The Journal of Immunology

Induction of CD83$^+$CD14$^+$ Nondendritic Antigen-Presenting Cells by Exposure of Monocytes to IFN-α

Gianni Gerlini, Giulia Mariotti, Alberto Chiarugi, Paola Di Gennaro, Roberto Caporale, Astrid Parenti, Leonardo Cavone, Adrian Tun-Kyi, Francesca Prignano, Riccardo Saccardi, Lorenzo Borgognoni and Nicola Pimpinelli

J Immunol 2008; 181:2999-3008; ;
doi: 10.4049/jimmunol.181.5.2999
http://www.jimmunol.org/content/181/5/2999

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Induction of CD83$^+$CD14$^+$ Nondendritic Antigen-Presenting Cells by Exposure of Monocytes to IFN-α[1]

Gianni Gerlini,[2]*† Giulia Mariotti,† Alberto Chiarugi,§ Paola Di Gennaro,† Roberto Caporale,‡ Astrid Parenti,§ Leonardo Cavone,§ Adrian Tun-Kyi,¶ Francesca Prignano,† Riccardo Saccardi,‡ Lorenzo Borgognoni,* and Nicola Pimpinelli†

IFN-α is a well-known agent for treatment of viral and malignant diseases. It has several modes of actions, including direct influence on the immune system. We investigated IFN-α effects on PBMC in terms of dendritic cell (DC) differentiation, as PBMC are exposed to high IFN-α levels during treatment of infections and cancers. We show that in vitro IFN-α exposure induced rapid and strong up-regulation of the DC-maturation markers CD80, CD86, and CD83 in bulk PBMC. Consistently, IFN-α induced up-regulation of these molecules on purified monocytes within 24 h. Up-regulation of CD80 and CD83 expression was IFN-α concentration-dependent. In contrast to GM-CSF + IL-4-generated DCs, most of the IFN-α-challenged CD83$^+$ cells coexpressed the monocyte marker CD14. Despite a typical mature DC immunophenotype, IFN-α-treated monocytes conserved phagocytic activity and never acquired a dendritic morphology. In mixed lymphocyte reactions IFN-α-treated monocytes were less potent than GM-CSF + IL-4-generated DCs but significantly more potent than untreated monocytes to induce T cell proliferation in bulk PBMC. However, only GM-CSF + IL-4-generated DCs were able to induce a significant proliferation of naive CD4$^+$ T cells. Notably, autologous memory CD4$^+$ T cells proliferated when exposed to tetanus toxoid-pulsed IFN-α-treated monocytes. At variance with untreated or GM-CSF + IL-4-exposed monocytes, those challenged with IFN-α showed long-lasting STAT-1 phosphorylation. Remarkably, CD83$^+$CD14$^+$ cells were present in varicella skin lesions in close contact with IFN-α-producing cells. The present findings suggest that IFN-α alone promptly generates nondendritic APCs able to stimulate memory immune responses. This may represent an additional mode of action of IFN-α in vivo. ***The Journal of Immunology,*** **2008, 181: 2999–3008.**

Dendritic cells (DCs)[3] are professional APCs able to generate strong primary immune responses (1, 2). Under normal conditions, they reside as immature DCs in peripheral tissues where, upon Ag uptake and exposure to proinflammatory cytokines, they undergo maturation and migrate to local lymph nodes (LNs) (3). This process is accompanied by functional and immunophenotypic changes characterized by up-regulation of MHC classes I–II and costimulatory molecules, as well as by neo-expression of CD83, a DC maturation marker (4). In the last years, different methods for the generation of large numbers of DCs from blood precursors such as CD14$^+$ monocytes (5) or CD34$^+$ cells (6) have been described, thus offering the opportunity to use DCs as natural adjuvants in cancer immunotherapy (7–10). Typically, monocyte-derived DCs are generated in vitro using GM-CSF and IL-4 (5).

Type I IFNs are potent antiviral cytokines produced in large amounts in response to viral infections by a subset of cells commonly known as plasmacytoid dendritic cells (pDCs) (11, 12) and, at a much lower extent, by B cells, monocytes, and macrophages (13). Beyond antiviral effects, type I IFNs have been shown to have important anticancer properties, including antiproliferative and antiangiogenic effects (14, 15). Clinically, type I IFNs, and particularly IFN-α, are widely used as immune response modifiers in several diseases, including hepatitis C and B virus infections (16, 17), as well as in malignancies such as chronic myeloid leukemia, cutaneous T cell lymphoma, renal cancer, and melanoma (18–20).

Increasing bodies of evidence shows that type I IFNs modulate DC biology at different levels. Used in combination with classic cytokines such as GM-CSF + IL-4 (21–24) or GM-CSF alone (25–27), type I IFNs influence DC generation and maturation. Numerous papers show a promoting effect of type I IFNs on DC generation (21, 23–27), but an inhibitory effect has also been reported (22, 28, 29). Even though blood and tissue resident cells can be exposed to high levels of type I IFNs during IFN-α therapy (14, 30, 31) or viral infections (11, 32, 33), to our knowledge the effects of type I IFNs on monocytes have never been studied in the absence of other cytokines. To this end, we investigated the effects of IFN-α on PBMC or on purified monocytes in terms of DC differentiation and function. The immunophenotype of monocytes infiltrating varicella skin lesions where IFN-α is highly produced was also investigated.

Materials and Methods

Antibodies

The following unconjugated or FITC- or PE-conjugated mouse mAbs were used: anti-HLA-DR (L243, IgG2a), anti-lineage cocktail 1 (Lin-1; CD3,

*Plastic Surgery Unit, Regional Melanoma Referral Center, Tuscan Tumor Institute (ITT), Santa Maria Annunziata Hospital, Florence, Italy; †Department of Dermatological Sciences, ‡Division of Hematology, and §Department of Pharmacology, University of Florence, Florence, Italy; and ¶Cancer Biology Program, Division of Hematology/Oncology, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215

Received for publication November 26, 2007. Accepted for publication June 22, 2008.

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

[1] G.G. is supported by a grant from the Fioravanti family. G.M. and P.D.G. are supported by a grant from the Ente Cassa di Risparmio di Firenze.

[2] Address correspondence and reprints request to Dr. Gianni Gerlini, Plastic Surgery Unit-Regional Melanoma Referral Center, Tuscan Tumor Institute (ITT), Santa Maria Annunziata Hospital, Via Antella, 58, I-50011, Florence, Italy. E-mail address: g.gerlini@dfc.unifi.it

[3] Abbreviations used in this paper: DC, dendritic cell; LN, lymph node; pDC, plasmacytoid dendritic cell; anti-Lin-1, anti-lineage cocktail 1; ST-DC, standard-DCs; IFN-MO, IFN-monocytes; 7-AAD, 7-aminoactinomycin D; IRF-1, interferon responsive factor-1; MFI, mean fluorescence intensity; SLE, systemic lupus erythematodes.

CD14, CD16, CD19, CD20, CD56), anti-CD3 (HIT3a, IgG2a), anti-CD4 (RPA-T4, IgG1), anti-CD8 (RPA-T8, IgG1), anti-CD11c (BLy6, IgG1), anti-CD14 (M5E2, IgG2a), anti-CD19 (HIB19, IgG1), anti-CD56 (NCAM; 16.2, IgG1), anti-CD80 (L307.4, IgG1), anti-CD83 (HB15e, IgG1), and anti-CD86 (2331 FUN-1, IgG1) from BD Biosciences; anti-ICAM-1 (P2A4, IgG1) from Chemicon International; anti-ICAM-3 (CBR-IC3/1, IgG1) and anti-CD11a (R7.1, IgG1) from Bender MedSystems; anti-CD11b (ICRF44, IgG1) from Serotec; and anti IFN-α (MMHA-2, IgG1) from PBL Biomedical Laboratories. For unconjugated primary mAbs, secondary FITC-, PE-, or Cy5-conjugated goat or rabbit anti-mouse Abs were from DakoCytomation.

Cell cultures

Complete medium was RPMI 1640 supplemented with 1% penicillin/streptomycin, 1% sodium pyruvate, 10% FCS (all from EuroClone), and 2% glutamine (Sigma-Aldrich). PBMC were isolated from buffy coats by density gradient centrifugation with Lymphoprep (Axis-Shield) and cryopreserved in 10% DMSO-FCS. Monocytes were isolated from PBMC using MACS anti-CD14 microbeads and a MidiMACS device (both from Miltenyi Biotec). Monocytes (88–95% purity) were cultured in petri dishes (Costar) at 37°C and 5% CO_2 in complete medium alone or with GM-CSF (10^3 U/ml; Leucomax, Sandoz-Wander Pharma) and IL-4 (10^3 U/ml; R&D Systems), indicated as standard-DC (ST-DC), or with different IFN-α-2a concentrations (10^1 to 10^5 U/ml; Roferon-A3, Roche), indicated as IFN-MO. LPS from *Escherichia coli* 026:B6 (1 μg/ml; Sigma-Aldrich) was used as DC maturation stimulus. Cell morphology was documented with an inverted microscope (Nikon TMS-F) and a Nikon F70 camera. Cell viability and recovery were assessed by the trypan blue exclusion test.

Human materials

All human tissue samples were obtained upon informed consent, according to the principles of the Helsinki Declaration. PBMC from peripheral blood of melanoma patients under IFN-α therapy were obtained as described above. Blood collection was performed the day after IFN-α injection (3 or 5×10^6 IU IFN-α-2a; Roferon-A3, Roche) three times per week. Varicella skin punch biopsies were performed on the edge of lesions.

