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Interferon-α and granulocyte-macrophage colony-stimulating factor differentiate peripheral blood monocytes into potent antigen-presenting cells

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Abstract: The diverse roles of interferon-α (IFN-α) in regulating the immune response to infectious agents suggested that it might affect dendritic cell (DC) development. Peripheral blood mononuclear cells cultured with IFN-α and granulocyte-macrophage colony-stimulating factor (GM-CSF) developed a dendritic morphology and expressed high levels of the class I and II human leukocyte antigens (HLA), B7 co-stimulatory molecules, adhesion proteins, and CD40. Elevated DC expression of B7-2 and HLA-DR was observed with increasing IFN-α concentrations up to 5000 U/mL. The effects of IFN-α on DC immunophenotype were not reversed by adding neutralizing antibodies against interleukin-4 (IL-4) or tumor necrosis factor α to the cell cultures or by eliminating lymphocytes from the cultures. The addition of IFN-α to cultures containing optimal concentrations of IL-4 and GM-CSF significantly increased the B7-2 and HLA-DR levels above those present on DCs grown in two cytokines. The DCs generated with IFN-α and GM-CSF were potent antigen-presenting cells in allogeneic mixed leukocyte reactions. They also were capable of taking up, processing, and presenting tetanus toxin to autologous T lymphocytes. These results demonstrate an important role for IFN-α in the generation of DCs with potent antigen-presenting capabilities from peripheral blood monocytes. *J. Leukoc. Biol.* 64: 358–367; 1998.

Key Words: *dendritic cell · interleukin-4 · mixed leukocyte reaction*

INTRODUCTION

The human interferon-α (IFN-α) family is comprised of a large group of homologous proteins encoded by at least 14 linked genes [1]. Virtually all somatic cells are thought to produce IFN-α, and the receptor for IFN-α is ubiquitously expressed. Cellular synthesis of IFN-α can be induced by viral infection or double-stranded RNA, infection by bacteria or mycoplasma, or exposure of the appropriate cell type to interleukin-1, interleukin-2, tumor necrosis factor, macrophage colony-stimulating factor, or platelet-derived growth factor [2]. IFN-α has multiple biological activities including antiviral, antiproliferative, and immunoregulatory effects. The effects of IFN-α on the immune system include activation of natural killer cells, stimulation of $CD8^+$ T lymphocyte proliferation and survival, promotion of $CD4^+$ Th1 cell differentiation, and up-regulation of class I major histocompatibility complex (MHC) expression [3–6]. The ability of IFN-α to orchestrate such a diverse and complementary range of immune activities against viral and bacterial pathogens suggested that it also might affect the development or function of antigen-presenting cells.

Dendritic cells (DCs) are highly potent antigen-presenting cells derived from hematopoietic progenitors [7, 8]. They have been identified in sites that favor their contact with foreign antigen including the skin (Langerhans cells), bronchial epithelium, and intestinal mucosa (interstitial DCs) [9, 10]. After they have encountered antigen, DCs migrate via the blood or lymph to lymphoid tissues where they present processed antigen to T lymphocytes. The transition of DCs from antigen-processing to antigen-presenting cells is accompanied by alterations in their surface membrane protein expression. Up-regulation of class I and class II MHC proteins, B7-1 and B7-2 co-stimulatory molecules, and CD40 expression enhances the ability of DCs to present antigen to T lymphocytes and induce their activation. Mature DCs also elaborate interleukin-12 (IL-12), which stimulates $CD4^+$ Th1 cell development [11–16].

We studied the effect of IFN-α on DC development and found that this cytokine, in combination with granulocyte-macrophage colony-stimulating factor (GM-CSF), was capable of inducing the differentiation of peripheral blood mononuclear cells into DCs with potent antigen-presenting and T cell-activating abilities.

MATERIALS AND METHODS

Culture methods

Peripheral blood mononuclear cells were obtained from heparinized blood of normal human donors by Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden)

Abbreviations: IFN-α, interferon-α; DC, dendritic cells; GM-CSF, granulocyte-macrophage colony-stimulating factor; HLA, human leukocyte antigen; IL-4, interleukin-4; TNF-α, tumor necrosis factor α; MHC, major histocompatibility complex; PBS, phosphate-buffered saline; FITC, fluorescein isothiocyanate; PE, phycoerythrin.

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Received December 4, 1997; revised April 7, 1998; accepted April 9, 1998.

density gradient centrifugation. The mononuclear cell fraction was washed three times with phosphate-buffered saline (PBS), resuspended in RPMI 1640 medium (GIBCO-BRL, Gaithersburg, MD) at 0.5 to 1.0 $\times$ 10^7 cells/mL, and seeded into 25-cm^2 flasks (Costar, Cambridge, MA) at 8 mL/flask. The flasks were incubated at 37°C for 2 h, then nonadherent cells were decanted and frozen for subsequent T lymphocyte purification. The adherent cell population was rinsed gently with RPMI 1640. Complete medium containing penicillin-streptomycin (100 IU/mL and 100 μg/mL, respectively; Mediatech, Herndon, VA) and either 10% heat-inactivated autologous serum or 10% AB serum (GIBCO-BRL) was added to the adherent cells.

Recombinant human cytokines and neutralizing antibodies

The following cytokines were added to mononuclear cell cultures either alone or in various combinations: GM-CSF 500 U/mL (10^4 U/mg; Pepro Tech, Rocky Hill, NJ), IL-4 1000 U/mL (2.9×10^4 U/mg; R & D Systems, Minneapolis, MN), tumor necrosis factor α (TNF-α) 10 ng/mL (>100 U/ng; Boehringer Mannheim, Indianapolis, IN), IFN-γ 100 U/mL (Boehringer Mannheim), and IFN-α-2b (Schering Corp., Kenilworth, NJ) at several concentrations. The optimal concentration of each cytokine was determined by titration experiments using flow cytometry to quantitate B7-2 and HLA-DR expression. Mouse monoclonal antibodies with neutralizing activity against human IL-4 (10 μg/mL; Endogen, Cambridge, MA), human IFN-α (25 μg/mL; Endogen), or human TNF-α (5 μg/mL; Genzyme Diagnostics, Cambridge, MA) were used in some of the culture experiments.

HL-60 myeloid leukemia cells were cultured in complete medium containing 10 ng/mL TNF-α with or without 5 μg/mL anti-TNF-α antibodies or in media alone for 7 days. TNF-α-induced up-regulation of CD14 expression on HL-60 was completely blocked by anti-TNF-α antibodies, thus confirming the biological activity of both reagents at the concentrations used in the DC experiments.

