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Type I IFNs Enhance the Terminal Differentiation of Dendritic Cells

Thomas Luft,[1,2]* Ken C. Pang,* Elisabeth Thomas,* Paul Hertzog,[†] Derek N. J. Hart,[‡] Joseph Trapani,[§] and Jonathan Cebon*

This study identifies type I IFNs as activating cytokines in a serum-free system in which human dendritic cells (DC) were generated from CD34$^+$ progenitor cells. After 14 days of culture in GM-CSF, TNF-α, and IL-4, CD34$^+$ progenitors gave rise to a population of large, immature DC expressing CD1a and CD11b but lacking CD14, CD80, CD83, CD86, and CMRF44. During the next 2 wk, this population spontaneously matured into nonadherent, CD1a$^{low/-}$, CD11b$^{low/-}$, CD14$^-$, CD80$^+$, CD83$^+$, CD86$^+$, CMRF44$^+$ DC with high allostimulatory activity in the MLR. To examine which factors influenced this maturation, 25 different cytokines or factors were added to the immature DC culture. Only type I IFNs (α or β) accelerated this maturation in a dose-dependent manner, so that after only 3 days the majority of large cells acquired the morphology, phenotype, and function characteristics of mature DC. Furthermore, supernatants from cultures containing spontaneously maturing DC revealed low levels of endogenous IFN production. Because of the similarity of the activation of DC in our culture system with the phenotypic and functional changes observed during Langerhans cells activation and migration in vivo, we investigated the effect of IFN-α on human Langerhans cell migration. IFN-α also activated the migration of human split skin-derived DC, demonstrating that this effect was not limited to DC derived in vitro from hemopoietic progenitor cells. DC activation by type I IFNs represents a novel mechanism of immunomodulation by these cytokines, which could be important during antiviral responses and autoimmune reactions. ***The Journal of Immunology,*** **1998, 161: 1947–1953.**

Dendritic cells (DC)[3] play a key role as APCs in the induction of immune responses (1). Similar to vaccination strategies using irradiated tumor cells in murine models (1), vaccination with peptide Ags probably also relies on the presence of intradermal APCs. Early results of clinical trials have shown immunologic and even antitumor responses in patients following intradermal and s.c. vaccination with tumor-associated peptide Ags (2, 3). In murine models, the efficiency of this vaccination has been increased using peptide-pulsed DC as cellular adjuvants (4, 5). Ag-loaded DC, either purified from blood (6) or produced from monocytes (7, 8) or CD34$^+$ hemopoietic progenitor cells ex vivo (9) might therefore constitute an attractive cellular adjuvant for future clinical vaccination trials.

These earlier in vitro studies of DC development and maturation were performed in the presence of FCS or human serum (HS). The presence of irrelevant Ags, undefined growth factors, and potential infectious agents in serum represent major impediments to the use of these DC in human disease, leading us to investigate cytokine and culture conditions required for the serum-free production of these cells.

We have previously described a serum-free culture model to produce human DC from adult CD34$^+$ hemopoietic progenitor cells (10). Progenitor cells purified from leukapheresis harvests of cancer patients with mobilizing treatments, from the bone marrow of rib fragments of patients with lung cancer undergoing thoracotomy, and from normal bone marrow donors were compared. These progenitor cells of various sources gave rise to similar DC cultures. The study reported the accumulation of a large-size, CD1a$^+$CD11b$^+$CD14$^-$ population of immature DC differentiating from myeloid CD33$^+$ precursor cells after 13 to 15 days in culture. No markers associated with activated DC (CD80, CD83, CD86) were expressed before day 15. During the following 14 days, this large-size population spontaneously matured into DC that displayed an activated phenotype (HLA-A,B,C^{bright}, HLA-DR^{bright}, CD80$^+$, CD83$^+$, and CD86$^+$). CD1a and CD11b expression was gradually lost. At day 28, mature and immature DC stages coexisted in cultures. However, CD1a$^+$CD11b$^+$ DC sorted at day 13 matured homogeneously, and there was no evidence for a direct precursor of mature DC in the small-size CD1a$^-$ population. Further, extending the observation period to 37 days showed that all large cells finally acquired the activated phenotype. This study suggested that in serum-free cultures DC differentiate and mature in an asynchronous manner. Homogeneity of phenotypes was achieved by allowing more time for differentiation or by sorting precursors of a defined stage of differentiation. These activated DC were shown to be potent APCs (10). Characterizing the differentiation and activation of DC revealed close similarities with the phenotypic and functional changes occurring during Langerhans cell activation and migration (11–24). This system has now enabled us to more precisely define the cytokines that regulate DC development. Type I IFNs were the only molecules of a large number studied that were capable of inducing DC maturation and

*Melbourne Tumor Biology Branch, Ludwig Institute for Cancer Research, Melbourne, Victoria, Australia; [†]Molecular Genetics and Development Group, Institute of Reproduction and Development, Monash University, Melbourne, Victoria, Australia; [‡]Christchurch Hospital, Christchurch, New Zealand; and [§]Austin Research Institute, Melbourne, Victoria, Australia

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[2] Address correspondence and reprint requests to Dr. Thomas Luft, Ludwig Institute Oncology Unit, Austin and Repatriation Medical Center, Studley Road, Heidelberg, Victoria 3084, Australia.

