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Mature Dendritic Cells Derived from Human Monocytes Within 48 Hours: A Novel Strategy for Dendritic Cell Differentiation from Blood Precursors

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It is widely believed that generation of mature dendritic cells (DCs) with full T cell stimulatory capacity from human monocytes in vitro requires 5–7 days of differentiation with GM-CSF and IL-4, followed by 2–3 days of activation. Here, we report a new strategy for differentiation and maturation of monocyte-derived DCs within only 48 h of in vitro culture. Monocytes acquire immature DC characteristics by day 2 of culture with GM-CSF and IL-4; they down-regulate CD14, increase dextran uptake, and respond to the inflammatory chemokine macrophage inflammatory protein-1α. To accelerate DC development and maturation, monocytes were incubated for 24 h with GM-CSF and IL-4, followed by activation with proinflammatory mediators for another 24 h (FastDC). FastDC expressed mature DC surface markers as well as chemokine receptor 7 and secreted IL-12 (p70) upon CD40 ligation in the presence of IFN-γ. The increase in intracellular calcium in response to 6Ckine showed that chemokine receptor 7 expression was functional. When FastDC were compared with mature monocyte-derived DCs generated by a standard 7-day protocol, they were equally potent in inducing Ag-specific T cell proliferation and IFN-γ production as well as in priming autologous naive T cells using tetanus toxoid as a model Ag. These findings indicate that FastDC are as effective as monocyte-derived DCs in stimulating primary, Ag-specific, Th 1-type immune responses. Generation of FastDC not only reduces labor, cost, and time required for in vitro DC development, but may also represent a model more closely resembling DC differentiation from monocytes in vivo. The Journal of Immunology, 2003, 170: 4069–4076.

Dendritic cells (DCs) are highly specialized APC with the unique capacity to establish and control primary immune responses. DCs reside in peripheral tissues in an immature state where they capture and process Ag for presentation in the context of MHC molecules. Ligation of receptors for inflammatory chemokines recruits immature DCs and their blood precursors to sites of inflammation or infection. Upon encounter with microbial, proinflammatory, or T cell-derived stimuli, characteristic phenotypic and functional changes are induced, a process referred to as maturation of DCs. Mature DCs exhibit reduced phagocytic activity and increased expression of MHC and costimulatory molecules, and secrete large amounts of immunostimulatory cytokines. Maturing DCs also change their pattern of chemokine receptor expression, rendering them sensitive to lymphoid chemokines. Thereby, mature DCs acquire the capacity to migrate to the T cell areas of draining secondary lymphoid organs, where they encounter naive T cells and initiate an adaptive immune response.

Human DCs originating from pluripotent hematopoietic stem cells can be divided into at least three subpopulations: interstitial DCs residing in the skin and lymphoid organs and two subsets of blood DCs, the CD11c+ myeloid and CD11c+ plasmacytoid DC (9). The two subsets of blood DCs not only differentially express CD11c and CD123 (IL-3R α-chain), but also show distinct patterns of Toll-like receptor expression and regulation by cytokines (10–12). Circulating blood DCs are rare (they account for <1% of human PBMCs) and are difficult to maintain in culture. Although in vivo expansion of blood DCs by administration of the hematopoietic growth factors Flt-3 ligand and GM-CSF can be used to increase the yield of isolated cells (13), most experimental and clinical studies currently rely on the in vitro development of DC-like cells from CD34+ progenitor cells or blood monocytes (14–16). Commonly, monocytes are cultured for 5–7 days with GM-CSF and IL-4 to generate immature DCs that have to be activated for another 2–3 days with microbial, proinflammatory, or T cell-derived stimuli to obtain mature DCs with full T stimulatory capacity. There is increasing evidence that maturation of DCs from monocyte precursors may also be relevant in vivo, although the conditions under which it occurs and the exact time span required for the differentiation process are not known. However, experimental data indicate that the kinetics of DC differentiation from monocytes under physiologic conditions may not be reflected by current protocols for the in vitro development of DCs. A subpopulation of monocytes differentiates into DCs within 48 h in a model simulating transendothelial migration into lymphatic vessels (17). More recently, spontaneous maturation of CD11c+ myeloid DCs from a subset of CD14+CD16+ monocytes after overnight culture of PBMCs has been described (18). Monocytes may give rise to a...
subset of DCs during infection or inflammation, when high levels of proinflammasory mediators such as TNF-α, IL-1β, PGE
2, and IFN-α are produced. IFN-α not only induces the development of mature DCs from monocytes in vitro within 3 days (19), but high serum levels of IFN-α have been shown to be associated with the acquisition of DC characteristics by monocytes isolated from peripheral blood in patients with systemic lupus erythematosus (20).