Immunofluorescent staining and flow cytometry

Cells were incubated with saturating concentrations of fluorochrome-conjugated monoclonal antibodies at 4°C for 20 min, then washed twice with PBS. Data were collected using a FACScan II flow cytometer and Lysys II acquisition software (Becton Dickinson, San Jose, CA); data analysis was performed using CellQuest software (Becton Dickinson). Calibration for three-color flow cytometry was conducted using CaliBRITE beads and Auto-COMP software (Becton Dickinson). The following fluorochrome-conjugated mouse monoclonal antibodies against human proteins were used for immunofluorescent staining: fluorescein isothiocyanate (FITC)-conjugated anti-CD1a, phycoerythrin (PE)-conjugated anti-CD11c, FITC-conjugated anti-CD32, FITC-conjugated anti-CD40, and PE-conjugated anti-CD86 (all from PharMingen, San Diego, CA); PE-conjugated anti-CD11a, FITC-conjugated anti-CD13, FITC-conjugated anti-CD14, and PE-conjugated anti-CD54, and PE-conjugated anti-HLA-A,B,C (all from Caltag, San Francisco, CA); FITC-conjugated anti-CD3, PE-conjugated anti-CD11b, PE-conjugated anti-CD19, FITC-conjugated anti-CD20, PE-conjugated anti-CD40 ligand, PE-conjugated anti-CD56, PE-conjugated anti-CD80, PerCP-conjugated anti-HLA-DR (all from Becton Dickinson); and PE-conjugated anti-CD83 (Immunotech, Westbrook, ME).

Quantitation of DC yield

After 7 days of culture the nonadherent cell density in each flask was enumerated using a hemacytometer. The cells were subjected to immunofluorescent staining using PE-conjugated anti-B7-2 and PerCP-conjugated anti-HLA-DR, and analyzed by flow cytometry, as described above. A total of 200,000 ungated events were acquired for each sample and the DCs were identified by their light scatter characteristics and high level of B7-2 and HLA-DR co-expression. The percentage of DCs per sample was determined and the total number of DCs per flask was calculated.

Preparation of purified DCs

Azide-free mouse monoclonal antibodies against human CD3, CD19, and CD56 (all from PharMingen) were used to deplete T lymphocytes, B lymphocytes, and NK cells, respectively, from DC cultures. The cultured cells were washed with PBS and incubated with the monoclonal antibodies (0.4 μg/10^6 cells) for 20 min at 4°C. The antibody-coated cells were incubated with paramagnetic polystyrene beads conjugated to sheep anti-mouse IgG (Dynabeads M-450; Dynal, Lake Success, NY) for 20 min. The DCs were then isolated by negative depletion using a magnet, as described [17]. The purified DCs were used directly in mixed leukocyte reactions or soluble antigen-presentation assays.

Isolation of $CD14^+$ monocytes

Peripheral blood mononuclear cells were isolated, then T and B lymphocytes and NK cells were depleted using monoclonal antibodies and paramagnetic beads, as described above. The remaining cells then were stained with FITC-conjugated anti-CD14 (Caltag, San Francisco, CA) and sorted using a $FACStar^{plus}$ flow cytometer and Lysys II software (Becton Dickinson). Gating was set to harvest viable cells that were strongly $CD14^+$.

Evaluation of mannose receptor-mediated endocytosis

Purified DCs (10^5/sample) were suspended in complete medium and placed at either 0 or 37°C. FITC-conjugated dextran (Molecular Probes Inc., Eugene, OR) was added to each sample at a final concentration of 1 mg/mL and incubation was continued for 45 min. Cells were rinsed twice with cold PBS containing 1% fetal calf serum and 0.01% NaN_3, rinsed once using PBS with 1% paraformaldehyde, and subjected to flow cytometry.

Mixed leukocyte reaction

T lymphocytes present in the nonadherent fraction of mononuclear cells isolated by Ficoll-Paque density gradient centrifugation were purified by passage over a T cell enrichment column (R & D Systems) according to the manufacturer's instructions. Purified T lymphocytes were counted and seeded into plates (96 round-bottomed wells/plate; Costar, Cambridge, MA) at 10^5 cells/well. Purified allogeneic DCs were added to the T cells at varying ratios in triplicate wells. The final volume of each well was adjusted to 200 mL of complete medium. After 4 days of culture, 1 mCi of methyl-[^{3}H]thymidine (1 mCi/mL; DuPont-NEN, Boston, MA) was added to each well and incubation was continued for an additional 18 h. Cells were collected on fiber disks using a PhD Cell Harvester (Cambridge Technology; Cambridge, MA) and thymidine uptake was quantitated by liquid scintillation counting.

Soluble antigen-presentation assay

Purified DCs were suspended in complete medium containing 40 mg/mL recombinant tetanus toxin fragment C (TT; Boehringer Mannheim) or complete medium alone and incubated overnight in polypropylene tubes at 37°C. The cells were then washed with RPMI 1640, counted, and placed in triplicate wells of a 96-well plate at 40–5000 cells/well. Purified autologous T lymphocytes, prepared as above, were added to the DCs at 10^5 cells/well in a final volume of 200 mL complete medium, and incubated for 4 days. Methyl-[^{3}H]thymidine 1 mCi was added to each well and incubation was continued for an additional 18 h. Thymidine uptake was quantitated as described above.

Statistical methods

Data from groups were analyzed using a paired *t* test and were considered significantly different for $P < 0.05$.

RESULTS

The effect of IFN-α on DC morphology

Peripheral blood mononuclear cells grown in GM-CSF alone or in combination with either IFN-α (IFN-α/GM-CSF) or IL-4 (IL-4/GM-CSF) were stained with Giemsa and examined by light microscopy. Cells grown in IFN-α/GM-CSF displayed lobulated nuclei and numerous fine cytoplasmic projections consistent with a DC phenotype (**Fig. 1**). They differed from

cells cultured in IL-4/GM-CSF by having more numerous fine cytoplasmic processes (Fig. 1). Cells treated with GM-CSF alone were larger and had round nuclei, voluminous cytoplasm, and rare cytoplasmic projections.

The immunophenotype of DCs grown in IFN-α/GM-CSF

The starting population of adherent mononuclear cells was heterogeneous, consisting of $CD14^+$ cells (24 ± 8%), $CD3^+$ cells (63 ± 9%), $CD20^+$ cells (9 ± 4%), and $CD56^+$ cells (12 ± 4%). DCs are derived from the $CD14^+$ cells in our culture system [17]. The effect of IFN-α/GM-CSF, IL-4/GM-CSF, or GM-CSF on the immunophenotype of the mononuclear cells was evaluated (**Table 1**). The cells grown in IFN-α/GM-CSF expressed high levels of class I and II HLA proteins, and B7-1 (CD80), B7-2 (CD86), and CD40 co-stimulatory proteins, consistent with their differentiation into DCs (**Fig. 2**). The expression of these immunoregulatory proteins was higher on DCs cultured in IFN-α/GM-CSF than on those prepared using IL-4/GM-CSF (Table 1). The adhesion molecules CD11a, CD11b, CD11c, and CD54 also were abundantly displayed on IFN-α/GM-CSF DCs. The CD1a protein, which is present on Langerhans cells, was expressed only at low levels by the cultured cells. The CD83 antigen, a marker of mature DCs, was expressed by a minority of IFN-α/GM-CSF DCs. The DCs grown in IFN-α/GM-CSF expressed CD14 at levels lower than the starting monocyte population. In contrast, cells grown in GM-CSF alone expressed very high levels of CD14, indicating that the addition of IFN-α to GM-CSF down-regulated the expression of this antigen by the mononuclear cells.