Flow cytometry analysis

All mAb dilutions and washing steps were performed in PBS containing 2% FCS and 2 mM EDTA. Isotype-matched mAbs were used as negative control. Flow cytometry was performed using FACSort and data were analyzed using CellQuest (both from BD Biosciences). 7-Aminoactinomycin D (7-AAD; Viaprobe, BD Biosciences) was used to exclude dead cells from analysis.

Immunohistochemistry and immunofluorescence

Lesional skin biopsies were snap-frozen in liquid nitrogen and stored at −80°C. Representative five to seven serial cryostat sections were stained with anti-IFN-α according to the APAAP (alkaline phosphatase anti-alkaline phosphatase) technique. For immunofluorescence studies, slides were preincubated 1 h with 20 mg/ml BSA in PBS/Triton X-100 0.3% (both from Sigma-Aldrich) and then 2 h with anti-CD83 mAb. A subsequent incubation (1 h) with Alexa-594 goat anti-mouse secondary Ab (Molecular Probes) allowed binding visualization. A subsequent incubation with FITC-conjugated anti-CD14 mAb was performed for double labeling. Immune detection specificity was established by omission of the primary Ab. Cells were observed with an inverted Nikon fluorescence microscope.

Western blotting

Monocytes in culture were exposed for different times (1–12 h) to saline solution, GM-CSF + IL-4, IFN-α, or IFN-γ. Cells were lysed with 50 mM Tris (pH 7.4), 1 mM EDTA, 1 mM PMSF, 4 μg/ml aprotinin and leupeptin, 1% SDS. Total proteins were measured according to standard techniques, and 20–40 μg of protein/lane was loaded. After 4–20% SDS-PAGE and blotting, membranes (Hybond-ECL, Amersham Biosciences) were blocked with PBS containing 0.1% Tween 20 and 5% skimmed milk (TPBS/5% milk) and incubated overnight with either polyclonal anti-phospho-STAT-1 (Tyr^{701}) (Cell Signaling Technology) or polyclonal anti-IFN responsive factor (IRF)-1 (Santa Cruz Biotechnology), both diluted 1/1000 in T-PBS/5% BSA. Membranes were then incubated 1 h with the corresponding peroxidase-conjugated secondary Ab (1/2000) in T-PBS/5% milk. Bands were visualized by ECL (Amersham Biosciences).

Electron microscopy

ST-DC and IFN-MO were fixed in Karnowsky's solution at room temperature, osmicated, and embedded in Epon 812. Ultrathin sections stained with uranyl acetate and lead citrate were examined under a JEM 1010 electron microscope (JEOL) at 80 kV.

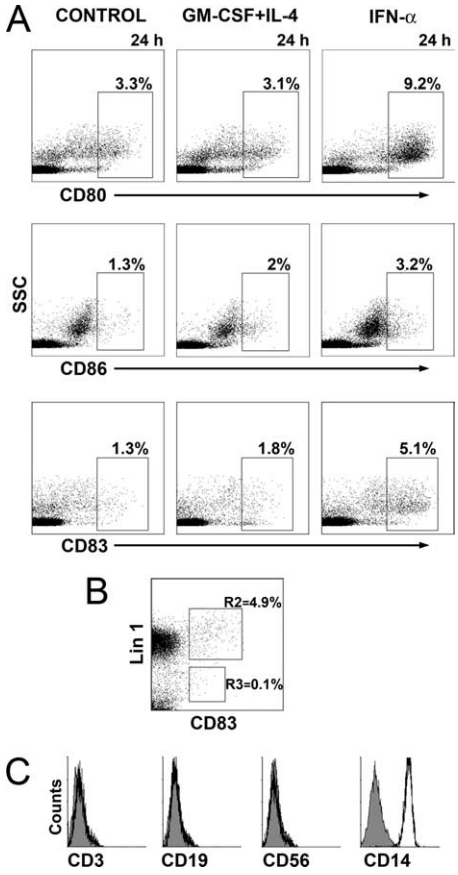

FIGURE 1. IFN-α up-regulates expression of CD80, CD86, and CD83 on PBMC. Total PBMC were cultured for 24 h in complete medium alone (control), in the presence of GM-CSF (10^3 U/ml) and IL-4 (10^3 U/ml), or in the presence of IFN-α (10^3 U/ml) and then analyzed by flow cytometry. *A*, Comparison of CD80, CD86, and CD83 expression on total PBMC. *B*, IFN-α-treated PBMC double stained with Abs against CD83 and Lin-1 revealed two populations of CD83-positive cells, one negative (R3) and the other positive for Lin-1 (R2). *C*, PBMC were double stained using an Ab against CD83 and the single-lineage markers as indicated. Gating on the $CD83^+$ cell populations, the analysis revealed that cells were negative for CD3, CD19, and CD56 and positive for CD14. One representative experiment out of three is shown. Filled histograms represent isotype controls.

Phagocytic assay

Phagocytic activity of ST-DC, IFN-MO, and monocytes was examined as follows: 4×10^5 cells were resuspended in 10% FCS RPMI 1640 medium and equilibrated at 37°C or 0°C for 10 min. Cells were then pulsed with FITC-conjugated dextran (1 mg/ml, 40,000 molecular mass; Molecular Probes) for 45 min at 37°C or 0°C. After incubation, cells were washed four times with cold PBS buffer containing 0.01% sodium azide (Sigma-Aldrich) and 1% FCS and analyzed by flow cytometry. Results are expressed as mean fluorescence intensity (MFI). Background is represented by cells incubated at 0°C.

T cell proliferation assay

ST-DC, IFN-MO, and monocytes were tested for their capability to induce proliferation of allogeneic T cells in a MLR. On day 3 cells were irradiated with 20 Gy and cocultured with allogeneic $CD14^+$-depleted PBMC (10^5 cells/well) in complete medium. After 5 days, cells were pulsed with 0.5 μCi/well [^{3}H]thymidine (Amersham Pharmacia Biotech), harvested after 18 h, and [^{3}H]thymidine uptake was measured using a scintillation counter.

T cell proliferation assays were also performed by coculturing ST-DC, IFN-MO and monocytes with CFSE (Molecular Probes Europe)-labeled allogeneic $CD14^+$-depleted PBMC (ratio 1:5) for 5 days. Cells

Table I. *The percentage of CD80$^+$, CD86$^+$, and CD83$^+$ cells increases in PBMC upon IFN-α treatment*[a]

	Control	GM-CSF + IL-4	IFN-α
CD80	2.9 ± 1.1	3.8 ± 1.3	8.3 ± 1.2**
CD86	1.4 ± 0.6	1.8 ± 0.7	3.2 ± 0.8*
CD83	1.2 ± 0.5	1.3 ± 0.5	4.3 ± 1.3**

[a] Cells were treated as described in Fig. 1 legend. Values represent percentages expressed as means ± SD. **, $p < 0.05$ *vs* GM-CSF + IL-4 and control. *, $p < 0.05$ only vs control.

were thereafter stained with anti-CD4 and anti-CD8 mAbs and analyzed by flow cytometry. CFSE-labeled PBMC alone were used as negative control and percentage of proliferating cells was calculated using CellQuest software. To verify whether the different APC subsets were also able to activate naive CD4$^+$ T cells, we performed MLR using purified CD4$^+$ naive T cells as responders (MACS naive CD4$^+$ T cell isolation kit; Miltenyi Biotec).

ST-DC, IFN-MO, and monocytes were tested for their capability to induce autologous CD4$^+$ T cell proliferation using tetanus toxoid (TT; Calbiochem) as recall Ag. Cells were pulsed overnight with TT (1 μg/ml) at different time points and cocultured with CFSE-labeled autologous purified CD4$^+$ T cells (MACS CD4$^+$ T cell isolation kit II; Miltenyi Biotec).

Statistical analysis

The values reported throughout the text are expressed as means ± SD of at least three different experiments. Data were analyzed using the two-sided Student's *t* test.

Results

IFN-α exposure increases the number of CD80$^+$, CD86$^+$, and CD83$^+$ cells in cultured PBMC

IFN-α has been previously used in combination with GM-CSF or GM-CSF + IL-4 to generate monocyte-derived DCs. To study the effects of IFN-α alone, total PBMC were cultured in absence or presence of GM-CSF + IL-4 or IFN-α. After 24 h, the percentage of CD80$^+$, CD86$^+$, and CD83$^+$ cells cultured in the presence of IFN-α was higher than that of cells cultured with GM-CSF + IL-4 or complete medium alone (Fig. 1*A* and Table I). By using anti-CD83 and a cocktail of mAbs directed against the lineage markers, two subpopulations of CD83$^+$ cells were identified in PBMC treated with IFN-α: a minor population (0.13 ± 0.04%) of lineage marker-negative cells corresponding to classic DCs, and a larger population (4.4 ± 0.92%) of lineage marker-positive cells (Fig. 1*B*). Further analysis showed that this latter cell population was negative for CD3, CD19, and CD56 but expressed the monocyte marker CD14 (Fig. 1*C*). Flow cytometry analysis performed on day 3 gave similar results (data not shown).

IFN-α induces CD80, CD86, and CD83 expression in cultured monocytes

To understand whether the IFN-α directly acted on monocytes or through other cells present in PBMC, we sorted CD14$^+$ monocytes and exposed them to different concentrations of IFN-α, defined as IFN-MO, or GM-CSF + IL-4, defined as ST-DC.