In the present study we describe a novel strategy for the development of mature DCs from monocytes within only 48 h of in vitro culture. DCs obtained by this new strategy were compared with monocyte-derived DCs (moDCs) generated by a standard 7-day protocol for the expression of DC activation markers, sensitivity to proinflammasory and lymph node-directing chemokines, and their ability to induce proliferation and Ag-specific IFN-γ production in autologous T cells as well as to prime autologous naive T lymphocytes.

Materials and Methods

Reagents and ELISA kits

Recombinant cytokines were obtained from the indicated sources. GM-CSF was purchased from Novartis (Basel, Switzerland), IL-4 from Promega (Madison, WI), TNF-α from R&D Systems (Wiesbaden, Germany), and IL-1β from Amersham International (Little Chalfont, U.K.). IFN-γ, IL-1β, IL-2, and IL-7 were obtained from Strathmann Biotech (Hannover, Germany). PGE
2, and FITC-dextran were purchased from Sigma-Aldrich (Steinheim, Germany), [1H]thymidine from Amersham Buchler (Freiburg, Germany), and tetanus toxoid (TT) from Statens Serum Institute (Copenhagen, Denmark). Soluble CD40 ligand trimer (CD40L) was a gift from Immunix (Seattle, WA). Total IL-12 was determined using an assay that detects both IL-12 (p40) and IL-12 (p70) (Bender Med Systems, Vienna, Austria). IL-12 (p70) was measured using the OptEIA human IL-12 (p70) set (from BD PhaRmingen, San Diego, CA). IL-4 and IFN-γ were quantified using ELISA kits from BD PhaRmingen.

Media

All cultures of human PBMC were maintained in RPMI 1640 medium (Biochrom, Berlin, Germany) supplemented with 2% human AB serum (BioWhittaker, Walkersville, MD), 2 mM L-glutamine (Life Technologies, Carlsbad, CA), 100 U/ml penicillin, and 100 μg/ml streptomycin (Sigma-Aldrich, Munich, Germany), hereafter referred to as complete medium.

Isolation and culture of cells

PBMC were isolated from peripheral blood of healthy donors by Ficoll-Hypaque gradient centrifugation. Monocytes were purified using the MACS CD14 isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany), and were subsequently cultured in six-well plates (0.5–1.5 × 10
6 cells/ml) in fresh complete medium supplemented with 1000 U/ml GM-CSF and 500 U/ml IL-4. To generate standard moDCs, cells were cultured for 6 days and subsequently incubated with a combination of proinflammasory mediators for 24 h (1000 U/ml TNF-α, 10 ng/ml IL-1β, 10 ng/ml IL-6, and 1 μM PGE
2). Alternatively, monocytes were cultured for 24 h with GM-CSF (1000 U/ml) and IL-4 (500 U/ml), followed by incubation with the same proinflammasory mediators for another 24 h (FastDC). In some experiments the different DCs were additionally stimulated with CD40L (500 ng/ml) or CD40L plus IFN-γ (1000 U/ml). For cocultures, a fraction of DCs was cryopreserved and thawed for restimulation. T cells were purified from PBMC by negative selection using the Pan T Cell Isolation kit (Miltenyi Biotec). To obtain CD45RA expressing cells were additionally depleted using CD45RO Micro Beads (Miltenyi Biotec).