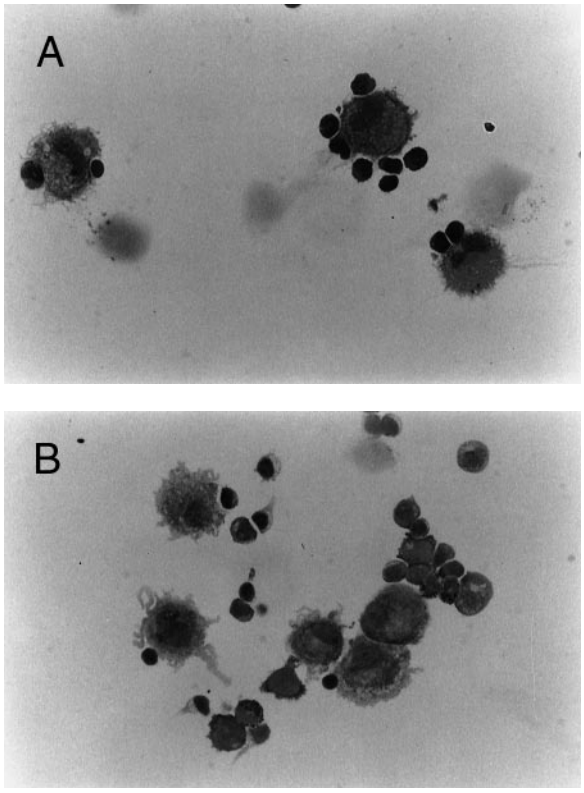

Fig. 1. Morphology of DCs grown in IFN-α/GM-CSF or IL-4/GM-CSF. Loosely adherent mononuclear cells were harvested after 7 days of culture in complete medium containing either (A) IFN-α/GM-CSF or (B) IL-4/GM-CSF. Cytospin preparations were performed and slides were stained with Giemsa. Cells were photographed at ×400 magnification.

TABLE 1. Immunophenotypes of Cultured Mononuclear Cells

Surface antigen	IFN-α + GM-CSF	IL-4 + GM-CSF	GM-CSF
CD1a	48%+ (271)	64%+ (197)	79%+ (245)
CD3 (TCR)	—	—	—
CD11a (LFA-1)	>90%+ (988)	>90%+ (284)	>90%+ (694)
CD11b (Mac-1)	>90%+ (4379)	>90%+ (7543)	>90%+ (5703)
CD11c (p150)	>90%+ (4386)	>90%+ (6403)	>90%+ (4479)
CD13	>90%+ (674)	>90%+ (475)	>90%+ (514)
CD14	56%+ (382)	—	>90%+ (3427)
CD20	—	—	—
CD32 (FcγRII)	83%+ (402)	74%+ (373)	>90%+ (802)
CD40	>90%+ (984)	>90%+ (555)	>90%+ (486)
CD54 (ICAM-1)	>90%+ (5244)	>90%+ (3705)	>90%+ (4714)
CD56	—	—	—
CD80 (B7-1)	>90%+ (776)	87%+ (208)	67%+ (82)
CD83	17%+ (286)	33%+ (240)	—
CD86 (B7-2)	>90%+ (2394)	>90%+ (2227)	>90%+ (815)
HLA-A, B, C	>90%+ (2822)	>90%+ (1135)	>90%+ (715)
HLA-DR	>90%+ (1890)	>90%+ (1162)	>90%+ (314)

Cells were considered negative if <10% expressed the indicated antigen. The net fluorescence intensities of the positive cell populations are given in parentheses.

The effect of IFN-α concentration on DC yield and immunophenotype

The relationship between IFN-α concentration and the expression of HLA-DR, B7-2, and CD40 by DCs was examined. The expression of all three immunoregulatory proteins was significantly enhanced by increasing concentrations of IFN-α, in the presence of GM-CSF, up to 5000 U/mL at which level a plateau was reached (**Fig. 3**). Cells cultured in IFN-α alone at 5000 U/mL had similar B7-2 and HLA-DR expression as those grown in IFN-α/GM-CSF at the same IFN-α concentration, suggesting a major role for IFN-α in generating the DC immunophenotype (Fig. 3).

Culture of peripheral blood mononuclear cells in media containing GM-CSF for 7 days resulted in the production of both loosely adherent mononuclear cells and firmly adherent macrophages. In contrast, macrophages were rarely observed in cultures containing IFN-α/GM-CSF. There was a dose-dependent inhibitory effect of IFN-α concentration on DC yield (**Fig. 4**). Nevertheless, the number of DCs obtained from cultures containing any concentration of IFN-α in combination with GM-CSF was not significantly different from that produced

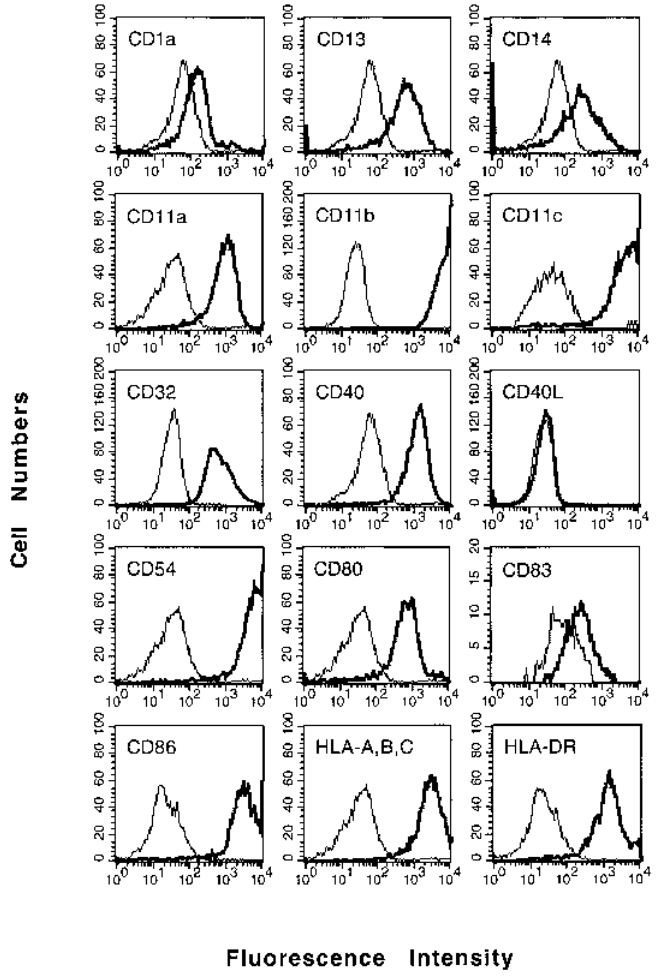

Fig. 2. Immunophenotype of mononuclear cells grown in IFN-α/GM-CSF. Peripheral blood mononuclear cells were grown in IFN-α 5000 U/mL and GM-CSF 500 U/mL for 7 days, then were stained with immunofluorescent antibodies and analyzed by flow cytometry. Dashed lines indicate background fluorescence from unstained cells and solid lines represent fluorescence from cells stained with fluorochrome-conjugated antibody to the indicated antigen.

by IL-4/GM-CSF cultures. Cultures containing IFN-α alone yielded low numbers of DCs and therefore subsequent experiments used the combination of IFN-α and GM-CSF. The variability of DC yield between experiments derived primarily from differences in the numbers of monocytes that adhered to the culture flask surface.