As low as 10 U/ml of IFN-α was sufficient to up-regulate CD80, CD86, and CD83 on IFN-MO. At this concentration the percentage of CD80$^+$CD86$^+$ IFN-MO was higher than that of ST-DC (61.4 ± 9.2% and 38 ± 7.6%, respectively, $p < 0.05$) (Fig. 2*A*). Similarly, the percentage of CD83$^+$HLA-DR$^+$ IFN-MO was significantly higher than that of ST-DC (16 ± 4.6% and 3.7 ± 2.2, respectively, $p < 0.05$). Higher IFN-α concentrations caused a gradual increase of double-positive cells (Fig. 2*A*), reaching a maximum at 10^3 U/ml IFN-α (77 ± 14.1% and 68 ± 12.3% for CD80/CD86 and CD83/HLA-DR double-positive cells, respectively). Indeed, the increase of double-positive cells was mainly due to the up-regulation of CD80 and CD83, as well as to a lesser extent of CD86 and HLA-DR (Fig. 2*A*).

Of note, IFN-α concentrations of 10^5 U/ml resulted in a decrease of mean fluorescence intensity and double-positive cells, as well as in a concomitant increase of 7-AAD incorporation, thereby suggesting cytotoxicity (data not shown). Therefore, subsequent experiments were conducted using 10^3 U/ml IFN-α.

CCR7 represents an additional DC maturation marker that mediates migration of DC toward lymph nodes in response to MIP-3β/EBI1-ligand chemokine (ELC) and 6Ckine/secondary lymphoid chemokine (SLC) (3). When culturing IFN-MO for 24 h, CCR7 up-regulation could be detected on 14.2 ± 8.8% of cells. In contrast, no CCR7 could be found on freshly isolated monocytes (Fig. 2*B*).

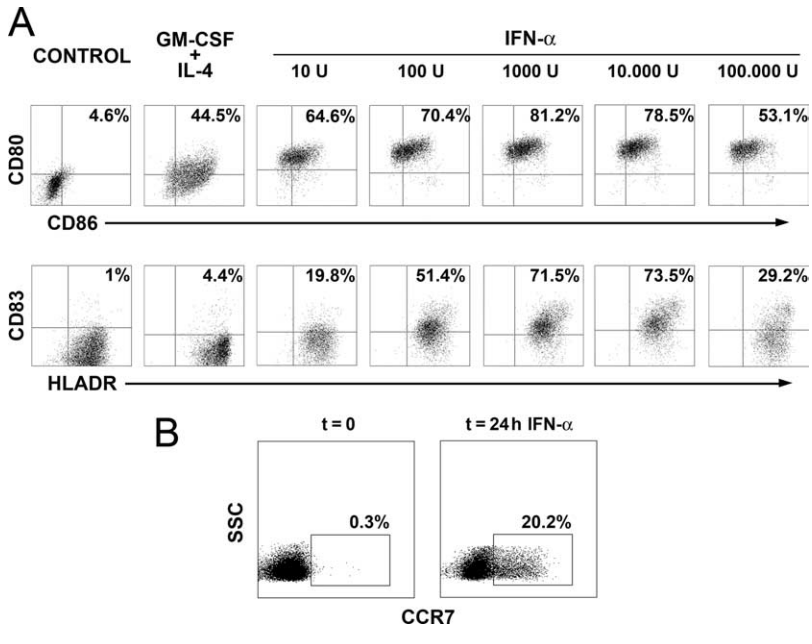

FIGURE 2. Expression of CD80 and CD83 molecules on IFN-MO is concentration-dependent. IFN-α induces expression of CCR7. *A*, CD14$^+$-purified monocytes were cultured in complete medium (control), GM-CSF + IL-4, or IFN-α at the indicated concentrations for 3 days. Thereafter, cells were double stained for CD80/CD86 and CD83/HLA-DR and analyzed by flow cytometry. Numbers represent the percentage of double-positive cells. One representative experiment out of three is shown. *B*, Flow cytometry analysis of CCR7 expression on fresh monocytes (T0) and 24 h-treated monocytes with 10^3 U/ml IFN-α. One representative experiment out of four is shown.

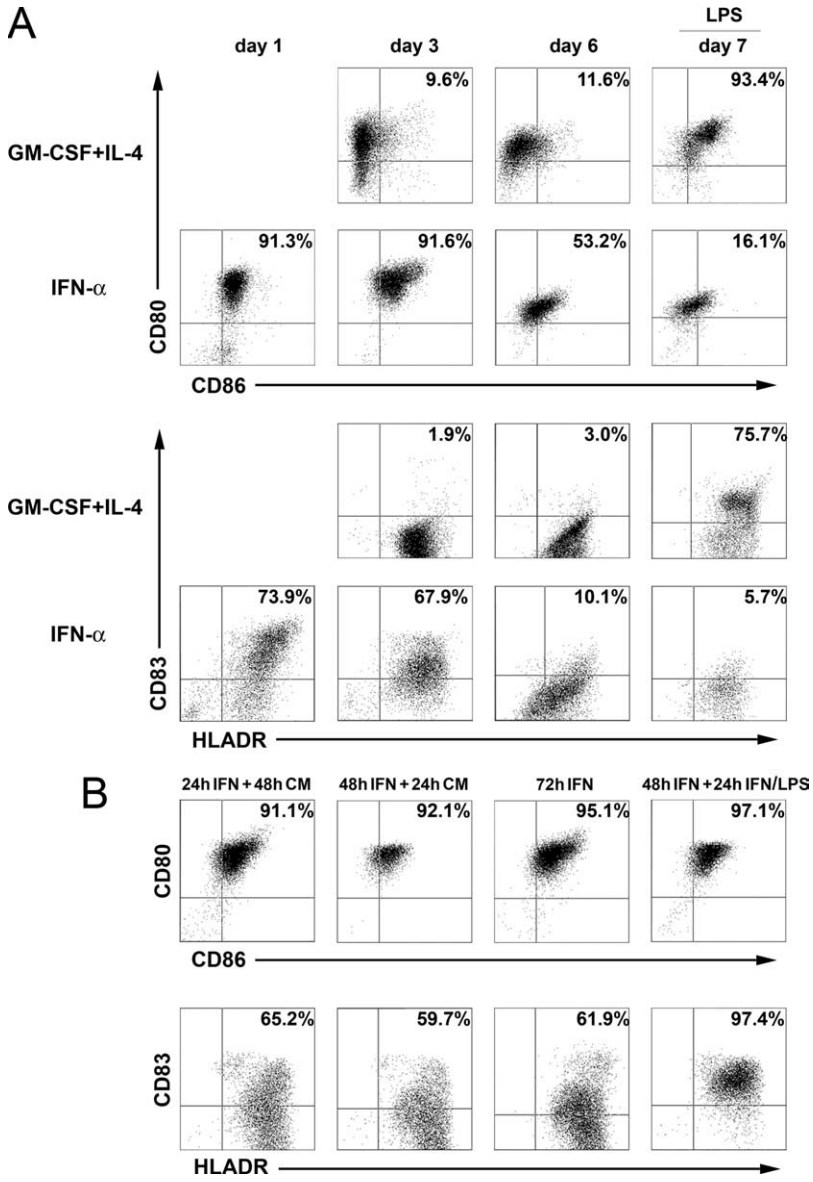

FIGURE 3. Time course analysis of costimulatory molecules, CD83 and HLA-DR expression on ST-DC and IFN-MO. *A*, Monocytes were cultured in GM-CSF + IL-4 (ST-DC) or IFN-α (10^3 U/ml; IFN-MO) for 6 days and thereafter stimulated with LPS for 24 h. IFN-MO (on days 1, 3, 6, and 7) and ST-DC (on days 3, 6, and 7) were double stained for CD80/CD86 and CD83/HLA-DR and analyzed by flow cytometry (see *Results*). Numbers indicate the percentages of double-positive cells. One representative experiment out of three is shown. *B*, Monocytes were primed for 24 or 48 h with IFN-α. After the priming, cells were washed in PBS and cultured in complete medium (CM) until day 3 and compared with cells cultured throughout 72 h with IFN-α. Additionally, cells treated for 48 h with IFN-α were stimulated for the last 24 h with LPS (1 μg/ml). On day 3 cells were double stained for CD80/CD86 and CD83/HLA-DR and analyzed by flow cytometry. One representative experiment out of three is shown.

Kinetics of IFN-α-induced expression of CD80, CD86, and CD83

We next studied the expression kinetics of CD80/CD86 and CD83/HLA-DR in ST-DC and IFN-MO during a 7-day culture. While on day 1 ST-DC were still adherent to the flask (and therefore not analyzed), the number of CD80/CD86- or CD83/HLA-DR-positive ST-DC was low on day 3, intermediate on day 6, and further augmented after LPS-induced maturation (Fig. 3*A* and Table II). Importantly, IFN-MO exhibited an opposite expression kinetic. As early as 24 h after the challenge, the number of CD80/CD86- or CD83/HLA-DR-positive cells highly increased. The number of double-positive cells remained high at day 3 and underwent a gradual down-regulation until day 7 (Fig. 3*A* and Table III). We then sought to understand whether the observed decrease of the number of CD80/CD86- and CD83/HLA-DR-positive cells was due to down-regulation of surface molecules or merely to cell death. We found that trypan blue-positive cells were similar in IFN-MO cultures compared with ST-DC on day 1 (74.5 ± 22.3% vs 75.8 ± 20.2%, respectively). However, a significant decrease of viable IFN-MO compared with ST-DC was observed on day 3 (24.9 ± 13.3% vs 60.9 ± 24.3%; $p < 0.01$), day 6 (18.8 ± 9.7% vs 52.8 ± 16.8%; $p < 0.05$), and day 7 (10.2 ± 4.4% vs 47.7 ± 22.5%; $p < 0.05$). Nevertheless, as shown

Table II. *Expression of CD83, HLA-DR, and costimulatory molecules on ST-DC upon 7 days culture analyzed by flow cytometry*[a]

	Day 1	Day 3	Day 6	Day 7
CD80/86	n.a.	9.1 ± 1.6	11.6 ± 0.9	88.1 ± 6.1*
CD83/HLADR	n.a.	1.3 ± 0.5	3 ± 0.3	80.1 ± 4.1*

[a] Cells were treated as described in Fig. 3 legend. Values are expressed as means ± SD. On day 1, cells were still adherent in the GM-CSF + IL-4 culture and were therefore not analyzed (n.a.). *, $p < 0.001$ vs ST-DC on day 3 and on day 6.