Flow cytometry and mAbs

The following mAbs (all from BD PhaRmingen) were used for FACS analysis: U39 (anti-HLA-DR, -DP, and -DQ, FITC-conjugated), L307.4 (anti-CD40, PE-conjugated), 233/1 (anti-CD86, allophycocyanin-conjugated), HB15e (anti-CD83, FITC-conjugated), ME2 (anti-CD14, allophycocyanin-conjugated), SC3 (anti-CD40, PE-conjugated), H17A3 (antiCD3, FITC-conjugated), RPA-T4 (anti-CD4, PE-conjugated), RPA-T8 (anti-CD8, allophycocyanin-conjugated), and HI100 (anti-CD45RA, PE-conjugated). Chemokine receptor 7 (CCR7) expression was determined by incubation with rat anti-CCR7 mAb (clone 3D12; provided by R. Forster, Hannover, Germany), followed by incubation with anti-rat IgG2a-biotinylated mAb (clone R7/203; BD PhaRmingen) and incubation with streptavidin-allophycocyanin (BD PhaRmingen). Cells were analyzed on a FACSCalibur flow cytometer (BD Biosciences, Heidelberg, Germany). Data were analyzed using CellQuest (BD Biosciences; version 3.2.1) and FlowJo (Tree Star, San Carlos, CA; version 2.7.8) software.

Scanning electron microscopy

Scanning electron microscopy was performed in cooperation with Prof. Dr. U. Welsch (Institute for Anatomy, University of Munich, Munich, Germany). Cells were spun onto standard microscopy glass slides and fixed with 2.5% glutaraldehyde in PBS (pH 7.4). After dehydration through graded ethanol, cells were critical point-dried in CO
2 and gold-coated by sputtering. Samples were examined using a JSM-35-CF scanning electron microscope (JEOL, Tokyo, Japan). After electron microscopy, prints were scanned using Adobe Photoshop software (Adobe Systems, Mountain View, CA; version 3.0).

Measurement of FITC-dextran uptake

Endocytic activity was assessed by incubating cells for 2 h with FITC-dextran (0.5 mg/ml) at 37°C. Cells were washed extensively with PBS, and FITC-dextran uptake was quantified as the mean fluorescence intensity (unspecific FITC-dextran binding to the cell surface was assessed by incubating cells on ice).

Cytometric measurement of changes in intracellular ionized calcium concentrations

MoDCs or FastDC were loaded for 40 min with the Ca
2+ -sensitive dyes Fluo-3 (3 μg/ml) and Fura Red (10 μg/ml) in the presence of 0.02% of the detergent Phoruno F-127 (all from Molecular Probes, Leiden, The Netherlands) as previously described (21). Cells were washed twice, and the chemokines macrophage inflammatory protein-1α (MIP-1α), 6Ckine (500 ng/ml; both from R&D Systems, Wiesbaden, Germany), and stromal cell-derived factor-1 (SDF-1; 250 ng/ml; R&D Systems) were added to activate chemokine receptors. Changes in intracellular calcium concentrations over time (time resolution, 200 ms) were determined by flow cytometry using the ratio of the fluorescence intensity of fluo-3 (increased intensity in the presence of Ca
2+ ) and Fura Red (reduced intensity in the presence of Ca
2+ ).

Coculture of DCs with autologous T cells

Coculture of DCs with autologous T cells was performed with minor modifications as previously described (22). To load the different DCs with Ag, immature moDCs or monocytes cultured with GM-CSF and IL-4 were incubated with TT (5 μg/ml) on day 5 or 1, respectively. After 24 h of incubation with TT, cells were stimulated to induce DC maturation. The different DC preparations were harvested on day 6 or 2, respectively, washed, and cocultured with autologous T cells at a ratio of 1:10 in the presence of soluble CD40L (500 ng/ml). Half the medium was replaced every second day by fresh culture medium containing IL-2 (25 U/ml) and IL-7 (10 ng/ml). On day 10, T cells were restimulated with DCs at a ratio of 10:1 in the presence of IL-7 (10 ng/ml), IL-2 (25 U/ml), and soluble CD40L (500 ng/ml). After 4 h of restimulation, culture supernatants were harvested for cytokine measurements.