The mechanism of IFN-α action

We examined whether the effect of IFN-α on DC development was secondary to induction of IL-4 production by T lymphocytes. The addition of neutralizing anti-human IL-4 antibodies to mononuclear cell cultures containing IL-4/GM-CSF decreased B7-2 and HLA-DR expression to levels expressed by cells grown in GM-CSF alone (**Fig. 5A**). In contrast, no effect of anti-IL-4 antibodies was seen on the immunophenotype of DCs grown in IFN-α/GM-CSF. A similar experiment using neutralizing antibodies against IFN-α showed that the antibodies could negate the effect of IFN-α on DC differentiation but not that of IL-4 (Fig. 5B). The potential role of TNF-α in mediating the effect of IFN-α on DC development also was investigated. Mononuclear cells grown in TNF-α (10 ng/mL) and GM-CSF for 7 days had an immunophenotype that was similar to that of cells grown in GM-CSF alone. The addition of anti-human TNF-α antibodies to mononuclear cell cultures containing IFN-α/GM-CSF failed to alter the immunophenotype of the resulting DCs compared to those cultured in IFN-α/GM-CSF without antibody.

The binding of CD40 ligand, expressed by activated T cells, to CD40 on hematopoietic progenitor cells can induce DC differentiation. T lymphocytes in DC cultures were studied to determine whether IFN-α was inducing the expression of CD40 ligand on these cells and thereby indirectly affecting DC development. T lymphocytes obtained from DC cultures containing IFN-α/GM-CSF did not express CD40 ligand, whereas the same T cells exposed to 10^{-6} M phorbol 12-myristate 13-acetate did express CD40 ligand, thus excluding an effect of IFN-α on CD40 ligand expression. Finally, the effect of

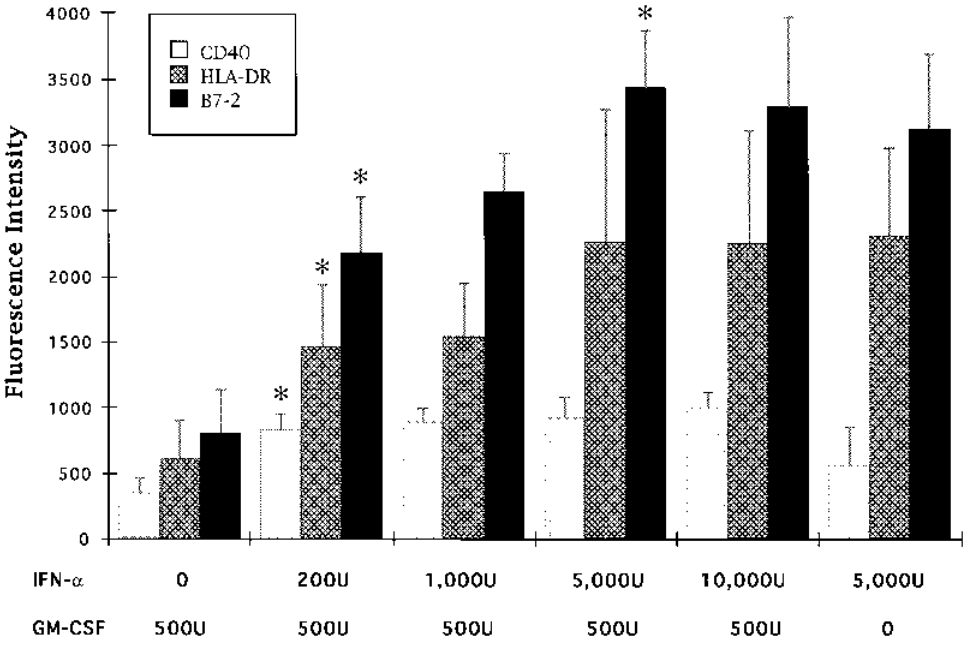

Fig. 3. Effect of increasing IFN-α concentrations on DC immunophenotype. Peripheral blood mononuclear cells were grown in complete medium containing the indicated concentrations of IFN-α and 500 U/mL GM-CSF for 7 days. The loosely adherent cells were harvested, subjected to staining with immunofluorescent antibodies, and analyzed by flow cytometry. *Level of fluorescence intensity for the indicated protein by the cell population was significantly higher ($P < 0.05$ by paired t test) than that of cells treated at the next lowest IFN-α concentration. The results represent the mean and standard deviation of data obtained from three experiments.

removing all potential accessory cells from the cultures was determined. Monocytes were extensively purified by negative depletion of T and B lymphocytes and natural killer cells, followed by sorting of $CD14^+$ cells. The DCs produced from purified monocytes had a similar immunophenotype as those produced from crude cultures of adherent mononuclear cells with the exception of a slightly lower B7-2 expression (**Fig. 6**). These results suggest that IFN-α has a direct effect on DC differentiation that is distinct from that of IL-4, TNF-α, or CD40 ligand.

The effects of IFN-α versus IFN-γ on DC immunophenotype

Peripheral blood mononuclear cells cultured in IFN-α/IL-4/GM-CSF displayed significantly higher levels of HLA-DR and B7-2 than cells grown in the same concentrations of either IFN-α/GM-CSF or IL-4/GM-CSF (**Fig. 7**). In contrast, the expression of these immunoregulatory molecules by DCs grown in IFN-γ/GM-CSF/IL-4 was not superior to that of DCs treated with either IFN-α/GM-CSF or IL-4/GM-CSF (Fig. 7). Therefore, IFN-α and IL-4 concentrations that have a maximal effect on DC differentiation can, in combination, further augment the expression of critical immunoregulatory proteins by DCs, indicating that these agents have unique and complimentary activities. In addition, the effects of IFN-α and IFN-γ on DCs are distinctly different.

The DCs generated with IFN-α/GM-CSF are potent activators of an allogeneic MLR

The DCs produced by culturing mononuclear cells in a variety of cytokine combinations were compared for their ability to stimulate an allogeneic MLR. DCs grown in IFN-α/GM-CSF or IL-4/GM-CSF stimulated allogeneic T lymphocyte proliferation to a similar degree in MLR experiments (**Fig. 8**). The most potent DCs were produced in cultures containing IFN-α/IL-4/GM-CSF (Fig. 8). In contrast, DCs cultured in IFN-γ/IL-4/GM-CSF did not perform better than those from cultures containing IL-4/GM-CSF in the MLR assay. These results demonstrate that DCs grown in IFN-α/GM-CSF are highly functional and that the activity of cultured DCs in the allogeneic MLR correlates closely with their expression of HLA-DR, B7-2, and CD40 immunoregulatory proteins.