[3] Abbreviations used in this paper: DC, dendritic cells; GM-CSF, granulocyte macrophage-CSF; HS, human serum.

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activation. This study, therefore, identifies a novel mechanism for the immunomodulatory effects of type I IFNs, which is important for further understanding the cytokine regulation of DC function.

Materials and Methods

Media

The serum-free medium X-Vivo 20 was purchased from BioWhittaker (Walkersville, MD). Cell lines were maintained in RPMI 1640 (Trace Biosciences, Melbourne, Australia) supplemented with 20 mM HEPES, 60 mg/L penicillin G, 12.6 mg/L streptomycin, 2 mM L-glutamine, 1% nonessential amino acids, and 10% heat-inactivated FCS (CSL, Melbourne, Australia).

Recombinant human cytokines, Abs, and peptides

The following cytokines were added to DC cultures: TNF-α (20 ng/ml) (R&D Systems, Minneapolis, MN); GM-CSF (40 ng/ml; Schering-Plough, Sydney, Australia), IL-4 (500 U/ml, Schering-Plough, Kenilworth, NJ), IFN-α2a (10–1000 U/ml, Roferon-A; Roche Products, Sydney, Australia), IFN-α8 (10–1000 U/ml, (Ciba-Geigy, Basel, Switzerland), and IFN-β (10–1000 U/ml, Berlex Biosciences, Richmond, CA). The cytokines used for screening experiments were: IL-1α (10 ng/ml, Dr. I. Campbell, Department of Medicine, Royal Melbourne Hospital, Melbourne, Australia); IL-1β (100 pg/ml; R&D Systems); IL-2 (100 U/ml, PreproTech, Rocky Hill, NJ); IL-3 (100 ng/ml, Dr. G. Begley, The Walter and Eliza Hall Institute (WEHI), Melbourne, Australia); IL-4 (1000 U/ml, Schering-Plough, Kenilworth, NJ); IL-6 (20 ng/ml, Dr. R. Simpson, Ludwig Institute for Cancer Research (LICR), Melbourne, Australia); IL-7 (20 ng/ml, Serotec, Oxford, U.K.); IL-8 (100 ng/ml, Dr. I. Campbell, Royal Melbourne Hospital); IL-10 (100 U/ml, Schering-Plough, Kenilworth, NJ); IL-12 (10 ng/ml, R&D Systems); IL-13 (20 ng/ml; PreproTech, London, U.K.); GM-CSF (50 ng/ml, Dr. G. Begley, WEHI); TNF-α (100 ng/ml, R&D Systems); TGF-β (1 ng/ml, Dr. S. Chandler, LICR), IFN-α2a (1000 U/ml, Roche Products, DeeWhy, Australia); IFN-γ (1000 U/ml, Boehringer, Ingelheim, Germany); platelet-derived growth factor (PDGF; 20 ng/ml, Dr. R. Whitehead, LICR); vascular endothelial growth factor (VEGF; 10 ng/ml, Dr. S. Stacker, LICR); insulin-like growth factor (IGF)-1 (LR3) (50 ng/ml, Dr. R. Whitehead, LICR); IGF-1 (50 ng/ml, Dr. R. Whitehead, LICR); leukemia inhibitory factor (LIF; 1000 U/ml, Dr. N. Nicola, WEHI); stem cell factor (SCF; 100 ng/ml, Dr. G. Begley, WEHI); FLT-3L (40 ng/ml, Genzyme Corp., Boston, MA); LPS serotype 0111:B4 (100 ng/ml, Sigma, St. Louis, MO); BSA (1%, Sigma); HS (10%, normal donors), and HS-LPS (10%, normal donors, serum filtered with Zetapor filter (Cuno-Life Sciences Division, Meriden, CT) to remove LPS). The following commercial mAbs were purchased: FITC-conjugated IgG1 isotype control, OKT6 (anti-CD1a), phycoerythrin-conjugated T4 (anti-CD4), and BB-1 (anti-CD80/B7/BB1) from Serotec; 5C3 (anti-CD40) and IT2.2 (anti-CD86/B70/B7-2) from PharMingen (San Diego, CA); and FITC-conjugated sheep anti-mouse mAb (Silenus, Boronia, Australia). The following mouse Ab were kindly provided by Dr. A. Boyd (WEHI): IAG-11 negative control, W6/32, anti-HLA-A,B,C; Ia, anti HLA-DR; OKM-1, anti-CD11b; FMC 17, anti-CD14. HB15a, anti-CD83, was a gift from Dr. T. Tedder, Duke University Medical Center (Durham, NC). CMRF44 were obtained from Dr. D. Hart, Department of Haematology, Christchurch Hospital (25).

Cell sources and cell lines

Bone marrow and leukapheresis harvest samples were obtained from normal donors and patients of the Department of Medical Oncology and Clinical Haematology, Royal Melbourne Hospital, Melbourne, Australia. Patients with lymphoma or solid tumors received stem cell-mobilizing chemotherapy and granulocyte colony-stimulating factor (G-CSF) as part of their treatment. Rib segments removed during thoracotomy from patients were obtained from the Department of Thoracic Surgery, Austin and Repatriation Medical Center. Informed consent was obtained. Protocols were approved by the Ethics Committee and conformed to the guidelines of the National Health and Medical Research Council of Australia.