Proliferative T cell response

Proliferative T cell responses were measured in cooperation with Prof. Dr. R. Wank (Institute of Immunology, University of Munich). T cell proliferation assay was performed with minor modifications as previously described (23). The different DC preparations were harvested and cocultured in complete medium supplemented with CD40L (500 ng/ml) with a constant number of autologous T cells or naive CD45RA
+ T cells (2 × 10
3 / 200 μl) in 96-well, round-bottom microtiter plates at ratios ranging from 1/10 to 1/320 in triplicate. On day 5 the cells were pulsed with [1H]thymidine (1 μCi/well) and harvested after 18 h onto a Filtermate (Wallac, Turku, Finland). The amount of incorporated [1H]thymidine was analyzed in a liquid scintillation counter (Wallac).

Statistical analysis

Data are expressed as the mean ± SEM. Statistical significance was determined using paired two-tailed Student’s t test. Differences were considered statistically significant for p < 0.05. Significance is presented for individual experiments (asterisks in figures). Statistical analysis was performed using StatView 4.5.1 software (Abacus Concepts, Calabasas, CA).

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Results

Within 48 h, monocytes incubated with GM-CSF and IL-4 plus proinflammatory mediators differentiate into mature DCs with distinct morphology (FastDC)

Monocytes were enriched from PBMC by CD14-positive selection using MACS and were subsequently cultured with GM-CSF and IL-4 for 48 h. Morphology, determined by light microscopy, and forward/side scatter intensity of the cells were similar to those in the initial population of monocytes (data not shown). However, cells already showed down-regulation of CD14 and high levels of MHC II expression consistent with early immature DC development (Fig. 1, top row). When proinflammatory mediators (TNF-α, IL-1β, IL-6, PGE₂) were added after 24 h of culture with GM-CSF and IL-4 to accelerate DC maturation, cells displayed a fully mature DC immunophenotype (CD83⁺, CD40⁺, CD86⁺, MHC II⁺) after only 48 h of total culture period (Fig. 1A, bottom row). Although a moderate increase in the forward/side scatter intensity of these stimulated cells compared with monocytes cultured for 48 h with GM-CSF and IL-4 alone could be observed, they were considerably smaller and less granular than moDCs (cultured for 7 days; data not shown). Scanning electron microscopy was performed to assess the morphology of cells more closely; while monocytes cultured with GM-CSF and IL-4 alone for 48 h maintained monocyte-like morphology, cells that were activated with proinflammatory mediators following 24 h of culture with GM-CSF and IL-4 formed long, fine cytoplasmatic protrusions typical of mature DC development (Fig. 1B). Using this two-step differentiation strategy, high numbers of mature and viable DCs (~30–40% of the initial population of monocytes) could be recovered after 48 h of culture. These cells will further be referred to as FastDC. Rapid DC maturation from monocytes could also be induced by adding the proinflammatory mediators together with GM-CSF and IL-4 at the initiation of culture. However, the expression of DC activation markers was less pronounced compared with the two-step differentiation and activation sequence (<60% CD83⁺ cells; data not shown). Monocytes cultured with GM-CSF alone or GM-CSF plus proinflammatory mediators in the absence of IL-4 for 48 h did not develop DC characteristics (data not shown).