The DCs generated with IFN-α/GM-CSF are capable of antigen uptake via mannose receptors

The DCs were evaluated for their ability to employ mannose receptors in antigen uptake by incubating them with FITC-conjugated dextran and subjecting them to flow cytometry. After 45 min of incubation at 37°C, DCs grown in IFN-α/GM-CSF or IL-4/GM-CSF both demonstrated high levels of dextran uptake (**Fig. 9**).

The DCs generated with IFN-α/GM-CSF are potent presenters of exogenous antigen

The DCs grown in IFN-α/GM-CSF were compared to those cultured in IL-4/GM-CSF or IFN-α/IL-4/GM-CSF for their ability to uptake, process, and present TT to autologous T lymphocytes. The IFN-α/GM-CSF-treated DCs were repeatedly superior to IL-4/GM-CSF-cultured DCs, but equivalent to IFN-α/IL-4/GM-CSF-treated cells in the TT assay (**Fig. 10**). DCs that were not pulsed with TT did not stimulate autologous T cell proliferation.

DISCUSSION

Peripheral blood monocytes cultured with IFN-α/GM-CSF differentiated into DCs as demonstrated by cell morphology, immunophenotype, and functional activity. These DCs dis-

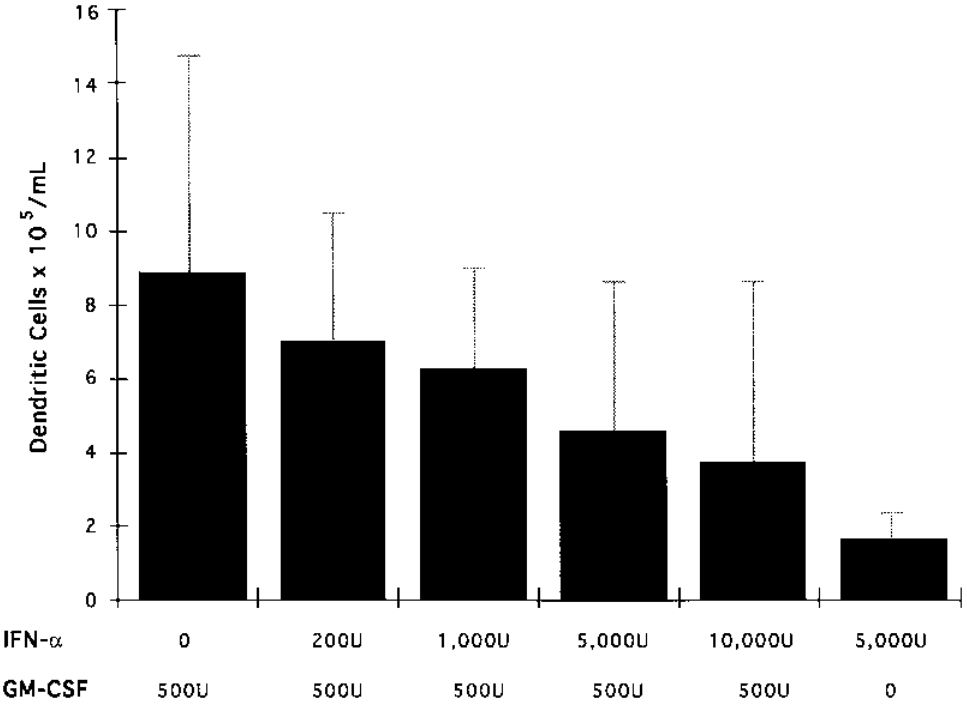

Fig. 4. Effect of increasing IFN-α concentrations on DC yield from mononuclear cell cultures. Peripheral blood mononuclear cells were cultured in complete medium containing the indicated cytokines for 7 days, then the DCs were enumerated, as described in the text. The results represent the mean and standard deviation of data obtained from five experiments.