Cell separation and DC cultures

Samples were separated on Ficoll-Hypaque (Pharmacia Biotech, Uppsala, Sweden); RBC were lysed using NH_4Cl, and $CD34^+$ cells were separated with the MACS CD34 progenitor cell isolation kit (Miltenyi Biotech, Sunnyvale, CA) following the manufacturer's instructions. Cells (10^6/ml, 4×10^6/ml) were cultured in 100 μl of X-Vivo 20 with cytokines in 96-well microcultures (Nunc, Roskilde, Denmark) or in 250 μl of medium in 24-well plates (Nunc); 50 to 100% fresh medium and cytokines were added twice weekly. Proliferating, crowded cultures were split or transferred with an Eppendorf pipette into progressively larger tissue culture plates (48-well plates (Falcon, Franklin Lakes, NJ), 24-well plates (Nunc), and 12-well plates (Flow Laboratories, McLean, VA)). The $CD34^-$ fraction was used for HLA typing (Victorian Tissue Typing Service, Royal Melbourne Hospital).

Morphology, flow cytometry, and FACS

Cytocentrifuge preparations were performed by applying 10^4 cells to glass slides spinning for 10 min at 300 rpm (Cytospin 2, Shandon, Pittsburgh, PA). The slides were air dried and stained with May-Grunwald/Giemsa. Cells for flow cytometry were resuspended in PBS + 10% HS, labeled with the primary Abs, washed, and labeled with the secondary Ab. Cells were fixed in PBS/2% formaldehyde/0.01% sodium azide/1% BSA. The immunophenotype was determined using a FACScan flow cytometer (Becton Dickinson, Franklin Lakes, NJ). Fluorescence-activated cell sorting (FACS) was performed on either a FACStar^{Plus} or a modified FACS II flow cytometer.

Mixed leukocyte reaction (MLR)

After irradiation (3000 rad), stimulator cells from DC cultures were plated in triplicate over a range of concentrations (10,000–3,000–1,000–300 per well) and overlaid with 10^5 allogeneic PBMC. PBMC of healthy laboratory volunteers were used as responders. Cells were cultured for 5 days in RPMI with 10% HS in 96-well round-bottom plates. After 5 days, cells were incubated with 1 μCi/well [^{3}H]thymidine (DuPont, Sydney, MA) for 20 h, transferred onto a glass fiber filter (Wallac, Turku, Finland), and [^{3}H]thymidine incorporation into DNA was measured using an LKB 1205 Betaplate scintillation counter (Wallac).

Migration of skin-derived DC

Split skin samples were obtained from the Department of Plastic Surgery, Austin and Repatriation Medical Centre, cut into paired pieces of equal size (1–2 cm^2), and cultured in X-Vivo 20 (2.5 ml, 6-well plate). Three thousand units per milliliter of IFN-α2a was added into one culture of each paired experiment. Two to three replicate paired experiments were performed for each donor skin sample. Numbers of migrating DC were assessed 12 hourly by morphologic criteria using a hemocytometer. Every 24 h, cells were harvested for FACS analysis and skin samples were transferred into fresh medium.

Assay for the antiviral activity of IFN

The antiviral activity of IFN was determined by cytopathic effect reduction assay using WISH cells (American Type Culture Collection (ATCC), Manassas, VA) as the target cells and Semliki forest virus (ATCC) as the challenge virus, as previously described (26). The amount of IFN in IU/ml was calculated by comparing the titer of a sample with that of the international reference standard, Ga 23901532 (National Institutes of Health, Bethesda, MD).

Results

Phenotypic analysis of maturing DC

In serum-free cultures of human $CD34^+$ progenitor cells, two stages of DC differentiation were observed. In the early phase of culture, (10–16 days), a population of large, adherent, Langerhans-like cells developed. These cells were $CD1a^{3+}$, $CD11b^{3+}$, $CD14^-$, $CD80^-$, $CD83^-$, $CD86^-$, HLA-A,B,C$^+$, and HLA-DR$^+$ cells (Fig. 1) and possessed a low allostimulatory capacity (Fig. 2). During the next 2 wk (late phase, days 17–28), this population spontaneously matured into nonadherent APC with up-regulation of HLA-A,B,C, HLA-DR, costimulatory molecules (CD80, CD86), and DC lineage-associated Ags (CD83, CMRF44) (Fig. 1). Simultaneously, down-regulation of CD11b and CD1a was seen (Fig. 1). The loss of these Ags was slow and was complete between days 28 and 40. No Birbeck granules were found in activated DC. This late phase (d14–28), now referred to as phenotypic maturation, was associated with a loss of adherence to plastic. The Ag-presenting capacity of cells from unsorted cultures was examined on days 14 and 28 (Fig. 2). Direct comparison of cells from both time points was possible because the same donor of responder cells was used within the paired experiments. Figure 2 shows the