Table III. *Expression of CD83, HLA-DR, and costimulatory molecules on IFN-MO upon 7 days culture analyzed by flow cytometry*[a]

	Day 1	Day 3	Day 6	Day 7
CD80/86	92.5 ± 2.2	91.5 ± 6.1	51 ± 2.5	15 ± 0.9*
CD83/HLADR	76.1 ± 5.3	67.7 ± 6.1	8.9 ± 1.2	4.5 ± 1.2*

[a] Cells were treated as described in Fig. 3 legend. Values are expressed as means ± SD. *, $p < 0.001$ vs IFN-MO on day 1 and on day 3 and $p < 0.05$ vs IFN-MO on day 6.

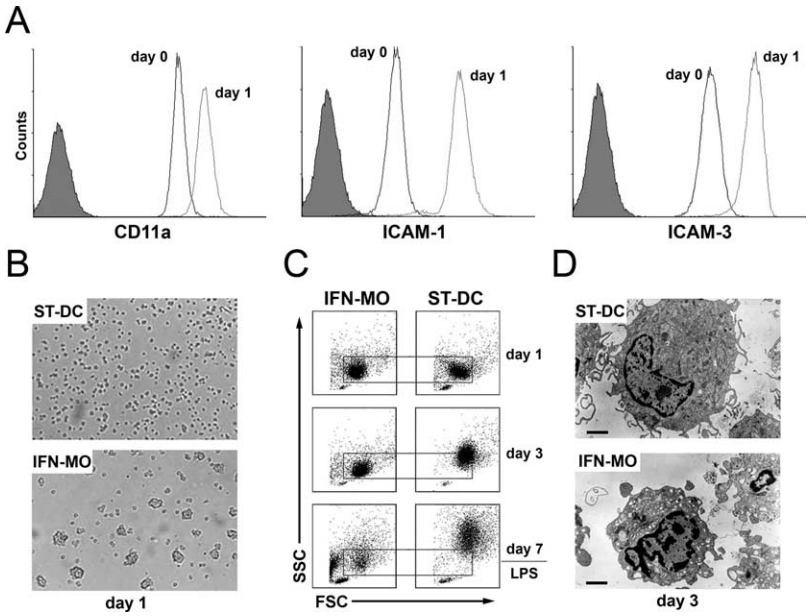

FIGURE 4. IFN-α-treated monocytes undergo a rapid cell aggregation but do not acquire a dendritic morphology. Monocytes were cultured in GM-CSF + IL-4 (ST-DC) or IFN-α (10^3 U/ml; IFN-MO) for 6 days and thereafter stimulated with LPS for 24 h. *A*, Flow cytometry analysis of adhesion molecules expression, CD11a, ICAM-1, and ICAM-3 on IFN-MO compared with fresh monocytes on day 1. One representative experiment out of three is shown. Filled histograms represent isotype controls. *B*, Representative phase-contrast images of ST-DC and IFN-MO cultures on day 1 (original magnification $\times$200). *C*, Flow cytometry analysis of ST-DC and IFN-MO showing the side scatter (SSC) and forward scatter (FSC) features on days 1 and 3 as well as after LPS-induced maturation on day 7. *D*, Representative electron microscopy images of ST-DC and IFN-MO on day 3 (original magnification $\times$5000; bar = 2 μm).

in Fig. 3*A*, where dot plots refer to viable cells, also a time-dependent down-regulation of surface molecules occurred in IFN-MO in addition to cell death.

Short IFN-α priming prompts long-standing maintenance of monocytes in a maturation state

It is known that in vitro-generated mature DCs maintain expression of costimulatory molecules and CD83 even after withdrawal of GM-CSF + IL-4. We therefore tested whether pulsed exposures to IFN-α also trigger long-lasting expression of these proteins. As shown in Fig. 3*B*, the challenge with IFN-α for 24, 48, or 72 h induced similar increases in the number of cells expressing CD80, CD86, CD83, and HLA-DR on day 3. Although LPS exposure did not modify the expression of DC maturation markers when added on day 6 to IFN-MO (Fig. 3*A*), we wondered whether earlier LPS stimulation (i.e., on day 3, after 48 h treatment of IFN-α) could

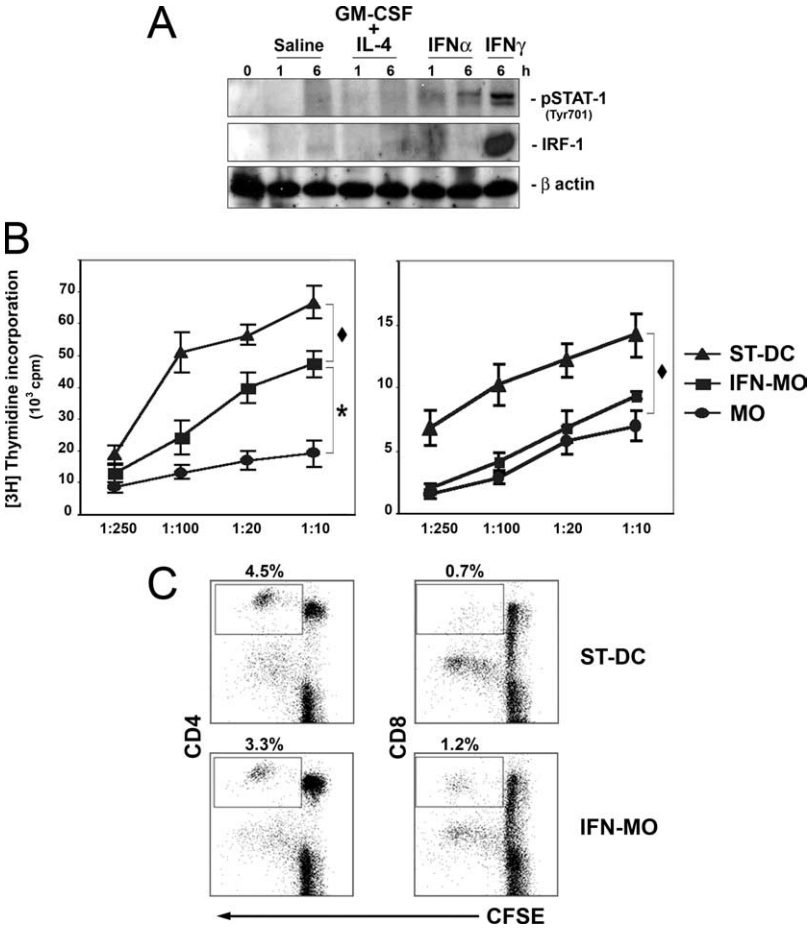

FIGURE 5. IFN-α enhances the stimulatory activity of monocytes and induces STAT-1 activation. *A*, STAT-1 phosphorylation and IRF-1 expression do not occur in freshly isolated (0) or saline solution- or GM-CSF + IL-4-treated monocytes. STAT-1 phosphorylation is detectable in IFN-α-treated monocytes at 1 and 6 h, while IRF-1 expression is not induced by IFN-α. IFN-γ triggers STAT-1 phosphorylation and strong expression of IRF-1 (used as positive control; β-actin as loading control). One blot representative of two separate experiments is shown. *B*, MLRs for ST-DC, IFN-MO, and monocytes (MO) performed with PBMC (*left panel*) or with naive $CD4^+$ T cells (*right panel*) as responders. Each point represents mean $\pm$ SD of a representative experiment conducted in triplicate. $\blacklozenge$ and $*$, Statistically significant difference ($p < 0.05$) between IFN-MO vs ST-DC and between IFN-MO vs MO, respectively. *C*, Cells were stained with anti-CD4 and anti-CD8 after coculturing with CFSE-labeled allogeneic CD14-depleted PBMC. CFSE fluorescence decrease was measured using flow cytometry, and proliferating cells were expressed as percentage of total cells. One representative experiment out of eight is shown.