FastDC are sensitive to the CCR7 ligand 6Ckine and to the CXCR4 ligand SDF-1

DC maturation is associated with a coordinated switch of chemokine receptor expression (2). While receptors for inflammatory chemokines are down-regulated, increased expression of CCR7 facilitates migration toward secondary lymphoid organs. CD14⁺ cells were cultured with GM-CSF and IL-4 alone for 48 h or were activated with proinflammatory mediators during the last 24 h. While monocytes cultured with GM-CSF and IL-4 alone remained CCR7⁻ after 48 h, FastDC homogeneously expressed CCR7 (see Fig. 1) to levels comparable to mature moDCs (data not shown). The functional activity of chemokine receptor expression was determined by measuring intracellular calcium mobilization in response to agonistic chemokines. GM-CSF/IL-4-cultured monocytes responded to the inflammatory chemokine MIP-1α, a CCR5 ligand, but were insensitive to the CCR7 ligand 6Ckine. In contrast, FastDC lost sensitivity to MIP-1α, but responded to 6Ckine, correlating with phenotypic DC maturation (Fig. 2). FastDC, but not GM-CSF/IL-4-cultured monocytes, also mobilized calcium in response to the CXCR4 ligand SDF-1 (Fig. 2, bottom). The difference in the pattern of chemokine response of GM-CSF/IL-4-cultured monocytes vs FastDC was similar to that of immature vs mature moDCs (both cultured for 7 days; data not shown).

PGE₂, TNF-α, and IL-1β are key mediators required for phenotypic maturation of FastDC and synergize to induce IL-12 production

To identify which of the proinflammatory mediators are required for maturation of FastDC, monocytes were cultured for 24 h with GM-CSF and IL-4 and subsequently activated for the following 24 h using different stimuli and combinations of stimuli. Only combinations of stimuli containing both PGE₂ and TNF-α were effective in inducing complete maturation of FastDC (> 50%
CD83 cells). Removal of PGE₂ from the combination of proinflammatory mediators used in prior experiments almost completely abolished FastDC development (Fig. 3, top; p < 0.05; n = 6). PGE₂ and TNF-α were not only required for maturation, but also synergized to induce production of total IL-12 by FastDC (Fig. 3, bottom; p < 0.05; n = 6). While IL-6 was completely dispensable in the combination of proinflammatory mediators, addition of IL-1β significantly enhanced IL-12 secretion of FastDC induced by PGE₂ and TNF-α and synergized with PGE₂ to induce DC maturation in the absence of TNF-α (Fig. 3). Neither PGE₂ nor any other stimulus used as a single agent (CD40L, TNF-α, IL-1β, IL-6) could induce DC maturation or IL-12 production (data not shown).

CD40L synergizes with proinflammatory mediators to maximize IL-12 production by FastDC, but a second signal is required for secretion of functional IL-12 (p70)

DCs stimulated by infectious organisms, proinflammatory mediators, or CD40L produce IL-12, a key cytokine in the induction of IFN-γ production by activated T cells (24). However, many of the stimuli known to induce DC maturation are not sufficient to induce the production of functional IL-12 (p70) by moDCs. To determine the capacity of FastDC to secrete the bioactive form of IL-12, CD14⁺ cells were cultured with either GM-CSF and IL-4 alone for 48 h or were activated with proinflammatory mediators during the last 24 h, and culture supernatants were harvested for measurement of total IL-12 and IL-12 (p70). While GM-CSF/IL-4-cultured monocytes did not secrete IL-12, FastDC produced moderate amounts of total IL-12, but failed to secrete IL-12 (p70) (Fig. 4). Soluble CD40 ligand, a stimulus known to induce IL-12 (p70) production in moDCs, was added after 12 h of proinflammatory stimulation to simulate encounter of CD40L-expressing activated T cells with CD40-expressing FastDC after migration to secondary lymphoid organs. CD40 ligation following proinflammatory stimulation enhanced production of total IL-12 by FastDC, but left secretion of IL-12 (p70) unaffected (Fig. 4, top). Although an ∼3-fold increase in IL-12-production by FastDC after additional CD40 ligation could be observed in each individual experiment performed (n = 6), this trend did not reach statistical significance due to the large differences in maximal levels of IL-12 detected. It is known that CD40 ligation requires the presence of T cell-derived cosignals, such as IFN-γ or IL-4, for maximal induction of IL-12 (p70) in moDCs (25, 26). If CD40 ligation of FastDC occurred in the additional presence of IFN-γ, the production of IL-12 (p70) could be induced (Fig. 4, bottom; p < 0.05; n = 6).

