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Dendritic Cells as the Terminal Stage of Monocyte Differentiation

Karolina A. Palucka, Nicolas Taquet, Françoise Sanchez-Chapuis, and Jean Claude Gluckman

Monocytes (MO) cultured for >5 days with either macrophage-CSF (M-CSF) or granulocyte macrophage (GM)-CSF and IL-4 differentiated without concomitant proliferation into CD14+ macrophages (Mφ) or CD1a+ dendritic cells (DC), respectively. When adherent and nonadherent CD14high Mφ from M-CSF cultures were separated and cultured further in cytokine-free medium or with GM-CSF/IL-4, most cells from both fractions that were exposed to GM-CSF/IL-4 acquired CD1a expression and DC morphology and function. Conversely, GM-CSF/IL-4 withdrawal at day 5 and additional culture of sorted CD1a+ DC for 2 to 7 days in cytokine-free medium led to cells rapidly becoming adherent CD1a-CD14+ Mφ. Replacing GM-CSF/IL-4 with M-CSF hastened the conversion of DC to Mφ without increasing cell numbers. CD1a+CD14+CD83+ mature DC were induced by a 2-2 day exposure to MO-conditioned medium, LPS, or TNF-α/IL-1β. Upon cytokine removal or culture with M-CSF, DC that had been pushed to maturation by conditioned medium or LPS remained stable or died in the new environment. TNF-α/IL-1β-driven DC displayed heterogeneous CD83 expression and could thus be sorted into CD83high and CD83low cells; in cytokine-free medium or in M-CSF, most CD83low cells converted to Mφ, whereas most CD83high cells remained nonadherent CD1a-CD14+ or died and thus appeared truly terminally differentiated. Hence, MO are precursors of Mφ as well as of DC, with each cell type having the capability to convert into the other until late in the differentiation/maturation process. Accordingly, the cytokine environment and the presence of differentiation and/or other stimulatory signals may be the “final decision-making factors” determining whether these cells will acquire DC or Mφ characteristics and function. The Journal of Immunology, 1998, 160: 4587–4595.

Materials and Methods

Media and reagents

Unless otherwise indicated, the culture medium used was composed of RPMI 1640, 1% L-glutamine, 1% penicillin/streptomycin (Life Technologies, Paisley, U.K.), 50 μM 2-ME (Sigma, St. Louis, MO), and 10% FCS.
DENDRITIC CELLS DIFFERENTIATE FROM MONOCYTES AND MACROPHAGES

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10^6 cells/3 ml culture medium supplemented with maturation-inducing factors (GM-CSF or M-CSF, respectively) (9, 15), that was conducted in Costar plastic plates (Cambridge, MA) unless otherwise indicated. In some experiments, Teflon dishes (Savillex, Minnetonka, MI) were used to culture otherwise ADH cells. Cultures were fed every 2 days by removing one-third of the supernatant and adding fresh medium (half of the culture volume) with full doses of cytokines.

To induce the maturation of MDDC, day 5 cells were collected, GM-CSF and IL-4 were washed away, and the cells were replated at 1 to 2 x 10^6 cells/ml culture medium supplemented with maturation-inducing factors (16, 17) (see Results).

Cytokine switch and separation of cell populations

Cytokine switch in GM-CSF/IL-4 cultures was performed as follows: cells were collected and, after a short centrifugation, supernatant was discarded; cells were replated and cultured further in cytokine-free medium or with M-CSF. MDDC were sorted according to CD1a and/or CD83 expression (routinely >95% purity after sort) with a FACStarPlus (Becton Dickinson) and used for additional studies. Alternatively, cells were separated by direct and/or indirect panning with plastic-immobilized primary or secondary Abs. Unlabeled cells and/or CD1a- (Ortho Diagnostic Systems, Raritan, NJ) or CD14- (LeuM3, Becton Dickinson) labeled cells were layered on Abs-coated plates in PBS plus 2% FCS and then incubated for 1 h at 4°C. Next, supernatant was collected, plates were gently rinsed, and residual cells were cultured in fresh medium and new cytokines (i.e., M-CSF or GM-CSF/IL-4 were added to CD1a+ or CD14+ cells, respectively). Incubation of cells with CD1a mAb did not result in additional activation, as no modification of the intracellular Ca^2+ concentration was found (data not shown in Fig. 2 C).