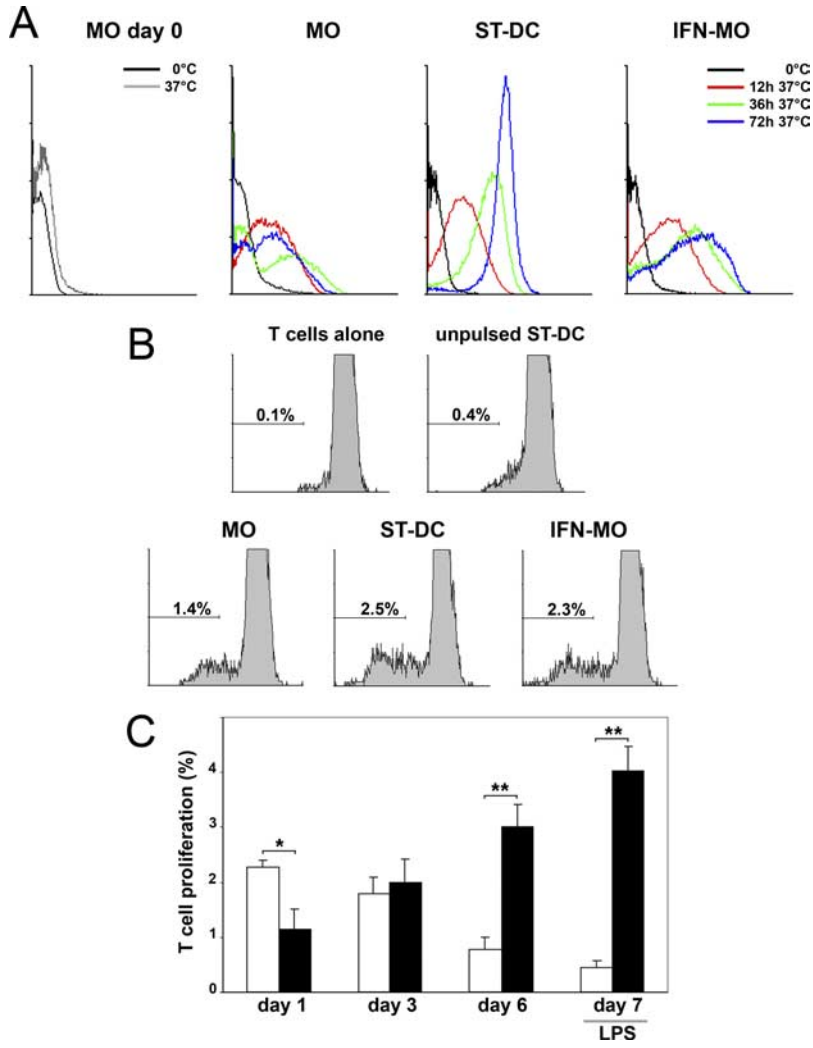

FIGURE 6. IFN-MO are able to uptake, process, and present exogenous Ags. *A*, Cells were challenged with FITC-dextran at 0°C and 37°C for 45 min at the time points indicated. Cells were then rinsed extensively in PBS and analyzed by flow cytometry. One representative experiment out of three is shown. *B* and *C*, APC were pulsed with tetanus toxoid (1 μg/ml) overnight at different time points of the culture. Cells were then cocultured with CFSE- labeled $CD4^+$ T cells for 5 days. Afterwards, cells were analyzed by flow cytometry, and results are expressed as percentage of proliferating T cells. *B*, To determine background staining and unspecific proliferation, T cells alone and T cells stimulated by unpulsed ST-DC were also analyzed. *B* refers to day 3. One representative experiment out of three is shown. *C*, T cell proliferation induced by IFN-MO (□) and ST-DC (■) at different days of cultures. Bars represent the means ± SEM of three independent experiments. * and **, Statistically significant difference ($p < 0.05$ and $p < 0.01$, respectively) between IFN-MO vs ST-DC.

modify the immunophenotype. Data shown in Fig. 3*B* demonstrate that earlier LPS stimulation significantly increased CD83 but not expression of costimulatory molecules.

IFN-α up-regulates adhesion molecules on monocytes

ST-DC undergoing in vitro maturation start to form clusters on days 3 and 4 thanks to the expression of several adhesion molecules. This is considered an important step toward the maturation stage (3). Hence, expression of integrins CD11a, CD11b, and CD11c as well as of ICAM-1 and ICAM-3 was examined on IFN-MO. Compared with freshly isolated monocytes, a significant MFI increase for CD11a (from 321.1 ± 85.2 to 581.7 ± 116.3; $p < 0.05$), ICAM-1 (from 62.7 ± 18.3 to 617.2 ± 173.2; $p < 0.01$), and ICAM-3 (from 396.8 ± 164.7 to 785.2 ± 140.2; $p < 0.05$) was observed in IFN-MO after 24 h incubation (Fig. 4*A*). Expression of CD11b and CD11c were not significantly increased (data not shown).

Morphology

We then analyzed possible morphological differences between ST-DC and IFN-MO during the in vitro maturation process (7 days). On days 1 and 2, most ST-DC were adherent to dishes (Fig. 4*B*), while on day 3, cells floating and clustered were observed (data not shown). Size and dendriticity of ST-DC gradually increased until day 7, indicated by the forward scatter (FSC) and side scatter (SSC) features, respectively (Fig. 4*C*). ST-DC showed a well-differentiated phenotype with long, multiple plasma membrane processes and a diameter of 12–15 μm (Fig. 4*D*). Consistent with expression of adhesion molecules, as early as 24 h after addition of IFN-α, most IFN-MO were in suspension joined in clusters on day 1 (Fig. 4*B*). At variance with ST-DC, no change in cell size and dendriticity could be observed throughout the 7 days (Fig. 4*C*). IFN-MO were smaller (9–11 μm in diameter) than ST-DC with only few, coarse membrane protrusions (Fig. 4*D*).

IFN-α triggers STAT-1 activation but not IRF-1 expression in cultured monocytes

JAK kinase-dependent STAT-1 transcription factor activation is central in IFN class I and II signaling. We therefore evaluated the effect of IFN-α or GM-CSF + IL-4 on STAT-1 phosphorylation. As shown in Fig. 5*A*, neither freshly isolated monocytes nor those maintained in saline solution for different times showed appreciable STAT-1 phosphorylation. Similarly, STAT-1 phosphorylation levels in monocytes exposed to GM-CSF + IL-4 were below detection limit. Conversely, in monocytes treated with IFN-α, STAT-1 phosphorylation occurred 30 min after exposure (data not shown) and was further augmented at 1 and 6 h (Fig. 5*A*); notably, STAT-1 phosphorylation was still present at 12 h (not shown). We next sought to determine whether IFN-α triggered expression of IRF-1, a transcription factor typically involved in IFN signaling downstream to STAT-1 phosphorylation. Consistent with lack of STAT-1 activation, IRF-1 was not expressed in saline- or GM-CSF +

IL-4-stimulated monocytes. Unexpectedly, however, IRF-1 expression was not detected even in cells treated with IFN-α for 6 and 12 h (Fig. 5*A* and data not shown). STAT-1 phosphorylation as well as IRF-1 induction occurred in monocytes exposed to IFN-γ, which was used as positive control (Fig. 5*A*).

IFN-α increases the stimulatory activity of monocytes

A hallmark of DCs is their ability to induce a strong proliferation of allogeneic T cells in a MLR. We therefore assessed in a MLR the stimulatory ability of IFN-MO in comparison to ST-DC and untreated monocytes using PBMC as responders. Remarkably, T cell proliferation triggered by IFN-MO was higher than that prompted by untreated monocytes, but lower than that triggered by ST-DC (Fig. 5*B*, *left panel*).

Next, we analyzed the ability of IFN-MO to stimulate proliferation of naive T cells, a unique ability of professional APCs. Of note, we found that only ST-DC induced significant cell proliferation, while that prompted by IFN-MO did not differ from that induced by untreated monocytes (Fig. 5*B*, *right panel*).

Although $CD4^+$ T cells are the predominant proliferating cells in MLR, IFN-α reportedly promotes DC-dependent $CD8^+$ T cell proliferation (24). We therefore analyzed the ability of IFN-MO and ST-DC to induce proliferation of $CD8^+$ or $CD4^+$ allogeneic T cell populations. Phenotype analysis of proliferating T cells revealed that ST-DC were more efficient than IFN-MO in inducing $CD4^+$ T cell proliferation (3.8 $\pm$ 1.1% and 2.5 $\pm$ 0.8%, respectively; $p < 0.05$) (Fig. 5*C*), whereas IFN-MO and ST-DC triggered equal proliferation of $CD8^+$ T cells (0.7 $\pm$ 0.5% and 0.6 $\pm$ 0.4%, respectively; $p = 0.66$) (Fig. 5*C*).

Ag uptake, processing, and presentation by IFN-α-treated monocytes

Next, we investigated whether IFN-MO were able to uptake, process, and present exogenous Ags to T cells. To this aim, we first analyzed their phagocytic activity by means of the FITC-dextran assay. Freshly isolated monocytes were not able to phagocytose (MFI = 3 $\pm$ 0.4; Fig. 6*A*), while phagocytic activity in cultured monocytes developed after 12 h and remained constant throughout the 3 days in culture (MFI = 6 $\pm$ 3.3, 5.7 $\pm$ 3.4, and 5.4 $\pm$ 3.2 at 12, 36, and 72 h, respectively; Fig. 6*A*). On the contrary, IFN-MO showed a more pronounced activity at 12 h, which increased until day 3 (MFI = 7.9 $\pm$ 3.3, 12.6 $\pm$ 5.6, and 18.6 $\pm$ 4.9 at 12, 36, and 72 h, respectively; $p < 0.01$; Fig. 6*A*). Phagocytic activity of ST-DC was even higher (MFI = 7.7 $\pm$ 0.9, 21.5 $\pm$ 0.8, and 35 $\pm$ 7 at 12, 36, and 72 h, respectively; $p < 0.001$; Fig. 6*A*). The phagocytic activity of IFN-MO decreased on day 6 and even more on day 7, after LPS addition, because of the high cell death rate (data not shown). As expected, LPS-treated ST-DC showed very low phagocytic activity (data not shown).