FastDC can be loaded with Ag: monocytes increase endocytosis of soluble dextran within 24 h of culture with GM-CSF and IL-4

The capacity to take up and process Ag for presentation of MHC molecules is a hallmark of immature DC function and is rapidly lost upon maturation of DCs (16). To assess the uptake of soluble Ag via endocytosis, CD14⁺ cells were cultured with GM-CSF and IL-4 alone for 48 h or were activated with proinflammatory mediators during the last 24 h and incubated with FITC-conjugated dextran at 37°C for 2 h at various time points. Dextran uptake was determined by FACS analysis. Monocytes increased FITC-dextran uptake by 12 h after initiation of culture with GM-CSF and IL-4 (Fig. 5). After 24 h, a >10-fold increase in mean fluorescence...
intensity could be observed, indicating a markedly enhanced capacity to internalize soluble dextran. While monocytes that were cultured with GM-CSF and IL-4 alone continued to increase endocytic activity, activation with proinflammatory mediators for the last 24 h lead to a rapid reduction of dextran uptake as a result of DC maturation (Fig. 5).

**FastDC loaded with TT induce Ag-specific autologous T cell proliferation and IFN-γ production**

Mature DCs are very effective stimulators of Ag-specific T cell responses. The T cell stimulatory capacity of FastDC was assessed and compared with that of moDCs using TT as a model Ag. To load FastDC with Ag, cells were incubated with TT during the first 24 h of culture with GM-CSF and IL-4 before they were activated with proinflammatory mediators for another 24 h. Immature moDCs were loaded with TT on day 5 and were subsequently stimulated with proinflammatory mediators for 24 h to induce DC maturation. FastDC or moDCs, each loaded with TT or left unloaded, were cocultured with autologous T cells following activation. CD40L was added to induce maximal levels of functional IL-12 secretion. Before the initiation of coculture, FastDC and activated mature moDCs were compared for their expression of surface markers. No differences in the expression of CD14, CD83, CD80, CD86, and MHC II were detected (data not shown). After 5 days of coculture, cells were pulsed with [³H]thymidine and harvested after 18 h to measure proliferation. Ag-loaded FastDC were equally effective in inducing the proliferation of autologous T cells compared with Ag-loaded, activated moDCs (Fig. 6, top row). In an additional set of experiments T cells were restimulated after 10 days of coculture with the same DCs (FastDC or moDCs, respectively, left unloaded or

**FIGURE 5.** Ag loading of FastDC. Monocytes show increased FITC-dextran uptake after 24 h of culture with GM-CSF and IL-4. Monocytes were cultured for 48 h with GM-CSF and IL-4 alone or were additionally stimulated with IL-6, IL-1β, TNF-α, and PGE₂ for the last 24 h. Cells were incubated with FITC-conjugated dextran at 37°C at the indicated time points of the differentiation process (controls on ice). After 2 h cells were washed, and dextran uptake was quantified as the mean fluorescence intensity (MFI) using FACS.

**FIGURE 6.** TT-loaded FastDC induce Ag-specific proliferation and IFN-γ production by autologous T cells. Monocytes were cultured for 24 h with GM-CSF and IL-4 in the presence of TT (5 μg/ml) and were stimulated with IL-6, IL-1β, TNF-α, and PGE₂ for another 24 h to generate Ag-loaded FastDC. Alternatively, cells were cultured for 5 days with GM-CSF and IL-4, loaded with TT, and stimulated for 24 h with proinflammatory mediators to generate mature Ag-loaded moDCs. Monocytes cultured with GM-CSF plus IL-4 alone for 48 h, immature moDCs, unloaded FastDC, and unloaded, stimulated moDCs were used as controls. DCs were cocultured with purified autologous T cells as described (see Materials and Methods). To determine T cell proliferation, cells were pulsed with [³H]thymidine on day 5, and incorporation was measured after 18 h. T cell proliferation alone (without DCs) measured as an additional control accounted for only 0.4 × 10³ cpm (data not shown). To assess cytokine production of T cells, supernatants were harvested for ELISA measurements after 4 h of restimulation with DCs on day 10 of coculture. One representative experiment of three performed with different donors is shown for T cell proliferation. The results of cytokine measurements are expressed as the mean ± SEM of these three experiments.
FIGURE 7. TT-loaded FastDC induce Ag-specific proliferation of autologous CD45RA+ T cells. TT-loaded FastDC and mature TT-loaded moDCs were generated as described. Monocytes cultured with GM-CSF plus IL-4 alone for 48 h, immature moDCs, unloaded FastDC, and unloaded, stimulated moDCs were used as controls. The different DCs were cocultured with purified autologous naive CD45RA+ T cells as described (see Materials and Methods). Cells were pulsed with [3H]thymidine on day 5 and harvested after 18 h to determine proliferation. One representative experiment of four is shown.