ADH and NA cells of M-CSF cultures were separated by the removal of NA cells that were transferred to new wells with new cytokines. Wells were rinsed thoroughly to collect all NA cells, and fresh medium with new cytokines was added on the remaining ADH cells. If necessary, ADH cells were detached by incubation for 10 min at 37°C in 0.02% EDTA/PBS.

Multiparameter flow cytometry analysis

Cell surface marker expression was evaluated by double or triple immunofluorescence staining, with the following Abs: CD1a, CD80, CD83, CD54 (LeuM3), CD20, CD80, and HLA-DR (all from Becton Dickinson); CD1a (Ortho Diagnostic); CD83 (Immunotech); and CD86 (PharMingen, San Diego, CA). After both a 45-min incubation at 4°C and washing, cells were either fixed in 1% paraformaldehyde/PBS or resuspended in PBS plus 0.5% propidium iodide (PI) to exclude dead cells from analysis.

The intracellular expression of CD1a, CD14, CD68, and myeloperoxidase (MPO; the latter two from Dakopatts, Glostrup, Denmark) was evaluated following cell membrane staining, PermeaFix (Ortho Diagnostic) treatment for both cell fixation and permeabilization (45 min at 20°C), and incubation (45 min at 20°C) with the relevant FITC-conjugated mAb at saturating concentrations. After final washing, cells were resuspended in 1% paraformaldehyde/PBS.

Analysis was performed with a FACScan or a FACScalibur (Becton Dickinson). Marker expression was evaluated as the percentage of positive cells among MDDC or Mø by defined by forward scatter/side scatter characteristics and/or CD1a or CD14 expression, respectively. The distinction between low and high expression was based on the evaluation of mean fluorescence intensity (MFI).

Functional studies

The MLR was performed using culture medium supplemented with 10% heat-inactivated normal human AB serum. Allogeneic T cells (5 x 10^4/well) were cultured for 5 to 6 days in 96-well culture microplates (Costar) as responder cells to 0.5 x 10^5 to 10 x 10^5 MDDC or Mø. [3H]Thd incorporation (specific activity = 1 Ci/mM) (Amersham, Little Chalfont, U.K.) was measured after 8- to 16-h pulses with 1 μCi/well. Results are shown either as mean cpm of triplicates or as stimulation indices (SI) calculated by dividing mean cpm in test wells by mean cpm in control wells without stimulating cells.

Dextran-FITC uptake (0.1–1 mg/ml; Sigma) was evaluated by flow cytometry following 15 to 30 min of incubation at 4°C and/or 37°C and extensive washing with cold PBS.

Results

M-CSF-driven Mϕ convert to MDDC in a GM-CSF/IL-4-dependent manner

We have previously shown that a fraction of Mϕ obtained by the culture of MO with GM-CSF or M-CSF can acquire CD1a expression and DC morphology upon delayed addition of IL-4 or replacement with GM-CSF/IL-4, respectively (15).

Here, we evaluated this Mϕ plasticity and the potential of Mϕ to convert into MDDC to a greater extent. To this end, we separated the ADH and NA fractions of M-CSF-driven Mϕ, which we recultured after switching cytokines. Table I summarizes the characteristics of Mϕ after 5 to 7 days of M-CSF culture in Costar wells. Upon 4 to 7 days of reculture in GM-CSF/IL-4, most NA cells of M-CSF cultures (initially 95% CD14low, membrane and intracellular CD1a-) down-regulated CD14 expression (only 12 ± 6% remained CD14low) and 45 ± 25% acquired de novo CD1a (Fig. 1). The conversion potential of ADH cells from the same cultures was lower, with only 27 ± 26% of cells becoming NA and CD1a+ CD14- upon reculture in GM-CSF/IL-4 (Fig. 1). In both fractions, CD14 expression remained stable when cultures were pursued with M-CSF or without the addition of cytokines (Fig. 1). Although the presence of both GM-CSF and IL-4 was necessary for Mϕ to convert into MDDC, Mϕ treated with GM-CSF or IL-4 alone also underwent morphologic and phenotypical changes, becoming round and CD1a- CD14low (Fig. 1 and data not shown).