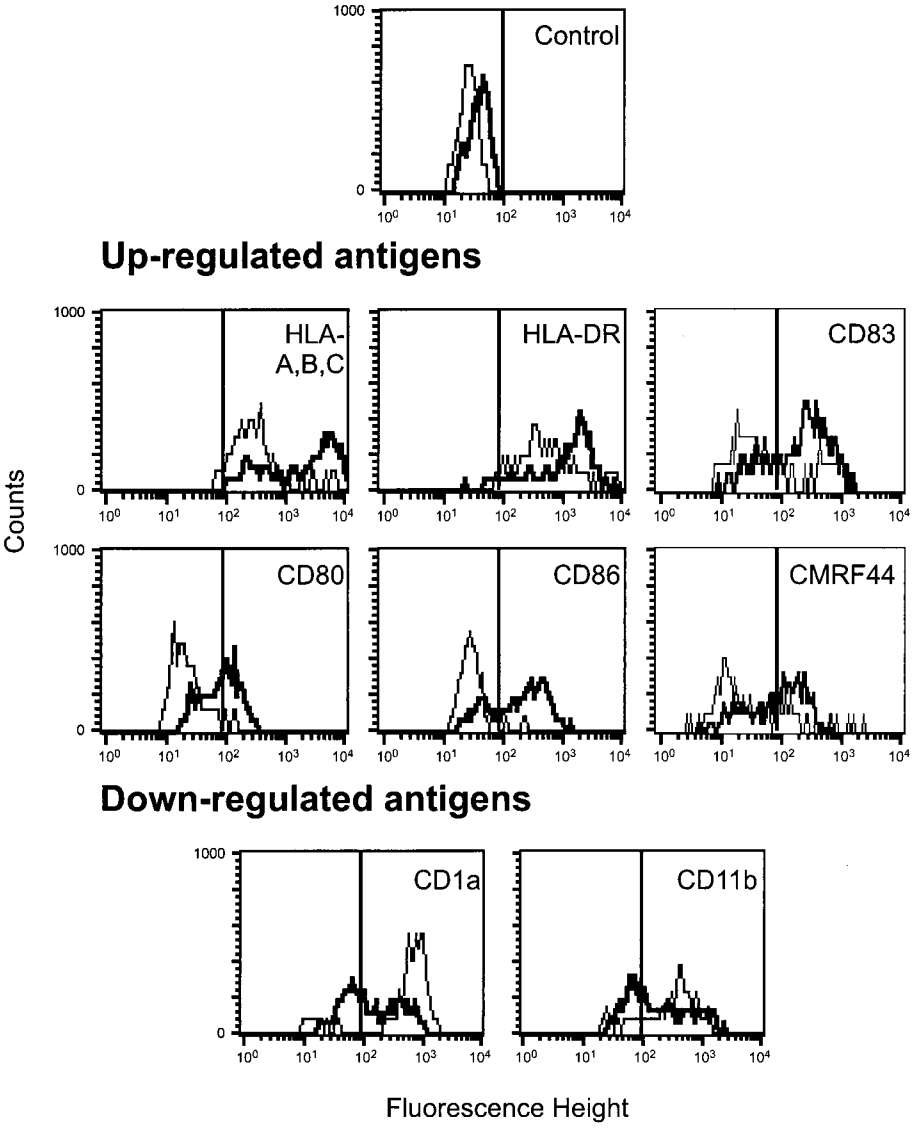

FIGURE 1. DC surface phenotype on days 14 and 28 by flow cytometry. Phenotypic changes during DC maturation: $CD34^+$ cells were cultured in GM-CSF, TNF-α, and IL-4 and analyzed by flow cytometry after 14 days and 28 days. Cultures were gated to examine only the large cells (20% of total cells). A comparison between day 14 (thin line) and day 28 (thick line) is shown in a representative experiment. The position of the control cells labeled with nonspecific Ab (IAG-11) is indicated by the horizontal line. *x*-axis, fluorescence height (log scale); *y*-axis, relative cell number.

significantly increased allostimulatory capacity of day 28 DC cultures compared with day 14 DC cultures. We have previously shown that mature DC in these cultures were derived solely from the $CD1a^{3+}$, $CD11b^{3+}$ population. These cells were capable of transient adherence to plastic and developed into nonadherent, $CD11b^{low/-}$, $CD14^+$, $CD86^{2+}$, $CMRF44^+$ DC. IL-4 was not required for this final maturation. Cell sources included leukapheresis harvests and bone marrow samples, predominantly of cancer patients but also normal bone marrow. No differences in differentiation and activation of progenitor-derived DC from different sources were observed (10).