Discussion

In the present study we report for the first time that mature DCs with full T stimulatory capacity can be derived from human monocytes within only 48 h of in vitro culture. Monocytes cultured for 24 h with GM-CSF and IL-4 followed by stimulation with the proinflammatory mediators TNF-α, IL-1β, IL-6, and PGE2 (27) for another 24 h rapidly undergo all phases of DC differentiation; cells transiently display immature DC characteristics (down-regulation of the monocytic marker CD14, increase in endocytotic uptake of soluble dextran and responsiveness to the inflammatory chemokine MIP-1α) before they develop into mature DCs expressing high levels of CD83, costimulatory proteins, and MHC II molecules as well as functional CCR7 (FastDC). FastDC loaded with TT induced equal rates of autologous T cell proliferation compared with TT-loaded, stimulated moDCs generated according to a 7-day standard protocol, indicating that they are capable of taking up and processing proteins for presentation on MHC molecules. Strong proliferative responses of autologous naive CD45RA+ cells indicated that Ag-loaded FastDC not only stimulate immune responses to recall Ags, but are also effective in initiating primary immune responses. Moreover, FastDC were equally capable of stimulating a Th1-type immune response as Ag-loaded, activated moDCs, as shown by IFN-γ production of autologous T cells cocultured with TT-loaded FastDC.

Our results confirm previous experimental evidence that circulating monocytes may represent a pool of stand-by precursor cells capable of rapid differentiation into DCs. Randolph et al. (17) showed that a subpopulation of monocytes, after migration across an endothelial barrier into a collagen matrix, reverse direction and migrate back. This reverse transmigration, simulating entry into lymphatic vessels, was associated with down-regulation of the monocytic marker CD14 and up-regulation of MHC II molecules. If monocytes encountered yeast cell walls or other phagocytic particles in the collagen matrix, reverse transmigrated cells acquired a mature DC phenotype and high allostimulatory capacity within 48 h. As we show here, monocytes efficiently take up and process Ag, undergo DC maturation, up-regulate the lymph node-directing chemokine receptor CCR7, and acquire T cell stimulatory within 48 h of incubation with GM-CSF and IL-4 plus proinflammatory mediators. During inflammation or infection, the production of GM-CSF by endothelial cells and the secretion of proinflammatory mediators such as TNF-α, IL-1β, and PGE2, by tissue-residing immune cells may cooperate with microbial stimuli to induce rapid differentiation and maturation of monocytes into DCs in vivo (9, 28, 29). Thus, monocytes may give rise to a subset of tissue-derived DCs capable of transit to secondary lymphoid tissues via lymphatic vessels to initiate or maintain adaptive immune responses.

