Nakazato Y: Immunohistochemical and immunoelectron microscopic localization of S-100 protein in the interdigitating reticulum cells of the human lymph node. Virchow Arch [B] (1981) **37**, 125 135.
8. Murphy GF, Messadi D, Fonferko E and Hancock WW: Phenotypic transformation of macrophages to Langerhans cells in the skin. Am J Pathol (1986) **123**, 401-406.
9. Bani D and Giannotti B: Differentiation of interdigitating reticulum cells and Langerhans cells in the human skin with T-lymphoid infiltrate: An immunocytochemical and ultrastructural study. Arch Histol Cytol (1989) **52**, 361-372.
10. Kaiserling E and Horny HP: Dermal Langerhans' cell tumor in chronic myelomonocytic leukemia. Ultrastruct Pathol (1988) **12**, 209-219.
11. Takahashi K, Miyatani K, Yanai H, Jeon HJ, Yoshino T, Hayashi K, Akagi T, Tsutsui K and Mizobuchi K: Induction of interdigitating reticulum cell-like differentiation in human monocytic leukemia cells by conditioned medium from IL-2-stimulated helper T-cells. Virchow Arch [B] (1992) **62**, 105-113.
12. Yoshino T, Mukuzono H, Aoki H, Takahashi K, Takeuchi T, Kubonishi I, Ohtsuki Y, Motoi M and Akagi T: A novel monoclonal antibody (OPD4) recognizing a helper/inducer T cell subset: Its application to paraffin-embedded tissues. Am J Pathol (1989) **134**, 1339-1346.
13. Inaba K, Steinman RM, Van Voorhis WC and Muramatsu S: Dendritic cells are critical accessory cells for thymus-dependent antibody responses in mouse and in man. Proc Natl Acad Sci USA (1983) **80**, 6041-6045.
14. Kunz M and Kunkel HG: Human dendritic cells-major stimulators of the autologous and allogeneic mixed leukocyte reactions. Clin Exp Immunol (1982) **49**, 338-346.
15. Messadi DV, Pober JS and Murphy GF: Effects of recombinant gamma-interferon on HLA-DR and DQ expression by skin cells in short-term organ culture. Lab Invest (1988) **58**, 61-67.
16. Schindler R, Mancilla J, Endres S, Ghorbani R, Clark SC and Dinarello CA: Correlations and interactions in the production of interleukin-6 (IL-6), IL-1, and tumor necrosis factor (TNF) in human blood mononuclear cells: IL-6 suppresses IL-1 and TNF. Blood (1990) **75**, 40-47.
17. Tosato G and Jones KD: Interleukin-1 induces Interleukin-6 production in peripheral blood monocyte. Blood (1990) **75**, 1305-1310.
18. Kohase M, Henriksen-DeStefano D, May LT, Vilcek J and Sehgal PB: Induction of β2-interferon by tumor necrosis factor: A homeostatic mechanism in the control of cell proliferation. Cell (1986) **45**, 659-666.
19. Schreiber S, Kilgus O, Payer E, Kutil R, Elbe A, Mueller C and Stingl G: Cytokine pattern of Langerhans cells isolated from murine epidermal cell cultures. J Immunol (1992) **149**, 3525-3534.

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