A similar conversion pattern was found when CD14high cells cultured with M-CSF in Teflon dishes under NA conditions were purified by panning and recultured with GM-CSF/IL-4 (data not shown). Overall, most cells recovered from M-CSF cultures that had been subsequently switched to GM-CSF/IL-4 became CD1a+ (Fig. 2 A) with increased allostimulatory capacity in the MLR (Fig. 2 B) and increased dextran uptake (2.8-fold higher MFI relative to M-CSF-treated cells), and they could differentiate into mature, strongly allostimulatory CD1a+ CD83+ DC upon LPS activation (Fig. 2, B and C).

Because culture of M-CSF-induced ADH Mϕ with GM-CSF/IL-4 consistently led to the appearance of two cell fractions, namely NA cells converting to DC and cells that remained ADH, we examined whether the latter fraction actually consisted of terminally differentiated Mϕ. To this end, we conducted bulk cultures of MO-enriched PBMC in Costar flasks (2 x 10^6 cells/20 ml) with M-CSF for 5 to 8 days, removed the NA cells, and added GM-CSF/IL-4 to the remaining ADH Mϕ. After 5 more days culture with GM-CSF/IL-4, two cell fractions were present, as seen previously. NA cells were collected, ADH cells were detached with EDTA-supplemented medium, and phenotypical evaluation revealed down-regulation of CD14 expression and the presence of 57% (range: 34–80%; n = 2) and 54% (range: 22–71%; n = 3)
CD1a+CD14- cells with high allostimulatory capacity in each fraction, respectively (Fig. 2D).

**Immature MDDC convert to Mϕ in a M-CSF-independent manner**

The in vitro instability of immature MDDC generated with GM-CSF/IL-4 has already been reported (11, 14, 17). We also found that upon removal of both GM-CSF and IL-4 and/or reculture with M-CSF, day 5 CD1a+CD14- MDDC (the characteristics of which are summarized in Table I) easily readhered and expressed membrane CD14 again (Fig. 3). In contrast to a previous report (11), this conversion was M-CSF independent (Fig. 3). Accordingly, we followed the kinetics of MDDC conversion to Mϕ in cytokine-free cultures (Fig. 4). From day 2 to day 5 of culture without cytokines

### Table I. Characteristics of Mϕ and MDDC from MO-enriched PBMC cultured for 5 to 7 days with M-CSF or GM-CSF/IL-4, respectively

<table>
<thead>
<tr>
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<th>Mϕ</th>
<th>MDDC</th>
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<tbody>
<tr>
<td>Cell recovery</td>
<td>0.90 ± 0.25 × 10^6 CD14+</td>
<td>0.95 ± 0.40 × 10^6 CD1a+</td>
</tr>
<tr>
<td>Morphology</td>
<td>Mixed: 1) ADH large, round and spindle-shaped cells; 2) minority of NA round, small cells (veiled in &lt;7-day cultures)</td>
<td>Mostly NA or loosely ADH large cells with prominent dendrites and small clusters</td>
</tr>
<tr>
<td>Phenotype</td>
<td>CD14+</td>
<td>CD14+</td>
</tr>
<tr>
<td></td>
<td>95 ± 5%</td>
<td>5 ± 5%</td>
</tr>
<tr>
<td></td>
<td>CD1a+</td>
<td>CD1a+</td>
</tr>
<tr>
<td></td>
<td>&lt;1%</td>
<td>75 ± 27%</td>
</tr>
<tr>
<td></td>
<td>CD68+</td>
<td>MFI index = 29</td>
</tr>
<tr>
<td></td>
<td>MFI index = 21</td>
<td>Low at day 5, negative at day 7</td>
</tr>
<tr>
<td>MPO</td>
<td>Negative at day 7</td>
<td></td>
</tr>
<tr>
<td>Function</td>
<td>MLR (cpm × 10^-3)</td>
<td>4.9 ± 3.7</td>
</tr>
<tr>
<td></td>
<td>dextran uptake</td>
<td></td>
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<td></td>
<td>++</td>
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</table>

* Mean ± SD (n = 7 and 6, respectively): 2 × 10^6 cells were plated on day 0; only NA cells were counted in M-CSF cultures. No cell proliferation was detected (lack of 5-bromodeoxyuridine incorporation, data not shown).