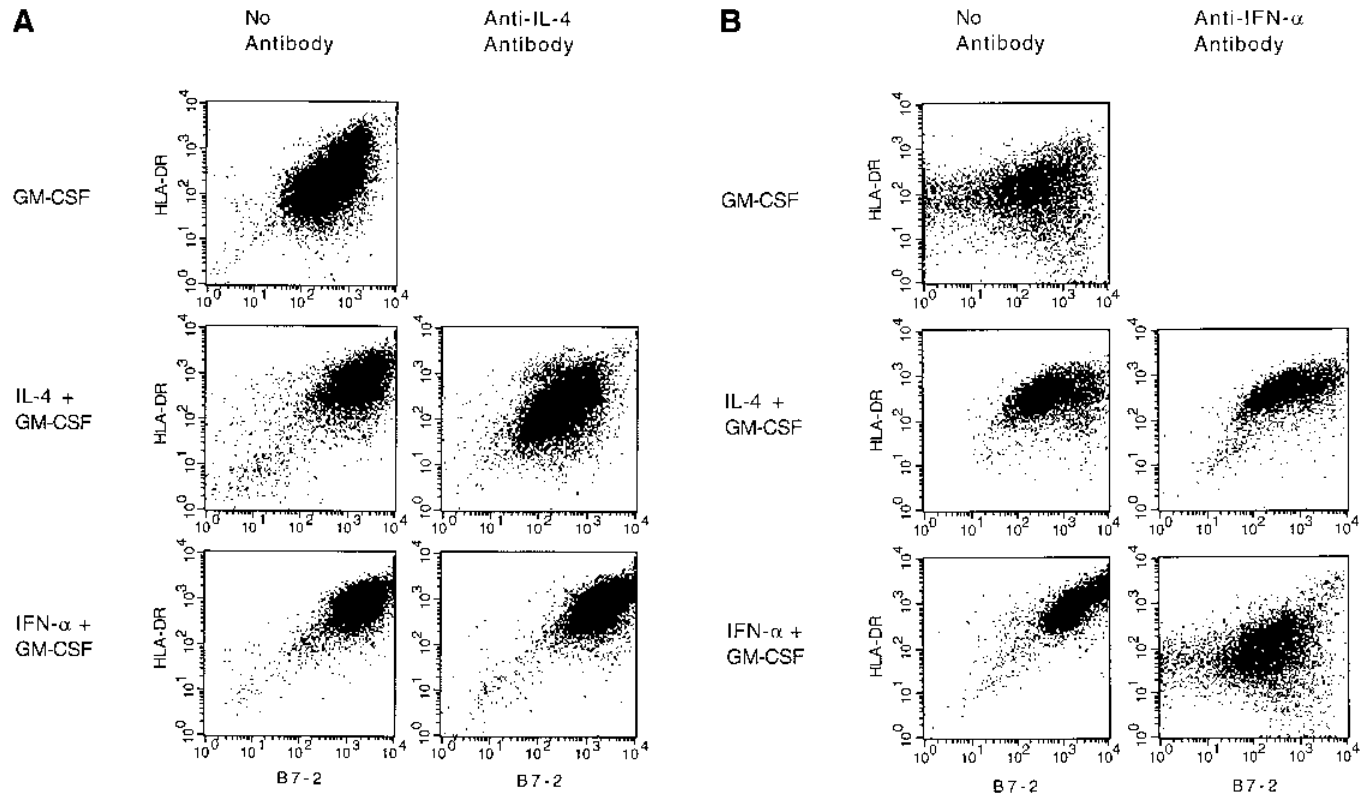

Fig. 5. The effect of anti-cytokine antibodies on the immunophenotype of DCs grown in IFN-α/GM-CSF or IL-4/GM-CSF. Mononuclear cells were grown in complete medium containing the indicated cytokines without or with (A) anti-IL-4 antibody or (B) anti-IFN-α antibody for 7 days. The DCs were harvested, incubated with immunofluorescent antibodies to B7-2 and HLA-DR and analyzed by flow cytometry. The addition of anti-IL-4 antibody to IL-4/GM-CSF cultures resulted in cells with the immunophenotype of cells grown in GM-CSF alone, but had no effect on IFN-α/GM-CSF cultures (A). Addition of anti-IFN-α antibody reversed the effects of IFN-α, but not IL-4, on DC development (B).

played a characteristic morphology with numerous fine cytoplasmic projections. The critical role of DCs as antigen-presenting cells was reflected by their high-level expression of class I and II HLA molecules and co-stimulatory molecules B7-1, B7-2, and CD40 [9, 17–23]. DCs generated with IFN-α/GM-CSF exhibited equivalent levels of these immunoregulatory proteins as DCs grown in IL-4/GM-CSF. Adhesion molecules important for DC and T lymphocyte interactions, including LFA-1 and ICAM-1 [18–21, 24–28], also were expressed at high levels by IFN-α/GM-CSF-treated DCs. The CD83 molecule, a marker for mature DCs, was expressed by only a minority of the DCs prepared using IFN-α/GM-CSF, suggesting that most of the DCs were not terminally differentiated. These results are consistent with previous studies in which DCs derived from peripheral blood monocytes expressed low levels of CD83 when grown in the absence of TNF-α or other DC-activating agents [29, 30].

The immunophenotypic characteristics of the DCs predicted their functional capabilities. DCs grown in IFN-α/GM-CSF stimulated allogeneic T lymphocyte proliferation to a similar degree as IL-4/GM-CSF-treated DCs in the MLR assay. DCs cultured in IFN-α/IL-4/GM-CSF demonstrated higher levels of HLA-DR and B7-2 expression and enhanced allogeneic T cell stimulating activity compared to those exposed to two cytokines. In contrast, the addition of IFN-γ to cultures containing IL-4/GM-CSF did not significantly increase HLA-DR and B7-2

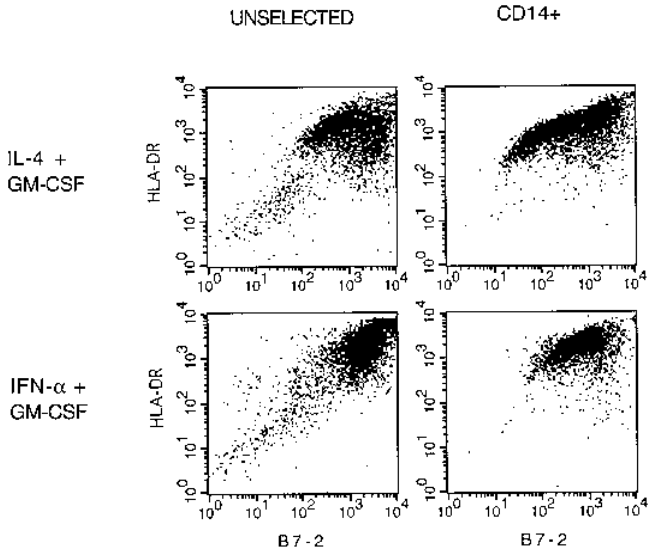

Fig. 6. Effect of monocyte purification on DC immunophenotype. The DCs were prepared using either unselected adherent mononuclear cells or highly purified monocytes from the same donor isolated by negative depletion of T, B, and NK cells followed by sorting of $CD14^+$ cells. The cells were grown in complete medium containing IFN-α/GM-CSF or IL-4/GM-CSF for 7 days, then stained with immunofluorescent antibodies to B7-2 and HLA-DR. Flow cytometry showed that DCs prepared from purified monocytes expressed slightly lower levels of B7-2, but similar amounts of HLA-DR compared to DCs derived from cultures of unselected mononuclear cells containing either cytokine combination.