The capacity of the proinflammatory mediators TNF-α and PGE2 to induce complete DC maturation of monocytes by 2 days of culture with GM-CSF and IL-4 is a novel finding of this study. However, the effects of some of the proinflammatory mediators used here on maturation, cytokine production, and T cell activation by FastDC confirm previous results obtained in studies with standard moDCs. PGE2 and a number of other stimuli tested, including CD40L, were not capable of inducing phenotypic maturation of FastDC by themselves; cooperation of PGE2 with TNF-α or IL-1β was required for complete maturation of FastDC (30–32). Stimulation with PGE2 rendered FastDC sensitive not only to the CCR7 ligand 6Ckine, but also to the CXCR4 ligand SDF-1 (CXCL12), as Luft and co-workers have very recently shown for moDCs (29). We also identified IL-1β as a key factor for optimal activation of FastDC. IL-1β synergized with PGE2 to induce phenotypic maturation in the absence of TNF-α, and removal of IL-1β from the proinflammatory mediators markedly reduced IL-12 production of FastDC. Failure of FastDC activated with proinflammatory cytokines plus PGE2 to secrete IL-12 (p70) is not surprising if recent reports on the effect of PGE2 on cytokine production by moDCs are taken into account; it has repeatedly been shown that PGE2 inhibits the secretion of IL-12 (p70) by moDCs even if potent stimuli, such as CD40L, are used for activation (29, 33). Nevertheless, activation with proinflammatory cytokines plus PGE2 does not affect the Th1-inducing capacity of FastDC or moDCs (29, 34). A marked increase in IL-12 production by
FastDC was observed if CD40 ligation followed proinflammatory signaling, indicating that CD40L synergizes with proinflammatory mediators to induce IL-12 production by DCs, a mechanism that has been described for IL-1β and LPS (35, 36). Increased levels of total IL-12 production were associated with secretion of the bioactive IL-12 (p70) heterodimer if IFN-γ was present during CD40 ligation. Thus, even after stimulation with PGE₂, low levels of IL-12 (p70) can be induced in FastDC if an additional signal is provided by T cell-produced cytokines such as IFN-γ (25, 26). We therefore hypothesize that FastDC matured with proinflammatory cytokines plus PGE₂ become capable of migrating to secondary lymphoid organs where encounter with activated T cells induces the secretion of IL-12 (p70) and facilitates the stimulation of Th1-type immune responses.

Historic aspects of the study of DC differentiation may help to explain why more rapid development of DC-like cells from monocyte precursors has not been attempted previously: when DCs were first derived in vitro from blood precursors, specific surface markers had yet to be discovered, and morphologic criteria had to be applied to identify DCs (14). Ever since, the concept has persisted that moDCs had to be of Langerhans cell-like morphology; increased cell size, development of granules, and formation of cytoplasmatic protrusions are some of the morphologic features attributed to the typical moDC. In vitro, culture of 5–7 days with GM-CSF and IL-4 is required to induce these morphologic changes and hence has become the standard protocol for generation of DCs from monocytes (15, 16). Although this regimen can be used for the generation of DC-like cells that have been proven to efficiently induce antitumoral immune responses in vivo in a number of vaccination trials (37–39), it may not reflect the time span required for DC differentiation from monocytes under physiologic conditions. Moreover, compared with the prolonged in vitro culture required in standard protocols, generation of FastDC not only saves time, but may also prove to be less sensitive to external disruptive factors such as microbial contamination. In our hands, generation of FastDC yielded high percentages of viable, fully mature DCs more reliably than the standard 7-day protocol used to generate moDCs.

In summary, we were able to demonstrate that the development of mature DCs from monocyte precursors does not require >2 days of in vitro culture. FastDC matured with proinflammatory mediators were compared with moDCs generated by a standard 7-day protocol and were found to possess equal capacity to induce Ag-specific proliferation of T cells as well as to prime autologous naive T lymphocytes. Moreover, FastDC are sensitive to chemokines agonistic to the lymph node-directing chemokine receptor CCR7 and induce a Th1 cytokine profile in autologous T cells, thus demonstrating all the qualities currently thought to be necessary for an effective DC-based, antitumor vaccine. This new protocol markedly reduces the time, work load, and cost associated with in vitro culture of DC precursors and thus may facilitate the use of DCs in clinical trials of cellular immunotherapy. Moreover, our findings support the hypothesis that monocytes represent a pool of circulating precursor cells capable of rapid differentiation into mature DCs after transit into inflamed or infected tissues.

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