Cytokine effects on the late phase of DC differentiation

To determine whether addition of exogenous cytokines could accelerate DC maturation and to identify those endogenous factors that might be acting, we screened a variety of cytokines for their effects on DC phenotype in serum-free cultures. HLA-DR and/or CD86 up-regulation were used as markers of maturation. For this assay, cytokines were used at a concentration previously found to be active in other systems (see *Materials and Methods*). Cytokines were added on day 14, and cultures were analyzed by flow cytometry for the presence of activated ($HLA\text{-}DR^{bright}$, $CD86^+$) DC on

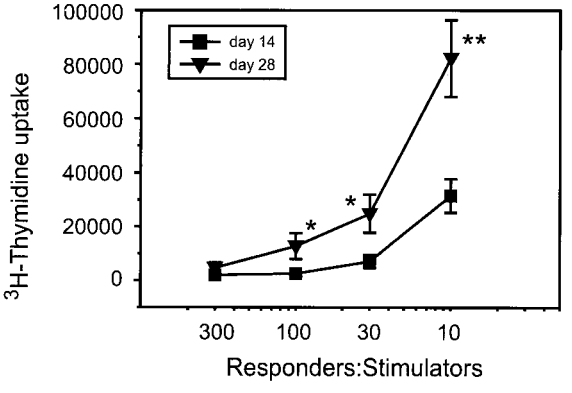

FIGURE 2. Allostimulatory capacity of DC cultured in GM-CSF, TNF-α, and IL-4 for days 14 and 28 using MLR. The allostimulatory capacity of 14 individual unsorted DC cultures was directly compared after 14 to 15 days (■) and 28 days (▼) using the same allogeneic responder cells for both time points. DC were cultured in GM-CSF, TNF-α, and IL-4, irradiated, and cocultured with 10^5 PBMC in triplicate wells. [^{3}H]thymidine uptake was measured after 5 days. Thymidine incorporation of responder cells alone was 1600 ± 350 cpm. Results are shown as the mean ± SE of 14 individual experiments (*$p < 0.05$, **$p < 0.01$).

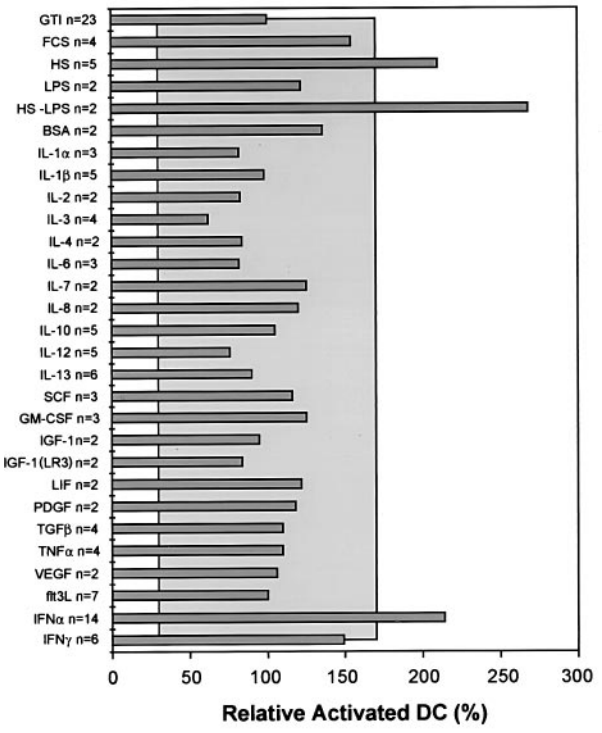

FIGURE 3. Screening of cytokines for activating effects on DC. $CD34^+$ cells were cultured in 96-well plates in GM-CSF, TNF-α, and IL-4 (GTI). At day 14, cytokines were added at the concentrations shown in *Materials and Methods.* Cultures were analyzed by flow cytometry at day 17. The proportion of activated DC ($HLA\text{-}DR^{3+}$ and/or $CD86^+$) was calculated referring to the mean expression in multiple control cultures (GTI) as 100%. SDs were calculated for all protocols and a no-difference interval of 2 SDs above control levels of expression was chosen as a cut-off level for increased expression (shaded area). Only HS and IFN-α2α were capable of inducing an increase >2 SD in the proportion of activated DC in culture.

day 17. Each set of experiments ($n = 23$) included control cells grown in standard conditions in GM-CSF, TNF-α, and IL-4. The percentage of activated DC in these control cultures on day 17 was referred to as 100%. SDs were calculated for all experiments to control for the variations between individual experiments. Twice the mean of all SD was used to define the no-difference interval (gray) in Figure 3. Only normal HS, HS filtered through a Zetapor filter to remove LPS (HS-LPS), and IFN-α2a were capable of increasing the percentage of activated DC above 2 SD of the control.

Effects of type I IFNs on DC maturation

To determine whether other type I IFNs could also accelerate DC maturation, IFN-α2a, IFN-α8, and IFN-β were added to cultures containing GM-CSF, IL-4, and TNF-α. Each of the three IFNs had a similar capacity to up-regulate HLA-A,B,C, CD80, and CD86 and to down-regulate CD1a and CD11b expression on the large cell population ($n = 3$). These changes occurred within 3 days and were concentration dependent (Fig. 4). Furthermore, up-regulation of HLA-DR, CD83, and CMRF44 expression was observed in response to IFN-α2a (shown for CD83 in Fig. 5). Cell numbers and percentages of large cells in culture did not significantly change during the 3 days of exposure to IFN-α. There was no evidence of increased cell death of large cells during this period.