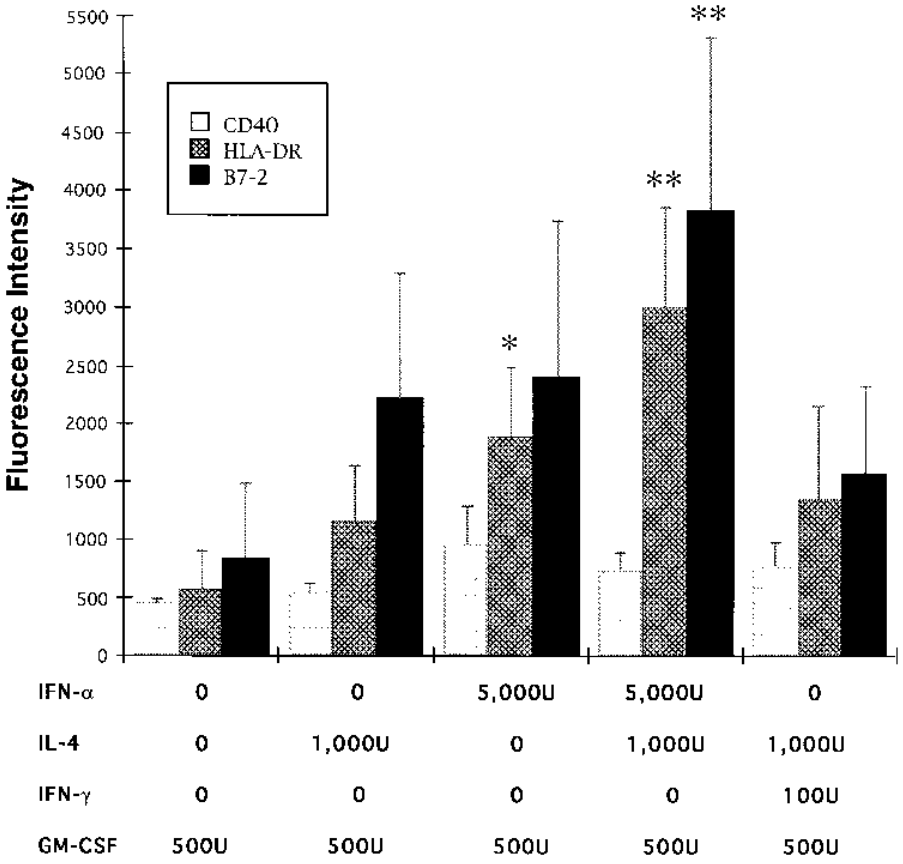

Fig. 7. IFN-α, but not IFN-γ, augments the expression of B7-2 and HLA-DR by DCs cultured in IL-4/GM-CSF. Mononuclear cells were grown in complete medium containing the indicated combinations of GM-CSF, IL-4, IFN-α, and IFN-γ that were previously found to be maximally active. The cells were harvested, incubated with immunofluorescent antibodies to CD40, B7-2, and HLA-DR, and analyzed by flow cytometry. The results represent the mean and standard deviation of data obtained from nine experiments. A single asterisk indicates that the expression of HLA-DR by IFN-α/GM-CSF-treated cells was significantly higher ($P < 0.05$ by paired t test) than that of DCs grown in IL-4/GM-CSF. The double asterisk indicates that the expression of B7-2 and HLA-DR by DCs cultured in IFN-α/IL-4/GM-CSF was significantly higher that that of cells grown in either IFN-α/GM-CSF or IL-4/GM-CSF.

expression or T lymphocyte proliferation in the MLR assay in comparison to IL-4/GM-CSF-treated DCs. Previously published data showed that IFN-γ does not increase HLA-DR or B7 expression by Langerhans cells and inhibits the ability of DCs to stimulate allogeneic T lymphocytes [31, 32]. Our results demonstrated that IFN-α, but not IFN-γ, induces monocyte differentiation into functional DCs.

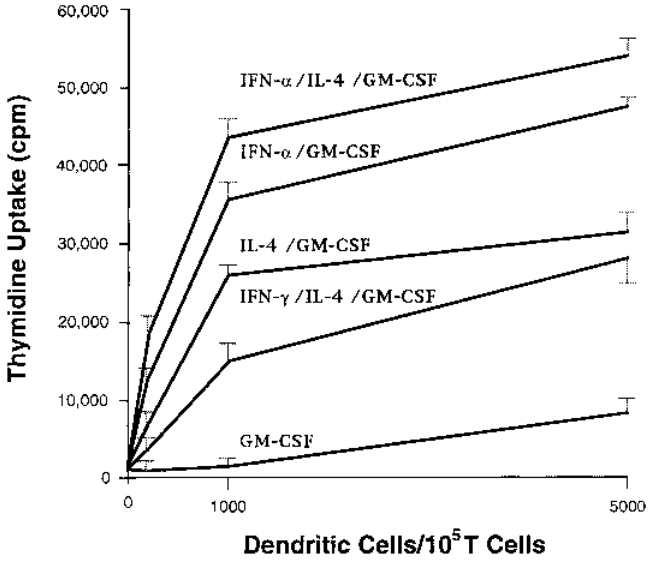

Fig. 8. DCs grown in IFN-α/GM-CSF are active in the allogeneic MLR assay. DCs prepared as in Figure 5 were purified by negative depletion and added in varying numbers (200, 1000, or 5000) to triplicate wells containing 10^5 allogeneic T lymphocytes in complete medium. After 4 days of co-culture, [^{3}H]thymidine was added for 18 h and the cells were analyzed for thymidine uptake. The figure shows an experiment that is representative of five similar assays.

DCs have the ability to take up, process, and present soluble antigen to autologous T lymphocytes [33–38]. Antigen uptake by DCs grown in IL-4/GM-CSF is mediated predominantly by macropinocytosis or binding to mannose receptors [39]. DCs grown in IFN-α/GM-CSF were capable of antigen uptake using mannose receptors and were potent presenters of tetanus toxin to autologous T lymphocytes. The T cell response generated by IFN-α/GM-CSF-treated DCs was superior to that engendered by DCs grown in IL-4/GM-CSF. This may be due to higher expression of class II HLA proteins or co-stimulatory molecules by the DCs prepared with IFN-α.

It is interesting that two cytokines with many opposing biological activities have similar and cooperative actions on the generation of DCs. Whereas IFN-α stimulates the development of $CD4^+$ T cells into Th1 cells which regulate cytotoxic and phagocytic immunity, IL-4 induces the differentiation of Th2 cells which mediate allergic and anti-helminthic responses [40, 41]. In mice, IFN-α administration increases IgG2a production in response to antigenic stimulation, and inhibits the secretion of IgE [42]. In contrast, IL-4 induces IgE production, which can be blocked by IFN-α injection. IFN-α also inhibits the synthesis of IL-4 by splenic lymphocytes [42]. IL-4 antagonizes the protective effect of IFN-α against viral infection of fibroblasts, and inhibits the IFN-α-induced transcription of the ISG-54 gene by monocytes [43, 44]. Finally, IFN-α and IL-4 have opposing effects on hematopoiesis: IFN-α inhibits the proliferation of multipotent, granulocyte-macrophage, erythroid and megakaryocyte progenitor cells, whereas IL-4 acts in

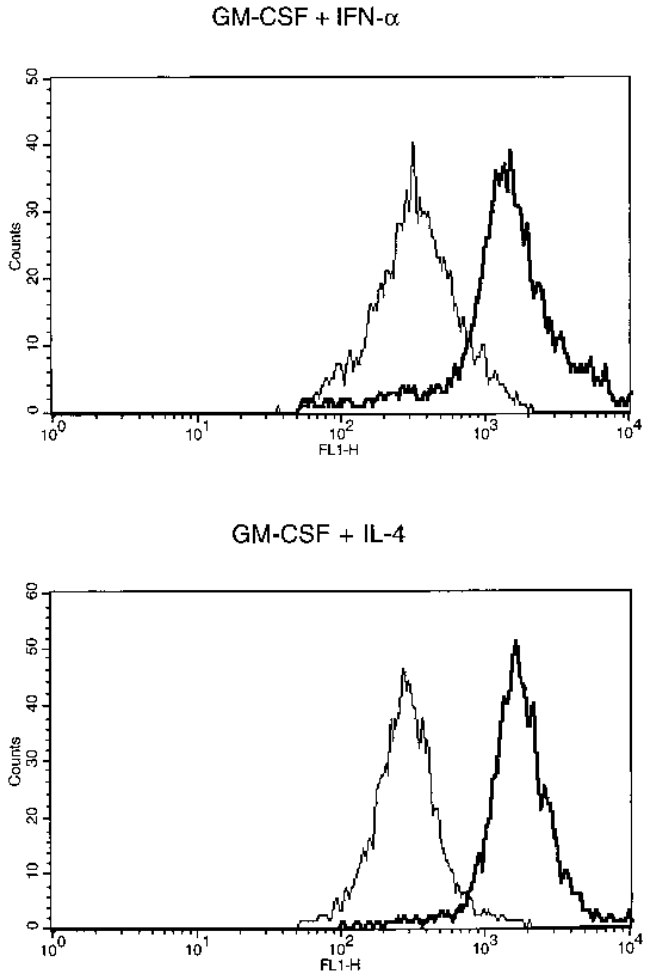

Fig. 9. DCs grown in IFN-α/GM-CSF take up antigen by use of mannose receptors. FITC-conjugated dextran was added to purified DCs (10^5 cells/sample) cultured in either IFN-α/GM-CSF or IL-4/GM-CSF and the DCs were incubated at either 0°C (fine line) or 37°C (dark line) for 45 min. DCs were then washed, fixed, and subjected to flow cytometry.