**FIGURE 1.** M-CSF-driven Mϕ convert into MDDC in a GM-CSF/IL-4-dependent manner. NA and ADH cells of day 5 M-CSF cultures were separated and cultured for 4 more days under the indicated conditions. Cell percentages (plots) and cell morphology (>400 magnification) were evaluated at the indicated time points. A, Cells of day 5 M-CSF cultures (arrows point to NA veiled cells). B, Culture of the day 5 NA fraction cells with GM-CSF/IL-4 (arrows point to DC). C, Culture of the day 5 ADH fraction cells with GM-CSF/IL-4 (the arrow points to DC). Cumulative data from five experiments are shown.
The conversion potential to MΦ relative to cells treated continuously with GM-CSF/IL-4).  

1 1/11 or 7 days after the switch from M-CSF to either cytokine-free medium (M-CSF−/−) or GM-CSF/IL-4 (M-CSF+/−GM-CSF/IL-4+/−). The first (+) and (−) indicate use or absence of the specified cytokine for the first 5 days, and the second sign refers to subsequent days; mean cell counts of five experiments starting from 2 × 10^6 MO on day 0 are shown. B, MLR-stimulating capacity of immature and LPS-driven MDDC converted from MΦ that were previously cultured for 7 days with M-CSF. Stimulating cells used were: NA+GI = day 7 NA cells cultured for 5 days with GM-CSF/IL-4; NA+GI+LPS = day 7 NA cells cultured for 5 days with GM-CSF/IL-4 and for 2 days with LPS; ADH+GI = day 7 ADH cells cultured for 5 days with GM-CSF/IL-4; ADH+GI+LPS: day 7 ADH cells cultured for 5 days with GM-CSF/IL-4 and for 2 days with LPS the responses of 5 × 10^6 allogeneic T lymphocytes (SI) are shown. Background incorporation was 20 cpm. One experiment representative of three is shown. C, Phenotype of day 5 M-CSF-induced MΦ cultured for an additional 5 days with GM-CSF/IL-4 and with LPS for the last 2 days: FITC-CD1a and phycoerythrin (PE)-CD83 labeling. D, ADH cells of day 5 bulk M-CSF cultures were cultured for 5 days with GM-CSF/IL-4; the resulting NA and ADH cells were evaluated for their capacity to stimulate 5 × 10^4 allogeneic T lymphocytes (cpm) as compared with day 5 MΦ. One experiment of two performed is shown.

The conversion potential to MΦ is a true property of CD1a+ MDDC

Next, we examined whether the changes in MDDC morphology, phenotype, and function described above were due to true conversion or whether they were the consequence of the heterogeneity of the day 5 cultured cells instead, resulting in the selection of a particular cell type upon cytokine withdrawal. To answer this question, CD1a^hi MDDC purified to >95% by panning (data not shown) or FACS sorting (Fig. 5) were cultured with either GM-CSF/IL-4 or M-CSF. Again, the majority of cells converted to MΦ phenotype and morphology (Fig. 5). Moreover, MDDC that had converted to ADH MΦ could again acquire CD1a expression and DC morphology upon reculture with GM-CSF/IL-4 (Fig. 5).