To examine whether IFN-α could also activate DC function, the allostimulatory capacity of DC-containing bulk cultures was compared with or without a 3-day exposure to IFN-α2a. Figure 6*A* shows the results of six individual experiments. Cultures were split

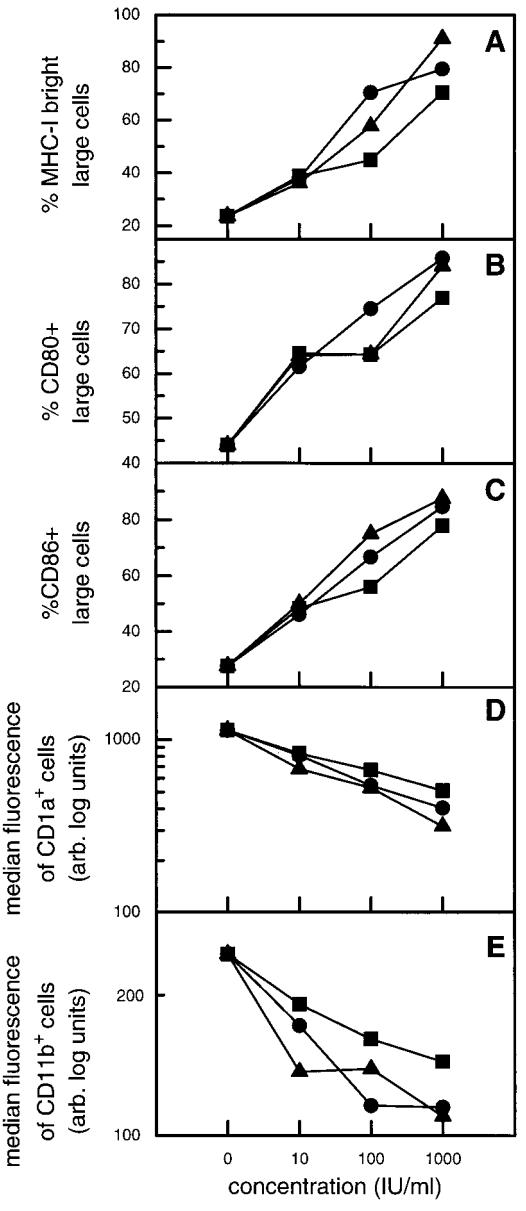

FIGURE 4. Effect of type I IFNs on DC maturation. Direct comparison of titrations of IFN-α2a (■) ($n = 3$), IFN-α8 (●) ($n = 1$), and IFN-β (itrif) ($n = 1$) on Ag expression of DC. DC cultured from a single donor were grown in GM-CSF, TNF-α, and IL-4 until day 14; type I IFNs were added daily between days 14 and 17. FACS analysis was performed on day 17. Cell populations were gated to include only the large cells (20% of total cells).

on day 14, and IFN-α2a (1000 U/ml) was added daily for 3 days into one-half of the cultures. Cultures exposed to IFN-α2a contained $23 \pm 3.4\%$ $CD86^+$ DC, and these cells showed a significantly increased allostimulatory capacity ($^*p < 0.05$). Cultures without IFN-α contained $9 \pm 1.5\%$ $CD86^+$ DC on day 17. These results were confirmed by an MLR using DC sorted on day 18 according to their high forward and side scatter characteristics (24). DC exposed to IFN-α2a for 3 days were more stimulatory than control DC (Fig. 6*B*).

Interaction of TNF-α and IFN-α

To investigate whether IFN-α could act alone or required TNF-α to induce DC maturation, cultures were washed on day 14 and continued until day 17 in serum-free medium containing GM-CSF

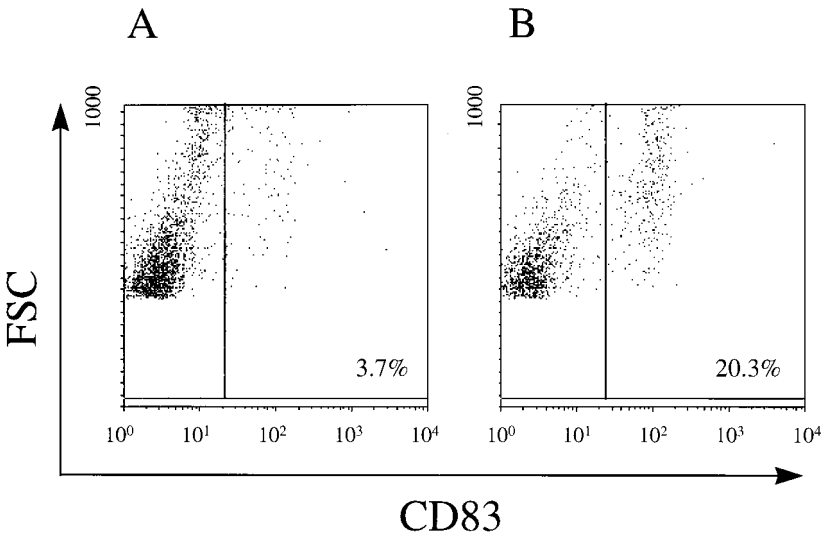

FIGURE 5. IFN-α2a induces CD83 expression in DC cultures. CD83 expression of DC cultures without (*A*) or with (*B*) IFN-α2a added for 3 days. Progenitor cells were cultured under standard serum-free conditions. On day 14, the culture was split and IFN-α2a (1000 U/ml) was added into one of the halves. FACS analysis was performed on day 17. Vertical gate indicates fluorescence of 98% of cells with control Ab.

and IL-4, without or with TNF-α (20 ng/ml, standard conditions), IFN-α, or both. IFN-α alone did not enhance DC maturation as compared with standard conditions. Both TNF-α and IFN-α were required for optimal maturation, as shown in Table I. Thus, the enhancement of DC activation by IFN-α under serum-free conditions required the presence of TNF-α.