concert with other growth factors to promote the growth of multiple hematopoietic lineages [45–56]. However, IL-4 inhibits macrophage progenitor proliferation in vitro [55–57]. The reason that IFN-α and IL-4 share the ability to induce DC differentiation of monocytes is unclear. The effects of IFN-α were not reversed by the addition of anti-IL-4 antibodies to DC cultures and the activity of IL-4 was not neutralized by the addition of anti-IFN-α antibodies, suggesting that these cytokines affect DC development by unique mechanisms. In addition, the combined effects of IFN-α and IL-4 on DC HLA-DR and B7-2 expression, and on MLR activity exceeded that achieved by maximally active concentrations of either cytokine. Therefore, in spite of their generally opposing biological activities, IFN-α and IL-4 share similar but complementary actions on DC development.

The induction of DC differentiation and activation by IFN-α indicates that it has a broader role in regulating the afferent arm of the immune response than was previously appreciated. In response to viral infection, IFN-α production induces the synthesis of antiviral proteins by a wide range of somatic cells, which can limit spread of the pathogen [1]. Class I HLA and β2 microglobulin expression is widely enhanced by IFN-α, thus encouraging the detection of infected cells. Numerous effector cell functions also are increased by IFN-α, including macrophage phagocytosis and cytotoxicity, NK cell activity, and antigen-induced $CD8^+$ cell proliferation [58–60]. IFN-α directs $CD4^+$ lymphocytes to differentiate into Th1 cells capable of elaborating IL-2, TNF-β, and IFN-γ which, in turn, stimulate cytotoxic T cell and macrophage activity. The Th1 cells also induce B cell production of antibodies capable of opsonization and complement fixation. The ability of IFN-α to stimulate DC development complements its other immunoregulatory functions and suggests that this action is physiologically relevant.

The activity of IFN-α appears to be distinct from that of previously described influences on DC development. The first preparation of human DCs from hematopoietic progenitors in vitro was achieved by growing $CD34^+$ cells in GM-CSF and TNF-α [61]. We found that the substitution of TNF-α for IFN-α in peripheral blood mononuclear cell cultures containing GM-CSF failed to result in cells with a DC immunophenotype. The addition of anti-TNF-α antibodies to cultures containing IFN-α and GM-CSF did not affect the generation of DCs. More recently, ligation of CD40 on $CD34^+$ hematopoietic progenitor cells was shown to induce them to differentiate into DCs [62]. Flow cytometry for CD40 ligand was performed on the lymphocyte population of the DC cultures in order to exclude the possibility that IFN-α induction of CD40 ligand expression on T cells was indirectly responsible for DC differentiation; no CD40 ligand expression was observed. The ability of IL-4 and

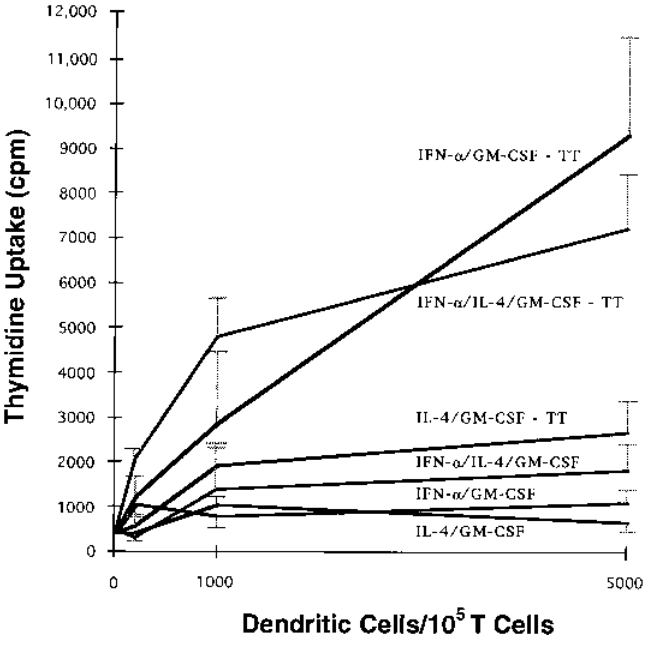

Fig. 10. DCs grown in IFN-α/GM-CSF take up and present exogenous antigen. DCs cultured in complete media containing IFN-α/GM-CSF, IL-4/GM-CSF, or all three cytokines for 7 days were purified by negative depletion and incubated overnight in complete medium containing TT or in medium alone. The DCs were added in varying numbers (200, 1000, or 5000) to triplicate wells containing 10^5 autologous T lymphocytes in medium with 10% autologous serum and incubated for 4 days. [^{3}H]thymidine was then added to each well and incubation was continued for an additional 18 h. The cells were harvested and assayed for thymidine uptake. The figure shows the results of an experiment that is representative of five similar assays.

GM-CSF to differentiate monocytes into DCs in vitro is well established [17, 38, 63]. The addition of neutralizing anti-IL-4 antibodies to monocyte cultures containing IFN-α/GM-CSF had no effect on DC development but completely eliminated the effect of IL-4 in a parallel culture containing IL-4/GM-CSF. Transforming growth factor-β1 is required for the generation of DCs from $CD34^+$ progenitor cells, but only in serum-free conditions [64]. The addition of either c-*kit* ligand or flt-3 ligand to $CD34^+$ cell cultures containing GM-CSF and TNF-α can augment the yield of DCs but neither affects DC immunophenotype or function [65–67]. Other cytokines have been shown to play a role in DC development in vitro. IL-3, like GM-CSF, can cooperate with TNF-α to generate DCs from $CD34^+$ cells [68]. This activity is distinct from that described here for IFN-α. Finally, extensive purification of monocytes before culture in IFN-α/GM-CSF had only a minor effect on cell immunophenotype, suggesting that the effect of IFN-α on DC development from monocytes was a direct one.

Therefore, the ability of IFN-α to induce peripheral blood monocyte differentiation into DCs appears to be distinct from that of previously studied cytokines. This biological action of IFN-α may partly explain its mechanism of action in the treatment of certain malignancies. Appreciation for the DC differentiating ability of IFN-α could lead to the development of new clinical applications for this cytokine.

ACKNOWLEDGMENTS

This research was supported in part by the Perry Cohan Fund, Public Health Service Grant K08-HL02919 (R. L. P.), the Breast Cancer Research Program of the University of California Grant 2CB-0160 (M. D. R.), an Interdisciplinary Grant from the Johnson Comprehensive Cancer Center (JCCC) Foundation (M. D. R.), and the JCCC Flow Cytometry Core Facility (Grant CA16042).

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