Mature CD1a+CD83^hi MDDC cannot convert to MΦ

MDDC maturation, as assessed by morphology and phenotype, was induced by culturing day 5 MDDC for >2 days with LPS, CM, or TNF-α/IL-1β; all of these conditions led to the induction of CD83 expression, increased the expression of costimulatory molecules, and increased the allostimulatory capacity of cells relative to control cultures (Table II and Fig. 6, A and B). Mature MDDC were then cultured in cytokine-free medium or with M-CSF. LPS-treated MDDC remained CD1a^−CD14^−CD83^+ (data not shown) or died in the new environment (70–80% PI+ cells). Similarly, CM-treated MDDC remained mostly NA and CD1a^lo/−CD14^−CD83^lo/− (Fig. 6, B and C), confirming the results of others (17). We took advantage of the fact that TNF-α/IL-1β-driven MDDC displayed heterogeneous CD83 expression to sort them into CD83^hi and CD83^lo/− cells (Fig. 7A). Sorted CD1a^+CD83^hi cells displayed much higher allostimulatory capacity than CD1a^+CD83^lo/− cells from the same cultures (Fig. 7B). Upon cytokine removal (data not shown) or in culture with
FIGURE 3. M-CSF-independent conversion of immature MDDC into Mφ. A, Morphology of cells cultured for 12 days with GM-CSF/IL-4; the conversion of MDDC to ADH Mφ upon cytokine withdrawal (B) or replacement with M-CSF from culture day 5 to 12 (C) (×400 magnification) is shown. D, CD1a$^+$ and CD14$^+$ cell counts at days 5 and 12 in culture with GM-CSF/IL-4 (GM-CSF/IL-4$^{+/+}$) or 7 days after switching from GM-CSF/IL-4 to either cytokine-free medium (GM-CSF/IL-4$^{-/-}$) or M-CSF (GM-CSF/IL-4$^{+/+}$/M-CSF$^{+/+}$). Cumulative data from six experiments (mean cell counts starting from $2 \times 10^6$ day 0 plated MO) are shown; see legend to Figure 2 for definition of (+) and (−).

FIGURE 4. Kinetics of the conversion of MDDC into Mφ. A, Recovered NA cell numbers after culture under the indicated conditions (see legend to Fig. 2) starting from $2 \times 10^6$ day 0 plated MO; mean values ± SD (n = 5) are given. B, Kinetics of CD1a and CD14 expression by cells cultured under the indicated conditions, as seen with two-color cytograms of cells labeled with FITC-CD1a and PE-CD14 mAbs; the horizontal and vertical lines indicate the level of autofluorescence in the FL-2 and FL-1 channels. Data from one representative experiment of five is shown. C, MLR-stimulating capacity for $5 \times 10^4$ allogeneic T lymphocytes (cpm) of the NA cells from the same culture seen in B, D, Kinetics of the MLR-stimulating capacity for $5 \times 10^4$ T lymphocytes (cpm) of $1 \times 10^5$ NA cells cultured under the indicated conditions; mean ± SD (n = 3) is given.
M-CSF, most CD83low cells converted to Mϕ (with <10% PI+ cells) (Fig. 7D); CD83high cells either remained NA CD1a–CD14+ DC or died (>45% PI+ cells) but did not convert to Mϕ (Fig. 7C).

Discussion

The blood is a reservoir from which MO can be recruited into tissues and differentiate into Mϕ and/or APC (20). Our data support the idea of MO being relatively immature precursors with a double differentiation potential that depends upon the cytokine environment. Indeed, MO-enriched PBMC (>80% CD14+CD68+MPOlow) cultured with either M-CSF or GM-CSF/IL-4 differentiated into Mϕ (CD14highCD1a–CD68+ MPO+) or MDDC (CD14low–CD1a-highCD68–MPOlow), respectively. The majority of NA and loosely ADH M-CSF-driven Mϕ could convert into MDDC if cultured anew with both GM-CSF and

Table II. Characteristics of MDDC cultured for 5 days with GM-CSF/IL-4 and cultured further for 2 days with maturation-inducing factors (7 days total culture) 

<table>
<thead>
<tr>
<th></th>
<th>TNF-α/IL-1β</th>
<th>LPS</th>
<th>CM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell recovery a</td>
<td>0.40 ± 0.15 × 10⁶</td>
<td>0.75 ± 0.30 × 10⁶</td>
<td>1.00 ± 0.20 × 10⁶</td>
</tr>
<tr>
<td>Morphology b</td>
<td>Heterogeneous clustered and nonclustered cells, partly ADH</td>
<td>Strongly clustered cells with prominent dendrites</td>
<td>&quot;Hairy&quot; cells with abundant short dendrites</td>
</tr>
<tr>
<td>Phenotype c,d</td>
<td>CD1a67-94%low/high CD14–CD83+</td>
<td>&gt;85%low/high</td>
<td>79-98%low</td>
</tr>
<tr>
<td></td>
<td>Heterogeneous: 9-33%high and 38-67%low CD83+</td>
<td>Mostly homogenous: &gt;90% CD83+</td>
<td>Low CD83 expression on all cells</td>
</tr>
<tr>
<td></td>
<td>CD80+CD68+ 20% 7%</td>
<td>&gt;84% 84 ± 19%</td>
<td>&gt;75% &gt;75% low/high</td>
</tr>
<tr>
<td></td>
<td>HLA-DR &gt;90%low/high</td>
<td>&gt;90%high</td>
<td>&gt;90%low/high</td>
</tr>
<tr>
<td>MLR index e</td>
<td>1.2</td>
<td>1.9</td>
<td>1.2</td>
</tr>
</tbody>
</table>