Then, we examined the ability of IFN-MO to process and present exogenous Ags to autologous T cells using tetanus toxoid, a common recall Ag. As shown in Fig. 6*B*, at 3 days IFN-MO and ST-DC were equally effective in inducing T cell proliferation and were more effective than untreated monocytes. Given that ST-DC and IFN-MO exhibited an opposite expression kinetic in terms of maturation marker and costimulatory molecules (Fig. 3*A*), we speculated that also the ability to stimulate T cells had different kinetics during a 7-day culture. Consistent with the immunophenotype profile, IFN-MO showed the highest stimulation activity on days 1 (2.3 $\pm$ 0.2%) and 3 (1.8 $\pm$ 0.6%), which then decreased significantly (0.8 $\pm$ 0.4% on day 6 and 0.4 $\pm$ 0.2% on day 7; $p <$ 0.05), while ST-DC were characterized by a low stimulation ability on days 1 (1.1 $\pm$ 0.6%) and 3 (2 $\pm$ 0.7%), which then increased significantly until day 7 after LPS addition (3 $\pm$ 0.7% on day 6 and 4 $\pm$ 0.9% on day 7; $p < 0.05$) (Fig. 6*C*).

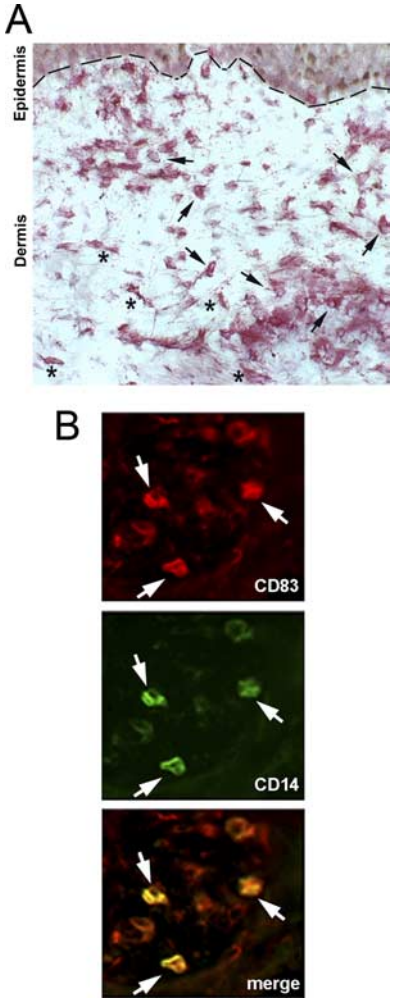

FIGURE 7. Detection of IFN-α proteins and $CD83^+CD14^+$ cells in varicella skin lesions. *A*, Representative cryostat serial sections of varicella skin lesions were stained with anti-IFN-α (MMHA-2) according to the APAAP (alkaline phosphatase anti- alkaline phosphatase) technique. Positive cells with a plasmacytoid, round shape morphology are indicated by arrows, while asterisks indicate the fibroblast- and macrophage-like ones (original magnification $\times$200). *B*, Sections of varicella skin lesions were double stained with anti CD83-PE and CD14-FITC and analyzed by fluorescence microscopy (original magnification $\times$400).

In vivo detection of $CD83^+CD14^+$ cells

To address the possible relevance of these findings in vivo, we analyzed two clinical settings where the presence of IFN-α might generate $CD83^+CD14^+$ cells: 1) PBMC from patients under IFN-α therapy, and 2) varicella skin lesions. FACS analysis of PBMC from melanoma patients under both low- and high-dose IFN-α therapy did not detect $CD83^+CD14^+$ cells (not shown). In varicella skin lesions there is a massive recruitment of pDCs producing a high amount of IFN-α/β as shown by the strong MxA staining (34). MxA is an IFN-α/β-inducible intracellular protein, well established as a surrogate marker for local type I IFN production (34–36). Herein, we analyzed the presence of IFN-α in varicella skin lesion sections by immunohistochemistry. A high number of IFN-α^+ cells were identified in the upper dermis (Fig. 7*A*). Consistently, several of these cells exhibited a plasmacytoid, round-shape morphology, whereas others showed a fibroblast- and

macrophage-like appearance (Fig. 7*A*). To identify the possible presence of $CD83^+CD14^+$ cells, sections of varicella skin lesions were double stained with mAb anti-CD83 and anti-CD14 and analyzed by fluorescence microscopy. Strikingly, coexpression of CD83 and CD14 was observed on numerous large, round cells localized in the dermis, in close proximity to IFN-α^+ cells (Fig. 7*B*).

Discussion

Several papers have demonstrated that type I IFNs, when added together with other cytokines, influence the DC compartment both in vitro (21, 23–29, 37) and in vivo (37, 38). Herein, we extended these observations by showing that in the absence of other cytokines, IFN-α per se prompts immunophenotypical and functional monocyte differentiation into nondendritic mature APCs. Indeed, upon IFN-α exposure, monocytes quickly differentiate into cells expressing CD80, CD86, HLA-DR, and CD83, a prototypical phenotype of mature DCs. Thus, IFN-α not only regulates DC differentiation triggered by other cytokines, but it suffices to prompt monocyte differentiation into functional APCs. As circulating DCs are estimated to be <1% of total PBMC, whereas monocytes are up to 10% of total PBMC, we reason that the effect of IFN-α on monocytes might be of pathophysiological relevance. To our knowledge, this is the first evidence that IFN-α suffices to differentiate monocytes into $CD83^+CD14^+$ nondendritic APCs.

We report herein that, in keeping with up-regulation of costimulatory molecules, the T cell stimulatory activity of IFN-MO was increased compared with untreated monocytes in MLRs using bulk PBMC as responders. However, given that IFN-MO did not stimulate adequate proliferation of naive $CD4^+$ T cells (a property that identifies professional APCs such as DCs), our data suggest that IFN-MO are not able to induce primary immune responses (1, 2). Consistently, it has been shown that the increased serum levels of IFN-α in patients affected by systemic lupus erythematodes (SLE) are responsible for the increased ability of their monocytes to induce strong MLR compared with monocytes from healthy donors (39). In apparent contrast with our observation, however, monocytes treated with SLE serum are able to induce proliferation of naive $CD4^+$ T cells and develop DC morphology (39). This discrepancy may be due to the numerous cytokines (i.e., GM-CSF) present in the SLE serum. Under our experimental settings, however, the possibility that exposure of monocytes to IFN-α resulted in the production of GM-CSF is unlikely, because this would have generated cells with a dendritic morphology (25–27).

Of note, we report herein that IFN-MO and ST-DC were equally effective in the uptake, processing, and presentation of tetanus toxoid to memory autologous $CD4^+$ T cells on day 3. Yet, the present evidence that memory $CD4^+$ T cell proliferation prompted by IFN-MO or DCs showed different temporal kinetics (Fig. 6*C*) points to specific roles of IFN-MO during Ag presentation to memory cells in vivo. It is tempting to speculate that IFN-MO play a key role in rapidly activating memory T cells in vivo, a crucial function in infective diseases.

The present finding that IFN-MO coexpress CD14 and CD83 is original and of importance. Indeed, expression of CD83, a prototypical maturation hallmark, is normally paralleled by complete down-regulation of CD14 in ST-DC. Thus, our finding along with evidence that DCs generated in the presence of GM-CSF + IL-4 plus IFN-α coexpress CD83 and CD14 (40) suggest that type I IFN allows monocyte maturation in the presence of CD14, and that down-regulation of CD14 is not a prerequisite for expression of maturation markers. Additionally, evidence that IFN-MO, at variance with ST-DC, coexpress CD14 with costimulatory molecules hints that these cells have specific immunocompetence and immunological roles. Similarly, while the expression of CD83 by ST-DC is paralleled by a drastic reduction of the phagocytic activity, $CD83^+$ IFN-MO showed an increased phagocytic activity until day 3. Later on, the high cell death rate is responsible for the observed reduction of phagocytosis.

In hematological malignancies IFN-α proved its efficacy by prolonging the survival of cancer patients and, at least for chronic myeloid leukemia, induced a significant clinical remission (41, 42). In the presence of tumors, IFN-MO could present tumor-derived Ags in LNs, generating (or boosting) tumor-specific CTLs. Consistent with this view, Molldrem et al. reported the presence of functional tumor-specific CTLs in chronic myeloid leukemia patients in cytogenetic remission after IFN-α treatment but not in IFN-resistant patients (43). These data are in line with the reported increased capability shown by IFN-α-treated DCs to stimulate $CD8^+$ effector T cells (24). In our study, IFN-MO and ST-DC induced a similar level of proliferation of $CD8^+$ T cells in a MLR. However, in three out of eight donors, IFN-MO induced a significantly stronger $CD8^+$ T cell proliferation than ST-DC (data not shown), suggesting that IFN-induced $CD8^+$ T cell proliferation may be donor-dependent.