IFN-α production in serum-free cultures of DC

Since type I IFNs were the only cytokines that could stimulate DC maturation, it appeared likely that autocrine or paracrine production of IFN was responsible for DC maturation in our serum-free cultures. This hypothesis was tested by assaying culture supernatants for IFN activity. Supernatants were collected at different times between days 14 and 30. IFN activity corresponding to 12 ± 2 IU/ml (range, 8–25 IU/ml) was detected in 10 samples in cultures from six patients. In supernatants from cultures from five other patients, IFN-like activity was not detectable. These data suggest that type I IFNs can be produced by the cells in these cultures and may act as autocrine or paracrine factors to regulate the final stages of DC maturation.

IFN-α and skin-derived DC

The similarity of phenotype and activation patterns of progenitor-derived DC and Langerhans cells led us to investigate whether type I IFNs were capable of activating skin-derived DC as well. Paired, split skin samples of similar sizes (1–2 cm^2) were floated in serum-free tissue culture medium, and cells migrating into the liquid phase were compared by assessing numbers of $CD1a^+CD80^+CD83^+$ cells with typical dendritic morphology. Cells were counted every 12 h and harvested for FACS analysis every 24 h, at which time the skin was transferred into fresh medium. In the presence of IFN-α (3000 U/ml), increased numbers of migrating DC were observed in 5 of 9 patients (Table II).

Discussion

The use of a novel serum-free culture system to produce DC from progenitor cells has enabled us to investigate the cytokines involved in DC maturation and activation. The main findings reported in this study are the accumulation of an immature DC population around day 14; the prolonged, spontaneous maturation of this immature population into activated DC during the following 14 days; and the capacity of type I IFNs to accelerate this maturation within 3 days.

Three different pathways have been described according to their intermediate (early) stages, which result in myeloid DC of similar phenotype. These include $CD14^+$ monocytic cells (7, 10), $CD14^-CD1a^+$ Langerhans cells (27–29), and $CD14^-CD1a^-$ peripheral blood-derived DC (6). Under the conditions described here, $CD34^+$ cells differentiated to an intermediate stage, which is

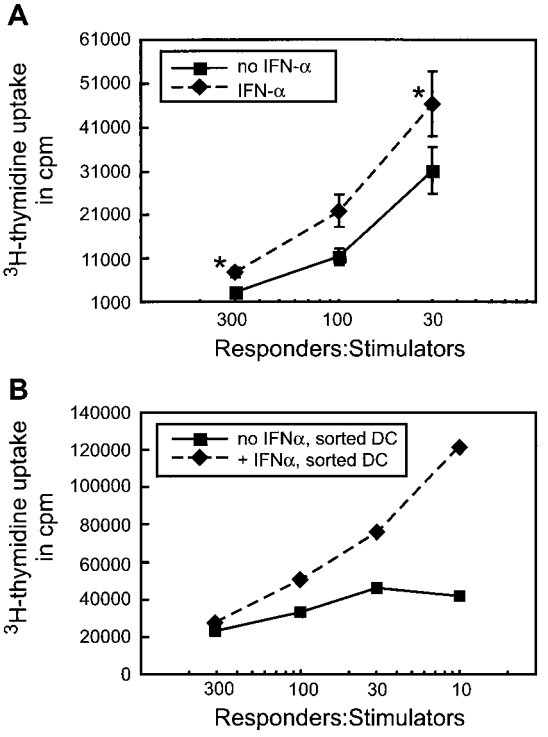

FIGURE 6. Allostimulatory capacity of DC activated by IFN-α. *A*, The effect of IFN-α2a on the allostimulatory capacity of unsorted cultures of DC was studied after 17 days culture in GM-CSF, TNF-α, and IL-4 (■) compared with paired cultures with addition of IFN-α2a (1000 U/ml) on days 14 through 17 (◆), ($n = 6$), $p < 0.05$. *B*, Allostimulatory function of DC sorted on day 18. Culture was split on day 14 and either continued in GM-CSF, TNF-α, and IL-4 (■) or in these three cytokines together with IFN-α2a (1000 U/ml) added on days 14 through 18 (◆). On day 18, large DC were sorted according to their high forward and side scatter, and the MLR was performed as described in *Materials and Methods*.

Table I. *Effects of TNF-α and IFN-α on DC activation between days 14 and 17*[a]

Ag	Cytokine (DC as % of large cells)		
	TNF-α	IFN-α	TNF-α + IFN-α
CD80	54 ± 7	44 ± 7	74 ± 5
CD83	50 ± 2	ND	72 ± 4
CD86	57 ± 10	47 ± 6	84 ± 2

[a] The proportion of activated ($CD80^+$, $CD83^+$, $CD86^+$) DC in cultures given as the percentage of large cells. Large cells represented 20 to 30% of total cells in culture. $CD34^+$ cells were cultured for 14 days in GM-CSF, TNF-α, and IL-4. Cultures were washed on day 14 and continued until day 17 without IL-4 in the presence of GM-CSF (40 ng/ml), TNF-α (20 ng/ml), and/or IFN-α (1000 U/ml). Results are shown as means ± SE of three to five individual experiments.