We report that the acquisition of a mature phenotype by IFN-MO was dependent on the concentration of IFN-α. Ten units per milliliter was sufficient to induce a significant expression of CD80, CD86, and CD83, which gradually increased, reaching the highest levels at 10^3 to 10^4 U/ml and decreased at 10^5 U/ml. The decreased expression at 10^5 U/ml could be due to IFN-α toxicity, as indicated by the increased incorporation of the cytotoxicity marker 7-AAD (see *Results*). Upon i.v. injection of IFN-α, serum levels can reach $5–6 \times 10^2$ U/ml (44). We report herein that monocytes exposed to these concentrations expressed high levels of costimulatory molecules, HLA-DR and CD83, but also underwent cell death. Interestingly, in solid tumors such as melanoma, a significant prolongation of both relapse-free survival and overall survival induced by IFN-α treatment occurs only with a high-dose regimen and associates to several toxic effects (18, 45). Collectively, these results suggest that upon IFN-α therapy, the high cytokine concentrations in blood and/or tissues generate large numbers of mature APCs, thereby supporting efficient antitumoral immune response and positive clinical outcome, but they also trigger cytotoxicity, which underlies the frequent toxic side effects.

Recently, it has been shown that CD83 plays a fundamental role both in DC maturation and DC-mediated T cell proliferation (46, 47). Of note, viruses can affect DC function by down-regulating CD83 expression (48, 49), thus escaping the immune system (50). In this regard, the rapid IFN-α production by pDCs in response to viruses could generate a high number of $CD83^+$ IFN-MO, which may represent a valid defense mechanism against viral infection. In good agreement with this hypothesis, we report that cells coexpressing CD14 and CD83 are present in the upper dermis of varicella skin lesions (Fig. 7*B*), where numerous IFN-α-producing pDCs are present (Fig. 7*A*) (34). Dermal $CD14^+$ cells are present in normal skin (51–54) and represent a pool of target cells that can rapidly differentiate into $CD83^+$ APC upon IFN-α exposure. Evidence for $CD14^+CD83^+$ cells in varicella skin lesions strengthens the clinical relevance of our findings. Although it is likely that also circulating monocytes during IFN-α therapy may differentiate into $CD83^+$ APCs, we failed to detect them. A possible explanation is that $CD83^+$ IFN-MO may leave the bloodstream and enter the lymph nodes as described for pDCs (11). This process is mediated by CD62L (L-selectin) (55) and by CCR7 (3), a molecule mediating LN homing. Interestingly, CD62L is constitutionally expressed by monocytes (56), and CCR7 is induced by IFN-α (as shown in Fig. 2*B*) and in monocyte-derived DCs cultured in the

presence of IFN-α (27). It is possible therefore that additional factors including adhesion molecules, which we found up-regulated on IFN-MO, may play a role in monocytes homing in LNs. In line with this hypothesis, the number of peripheral blood monocytes decreases while serum neopterin (a monocyte activation marker) increases upon IFN-α injection (31). Notably, the "maturing" effect of IFN-α we report herein was rapid, and 24 h priming was sufficient to maintain the mature phenotype for 3 days. On these bases, it is tempting to speculate that IFN-α-dependent induction of $CD14^+CD83^+$ monocytes may represent a fast, long-lasting response to pathogens. In addition to viruses, bacterial components such as LPS may further enhance the maturation of monocytes. Indeed, LPS was able to increase CD83 expression if added to the culture at an early differentiation stage (Fig. 3*B*). As for the signaling pathway(s) through which IFN-α triggers expression of costimulatory molecules, we report that STAT-1 transcription factor was activated in monocytes exposed to IFN-α. Remarkably, STAT-1 binds the 5′-regulatory regions of the human CD86 gene (57) and promotes expression of costimulatory molecules (58). These findings suggest that STAT-1 activation underpins, at least in part, IFN-α-dependent monocyte differentiation into APCs.

In conclusion, this study originally shows that exposure to IFN-α alone promptly generates a large pool of nondendritic mature APCs from monocytic precursors. The present results may be of relevance to viral infections as well as IFN-α therapy, and they represent a novel mechanism of action of IFN-α that could be exploited for therapeutic strategies.

Disclosures

The authors have no financial conflicts of interest.

References

1. Cella, M., F. Sallusto, and A. Lanzavecchia. 1997. Origin, maturation and antigen presenting function of dendritic cells. *Curr. Opin. Immunol.* 9: 10–16.
2. Banchereau, J., and R. M. Steinman. 1998. Dendritic cells and the control of immunity. *Nature* 392: 245–252.
3. Banchereau, J., F. Briere, C. Caux, J. Davoust, S. Lebecque, Y. J. Liu, B. Pulendran, and K. Palucka. 2000. Immunobiology of dendritic cells. *Annu. Rev. Immunol.* 18: 767–811.
4. Zhou, L. J., and T. F. Tedder. 1995. Human blood dendritic cells selectively express CD83, a member of the immunoglobulin superfamily. *J. Immunol.* 154: 3821–3835.
5. Sallusto, F., and A. Lanzavecchia. 1994. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. *J. Exp. Med.* 179: 1109–1118.
6. Romani, N., D. Reider, M. Heuer, S. Ebner, E. Kampgen, B. Eibl, D. Niederwieser, and G. Schuler. 1996. Generation of mature dendritic cells from human blood: an improved method with special regard to clinical applicability. *J. Immunol. Methods* 196: 137–151.
7. Timmerman, J. M., and R. Levy. 1999. Dendritic cell vaccines for cancer immunotherapy. *Annu. Rev. Med.* 50: 507–529.
8. Fong, L., and E. G. Engleman. 2000. Dendritic cells in cancer immunotherapy. *Annu. Rev. Immunol.* 18: 245–273.
9. Nestle, F. O., S. Alijagic, M. Gilliet, Y. Sun, S. Grabbe, R. Dummer, G. Burg, and D. Schadendorf. 1998. Vaccination of melanoma patients with peptide- or tumor lysate-pulsed dendritic cells. *Nat. Med.* 4: 328–332.
10. Hsu, F. J., C. Benike, F. Fagnoni, T. M. Liles, D. Czerwinski, B. Taidi, E. G. Engleman, and R. Levy. 1996. Vaccination of patients with B-cell lymphoma using autologous antigen-pulsed dendritic cells. *Nat. Med.* 2: 52–58.
11. Cella, M., D. Jarrossay, F. Facchetti, O. Alebardi, H. Nakajima, A. Lanzavecchia, and M. Colonna. 1999. Plasmacytoid monocytes migrate to inflamed lymph nodes and produce large amounts of type I interferon. *Nat. Med.* 5: 919–923.
12. Siegal, F. P., N. Kadowaki, M. Shodell, P. A. Fitzgerald-Bocarsly, K. Shah, S. Ho, S. Antonenko, and Y. J. Liu. 1999. The nature of the principal type 1 interferon-producing cells in human blood. *Science* 284: 1835–1837.
13. Ferbas, J. J., J. F. Toso, A. J. Logar, J. S. Navratil, and C. R. Rinaldo, Jr. 1994. $CD4^+$ blood dendritic cells are potent producers of IFN-alpha in response to in vitro HIV-1 infection. *J. Immunol.* 152: 4649–4662.
14. Corssmit, E. P., J. de Metz, H. P. Sauerwein, and J. A. Romijn. 2000. Biologic responses to IFN-α administration in humans. *J. Interferon Cytokine Res.* 20: 1039–1047.
15. Belardelli, F., and M. Ferrantini. 2002. Cytokines as a link between innate and adaptive antitumor immunity. *Trends Immunol.* 23: 201–208.
16. Schalm, S., R. De Man, and H. Janssen. 2002. Combination and newer therapies for chronic hepatitis B. *J. Gastroenterol. Hepatol.* 17 (*Suppl.* 3): S338–S341.
17. Luxon, B. A., M. Grace, D. Brassard, and R. Bordens. 2002. Pegylated interferons for the treatment of chronic hepatitis C infection. *Clin. Ther.* 24: 1363–1383.
18. Kirkwood, J. 2002. Cancer immunotherapy: the interferon-α experience. *Semin. Oncol.* 29: 18–26.
19. Agarwala, S. S., and J. M. Kirkwood. 2002. Update on adjuvant interferon therapy for high-risk melanoma. *Oncology* 16: 1177–1187.
20. Olsen, E. A. 2003. Interferon in the treatment of cutaneous T-cell lymphoma. *Dermatol. Ther.* 16: 311–321.
21. Radvanyi, L. G., A. Banerjee, M. Weir, and H. Messner. 1999. Low levels of interferon-alpha induce CD86 (B7.2) expression and accelerates dendritic cell maturation from human peripheral blood mononuclear cells. *Scand. J. Immunol.* 50: 499–509.
22. McRae, B. L., T. Nagai, R. T. Semnani, J. M. van Seventer, and G. A. van Seventer. 2000. Interferon-α and -β inhibit the in vitro differentiation of immunocompetent human dendritic cells from $CD14^+$ precursors. *Blood* 96: 210–217.
23. Luft, T., K. C. Pang, E. Thomas, P. Hertzog, D. N. Hart, J. Trapani, and J. Cebon. 1998. Type I IFNs enhance the terminal differentiation of dendritic cells. *J. Immunol.* 161: 1947–1953.
24. Padovan, E., G. C. Spagnoli, M. Ferrantini, and M. Heberer. 2002. IFN-α2a induces IP-10/CXCL10 and MIG/CXCL9 production in monocyte-derived dendritic cells and enhances their capacity to attract and stimulate $CD8^+$ effector T cells. *J. Leukocyte Biol.* 71: 669–676.
25. Santini, S. M., C. Lapenta, M. Logozzi, S. Parlato, M. Spada, T. Di Pucchio, and F. Belardelli. 2000. Type I interferon as a powerful adjuvant for monocyte-derived dendritic cell development and activity in vitro and in Hu-PBL-SCID mice. *J. Exp. Med.* 191: 1777–1788.
26. Paquette, R. L., N. C. Hsu, S. M. Kiertscher, A. N. Park, L. Tran, M. D. Roth, and J. A. Glaspy. 1998. Interferon-alpha and granulocyte-macrophage colony-stimulating factor differentiate peripheral blood monocytes into potent antigen-presenting cells. *J. Leukocyte Biol.* 64: 358–367.
27. Parlato, S., S. M. Santini, C. Lapenta, T. Di Pucchio, M. Logozzi, M. Spada, A. M. Giammarioli, W. Malorni, S. Fais, and F. Belardelli. 2001. Expression of CCR-7, MIP-3β, and Th-1 chemokines in type I IFN-induced monocyte-derived dendritic cells: importance for the rapid acquisition of potent migratory and functional activities. *Blood* 98: 3022–3029.
28. McRae, B. L., R. T. Semnani, M. P. Hayes, and G. A. van Seventer. 1998. Type I IFNs inhibit human dendritic cell IL-12 production and Th1 cell development. *J. Immunol.* 160: 4298–4304.
29. Lehner, M., T. Felzmann, K. Clodi, and W. Holter. 2001. Type I interferons in combination with bacterial stimuli induce apoptosis of monocyte-derived dendritic cells. *Blood* 98: 736–742.
30. Bukowski, R. M., C. Tendler, D. Cutler, E. Rose, M. M. Laughlin, and P. Statkevich. 2002. Treating cancer with PEG Intron: pharmacokinetic profile and dosing guidelines for an improved interferon-α-2b formulation. *Cancer* 95: 389–396.
31. Corssmit, E. P., R. Heijligenberg, C. E. Hack, E. Endert, H. P. Sauerwein, and J. A. Romijn. 1997. Effects of interferon-alpha (IFN-alpha) administration on leucocytes in healthy humans. *Clin. Exp. Immunol.* 107: 359–363.
32. Cella, M., F. Facchetti, A. Lanzavecchia, and M. Colonna. 2000. Plasmacytoid dendritic cells activated by influenza virus and CD40L drive a potent TH1 polarization. *Nat. Immunol.* 1: 305–310.
33. Grunfeld, C., D. P. Kotler, J. K. Shigenaga, W. Doerrler, A. Tierney, J. Wang, R. N. Pierson, Jr., and K. R. Feingold. 1991. Circulating interferon-alpha levels and hypertriglyceridemia in the acquired immunodeficiency syndrome. *Am. J. Med.* 90: 154–162.
34. Gerlini, G., G. Mariotti, B. Bianchi, and N. Pimpinelli. 2006. Massive recruitment of type I interferon producing plasmacytoid dendritic cells in varicella skin lesions. *J. Invest. Dermatol.* 126: 507–509.
35. Fah, J., J. Pavlovic, and G. Burg. 1995. Expression of MxA protein in inflammatory dermatoses. *J. Histochem. Cytochem.* 43: 47–52.
36. Jahnsen, F. L., F. Lund-Johansen, J. F. Dunne, L. Farkas, R. Haye, and P. Brandtzaeg. 2000. Experimentally induced recruitment of plasmacytoid ($CD123^{high}$) dendritic cells in human nasal allergy. *J. Immunol.* 165: 4062–4068.
37. Ito, T., R. Amakawa, M. Inaba, S. Ikehara, K. Inaba, and S. Fukuhara. 2001. Differential regulation of human blood dendritic cell subsets by IFNs. *J. Immunol.* 166: 2961–2969.
38. Paquette, R. L., N. Hsu, J. Said, M. Mohammed, N. P. Rao, G. Shih, G. Schiller, C. Sawyers, and J. A. Glaspy. 2002. Interferon-α induces dendritic cell differentiation of CML mononuclear cells in vitro and in vivo. *Leukemia* 16: 1484–1489.
39. Blanco, P., A. K. Palucka, M. Gill, V. Pascual, and J. Banchereau. 2001. Induction of dendritic cell differentiation by IFN-α in systemic lupus erythematosus. *Science* 294: 1540–1543.
40. Di Pucchio, T., C. Lapenta, S. M. Santini, M. Logozzi, S. Parlato, and F. Belardelli. 2003. $CD2^+/CD14^+$ monocytes rapidly differentiate into $CD83^+$ dendritic cells. *Eur. J. Immunol.* 33: 358–367.
41. Ozer, H., S. L. George, C. A. Schiffer, K. Rao, P. N. Rao, D. H. Wurster-Hill, D. D. Arthur, B. Powell, A. Gottlieb, B. A. Peterson, et al. 1993. Prolonged subcutaneous administration of recombinant alpha 2b interferon in patients with previously untreated Philadelphia chromosome-positive chronic-phase chronic myelogenous leukemia: effect on remission duration and survival: Cancer and Leukemia Group B study 8583. *Blood* 82: 2975–2984.
42. Sawyers, C. L. 1999. Chronic myeloid leukemia. *N. Engl. J. Med.* 340: 1330–1340.
43. Molldrem, J. J., P. P. Lee, S. Kant, E. Wieder, W. Jiang, S. Lu, C. Wang, and M. M. Davis. 2003. Chronic myelogenous leukemia shapes host immunity by

selective deletion of high-avidity leukemia-specific T cells. *J. Clin. Invest.* 111: 639–647.
44. Wetzler, M., H. Kantarjian, R. Kurzrock, and M. Talpaz. 1995. Interferon-alpha therapy for chronic myelogenous leukemia. *Am. J. Med.* 99: 402–411.
45. Kirkwood, J. M., M. H. Strawderman, M. S. Ernstoff, T. J. Smith, E. C. Borden, and R. H. Blum. 1996. Interferon alfa-2b adjuvant therapy of high-risk resected cutaneous melanoma: the Eastern Cooperative Oncology Group Trial EST 1684. *J. Clin. Oncol.* 14: 7–17.
46. Fujimoto, Y., L. Tu, A. S. Miller, C. Bock, M. Fujimoto, C. Doyle, D. A. Steeber, and T. F. Tedder. 2002. CD83 expression influences $CD4^+$ T cell development in the thymus. *Cell* 108: 755–767.
47. Lechmann, M., D. J. Krooshoop, D. Dudziak, E. Kremmer, C. Kuhnt, C. G. Figdor, G. Schuler, and A. Steinkasserer. 2001. The extracellular domain of CD83 inhibits dendritic cell-mediated T cell stimulation and binds to a ligand on dendritic cells. *J. Exp. Med.* 194: 1813–1821.
48. Kruse, M., O. Rosorius, F. Kratzer, G. Stelz, C. Kuhnt, G. Schuler, J. Hauber, and A. Steinkasserer. 2000. Mature dendritic cells infected with herpes simplex virus type 1 exhibit inhibited T-cell stimulatory capacity. *J. Virol.* 74: 7127–7136.
49. Arrode, G., C. Boccaccio, J. P. Abastado, and C. Davrinche. 2002. Cross-presentation of human cytomegalovirus pp65 (UL83) to $CD8^+$ T cells is regulated by virus-induced, soluble-mediator-dependent maturation of dendritic cells. *J. Virol.* 76: 142–150.
50. Lechmann, M., S. Berchtold, J. Hauber, and A. Steinkasserer. 2002. CD83 on dendritic cells: more than just a marker for maturation. *Trends Immunol.* 23: 273–275.
51. Nestle, F. O., X. G. Zheng, C. B. Thompson, L. A. Turka, and B. J. Nickoloff. 1993. Characterization of dermal dendritic cells obtained from normal human skin reveals phenotypic and functionally distinctive subsets. *J. Immunol.* 151: 6535–6545.
52. Gerlini, G., H. P. Hefti, M. Kleinhans, B. J. Nickoloff, G. Burg, and F. O. Nestle. 2001. CD1d is expressed on dermal dendritic cells and monocyte-derived dendritic cells. *J. Invest. Dermatol.* 117: 576–582.
53. Larregina, A. T., A. E. Morelli, L. A. Spencer, A. J. Logar, S. C. Watkins, A. W. Thomson, and L. D. Falo, Jr. 2001. Dermal-resident $CD14^+$ cells differentiate into Langerhans cells. *Nat. Immunol.* 2: 1151–1158.
54. Meunier, L., A. Gonzalez-Ramos, and K. D. Cooper. 1993. Heterogeneous populations of class II MHC^+ cells in human dermal cell suspensions. Identification of a small subset responsible for potent dermal antigen-presenting cell activity with features analogous to Langerhans cells. *J. Immunol.* 151: 4067–4080.
55. Girard, J. P., and T. A. Springer. 1995. High endothelial venules (HEVs): specialized endothelium for lymphocyte migration. *Immunol. Today* 16: 449–457.
56. Giuffre, L., A. S. Cordey, N. Monai, Y. Tardy, M. Schapira, and O. Spertini. 1997. Monocyte adhesion to activated aortic endothelium: role of L-selectin and heparan sulfate proteoglycans. *J. Cell Biol.* 136: 945–956.
57. Li, J., A. I. Colovai, R. Cortesini, and N. Suciu-Foca. 2000. Cloning and functional characterization of the 5′-regulatory region of the human CD86 gene. *Hum. Immunol.* 61: 486–498.
58. Tomic, J., D. White, Y. Shi, J. Mena, C. Hammond, L. He, R. L. Miller, and D. E. Spaner. 2006. Sensitization of IL-2 signaling through TLR-7 enhances B lymphoma cell immunogenicity. *J. Immunol.* 176: 3830–3839.