Table II. *Effect of IFN-α on skin-derived DC migration*[a]

Protocol	Time (DC × 10^3)	
	24 h	48 h
X-Vivo 20, no IFN-α	7 ± 2	13 ± 4
X-Vivo 20 + IFN-α	44 ± 2	41 ± 1

[a] Cumulative numbers of skin-derived DC identified by morphology after 24 and 48 h in serum-free medium (X-Vivo 20) with or without IFN-α (3000 U/ml). Shown are the medium ± SE ($n = 5$). Method is described in *Materials and Methods*.

$CD14^-$ and $CD1a^+$. This is consistent with the Langerhans cell phenotype. As with freshly isolated Langerhans cells (30), these early DC in serum-free cultures were characterized by intermediate HLA-A,B,C and HLA-DR expression, high expression of CD1a, expression of CD11b, and the lack of accessory molecules (CD80, CD86) as well as DC-associated molecules CD83 and CMRF44. In the presence of TNF-α, these immature DC took another 14 days to acquire the phenotypic and functional characteristics typical of activated DC. The phenotypic and functional changes observed during this process were similar to those seen in other studies and involved the up-regulation of HLA-A,B,C, HLA-DR (18), CD80, CD86 (19, 20), CD83, and CMRF44, down-regulation of CD1a and CD11b (18), and functional maturation (7, 10) into highly allostimulatory cells. In parallel, cells lost their ability to adhere to plastic and became nonadherent round cells with a corona of thin dendrites.

The spontaneous maturation in these serum-free cultures suggested that the production of autocrine or paracrine factors might be involved in this process. We therefore screened a number of cytokines and other molecules for their action in this system. Apart from HS, only type I IFNs (α or β) were capable of accelerating maturation, so that within 3 days a majority of the large-size cells in culture expressed CD80, CD83, and CD86 and started to down-regulate CD1a and CD11b. This effect of IFNs added into cultures containing GM-CSF, IL-4, and TNF-α was concentration dependent in a range between 10 and 1000 U/ml and was similar for three different type I IFNs (α2a, α8, and β) (Fig. 4). In parallel with the phenotypic changes, DC exposed to IFN-α had increased T cell stimulatory capacity. Both IFN-α and TNF-α were required for DC activation between days 14 and 17. Synergy of TNF-α and IFN on HLA class I expression has been previously reported at the level of transcriptional regulation (31). The nature of the effector in HS activating DC remains unknown. It has recently been shown that HS contains soluble CD14, which forms complexes with LPS and as a complex can activate $CD14^-$ DC (32). Therefore, these results also provide an explanation as to why LPS in the absence of serum did not activate our $CD14^-$ DC.

We have shown that IFN-like activity was produced in most spontaneously maturing serum-free DC cultures. Since autocrine production of IFN-α can cause significant biologic effects even in the absence of detectable activity in the supernatant (26), we regard the presence of measurable activity in cultures from 6 of 11 patients as significant. The clear effect of exogenously added IFN-α, the low level production of IFN-like activity in the cultures, and the prolonged period of immaturity, even in the presence of a high dose of TNF-α, all suggest that type I IFNs provide a necessary signal for the induction of DC maturation and activation.

In addition to the effects of IFN-α on in vitro-derived DC, we showed that IFN-α activated migration of resident DC from split skin samples floating in serum-free medium. These results suggest that the adherent, immature DC in our serum-free cultures are similar to skin-derived DC in their response to type I IFN as well as in phenotype.

These experiments open a new perspective on the events involved in DC maturation and activation, showing that type I IFN can enhance the effect of TNF-α in the induction of this process. This suggests that in addition to the multiple immunomodulatory effects, type I IFNs may also regulate immune responses at the level of the APC. Type I IFNs have a well-established role in the response to infections with viruses (33–35). Immunomodulatory effects include the promotion of Th1 responses by inhibition of IL-4 and IL-5 secretion (36, 37), increase in IFN-γ-producing cells (38), and effects on IgG production (13). Our studies show a novel mechanism, which is that type I IFNs also mediate effects by inducing maturation and activation of DC. This may help to explain the autoimmune phenomena associated with the use of IFN in hepatitis and cancer patients (33, 39–41). It may also provide an additional mechanism for the anticancer effects of IFN-α in a variety of tumors (42–47).

Importantly, these results may have an impact on clinical strategies for developing cancer vaccines. While IFNs are being used as adjuvant therapy for cancer and as antiviral therapy, the effect of IFNs on DC function has not previously been defined. IFN-α might therefore be a useful candidate as a vaccine adjuvant in clinical trials using tumor Ags as vaccines. Furthermore, this serum-free system should assist the further study of events associated with DC activation, as well as providing clinical opportunities for using IFN-activated DC as cellular adjuvants.

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