a Data from ≥ three independent experiments are given if not otherwise indicated.
b Mean ± SD (n = 5); 2 × 10⁶ cells were plated on day 0.
c Evaluated with an inverted microscope; some adherent cells resulting from GM-CSF/IL-4 removal could be seen in all cultures.
d Percentage of positive cells is shown (n = 5).
e Allo-MLR response induced by day 7 mature MDDC relative to day 7 immature MDDC cultured with GM-CSF/IL-4 only in the same experiment (ratios of cpm); the response of 5 × 10⁴ T lymphocytes to 5 × 10³ stimulating cells is shown and data are representative of three experiments.
IL-4 as determined by morphologic, phenotypical, and functional criteria. The conversion potential of strongly ADH Mφ varied depending upon the culture condition; while the majority of ADH cells generated in Costar plates appeared irreversibly committed, 50% of ADH Mφ generated in bulk cultures readily converted to MDDC. Moreover, even cells that remained ADH in the presence of GM-CSF/IL-4 did acquire CD1a expression and were capable of stimulating allogeneic T cells.

The necessity of both GM-CSF and IL-4 for the conversion of Mφ to MDDC to occur is in line with our earlier results showing that both cytokines are needed for MO to differentiate into DC (15). GM-CSF is known to down-regulate CD14 expression at the transcriptional level in MO (21), and this effect is potentiated by IL-4 (22). The previously reported formation of multinucleated giant cells when IL-4 was added to M-CSF-driven Mφ (11, 23) was not a major phenomenon in our cultures, although we could sporadically observe cell fusions with multinucleated cells that never involved more than three nuclei (data not shown).

Alternatively, immature MDDC converted to CD14+CD1a<sup>low</sup>/MPO<sup>−</sup> Mφ upon cytokine removal, regardless of the presence of M-CSF. However, in some donors, adding M-CSF could hasten CD14 reexpression and increase the proportion of ADH spindle-shaped cells. In another study, the conversion of MDDC to Mφ was ascribed to M-CSF, since the cells died upon GM-CSF/IL-4 withdrawal without cytokine replacement (11). We observed neither selective death of MDDC cultured in cytokine-free medium nor M-CSF rescue of cells, and the total numbers of recovered CD1a<sup>+</sup> and/or CD14<sup>+</sup> cells in the populations recovered at the indicated time point are shown. Combined results from two of four experiments are shown.

Interestingly, MDDC driven to maturation by TNF-α/IL-1β were heterogeneous with regard to CD83 expression, and all were not terminally differentiated, as most of CD83<sup>low</sup> cells easily converted to Mφ in cultures with M-CSF. Whether irreversible MDDC maturation is a random process or only involves MDDC subsets primed for terminal differentiation and maturation remains to be determined. In our study, day 5 cells of GM-CSF/IL-4 cultures were heterogeneous with regard to CD86 expression; and there consistently were ≤15% CD1a<sup>−</sup>CD86<sup>+</sup> MDDC, which could represent a cell subset primed for terminal maturation. Nevertheless, our results support the current concept that DC require a second signal (optimally CD40 ligation) for terminal maturation (our manuscript in preparation and (5, 8, 24, 25)). In contrast to

**FIGURE 6.** MDDC induced to maturation by CM do not convert into Mφ if cultured under switched cytokine condition or in cytokine-free medium. A, A two-color cytogram of day 5 MDDC labeled with FITC-CD1a and PE-CD14 mAbs showing the CD1a<sup>+</sup> cell sort gate. B, Histograms illustrate the phenotype of CD1a-sorted MDDC cultured for 5 more days with GM-CSF/IL-4 (upper panels) or CM (lower panels). Open histograms indicate control labeling with an irrelevant mAb, while solid histograms indicate staining with the relevant mAb against the indicated marker. C, Cells cultured in GM-CSF/IL-4 for 7 days (day 7) and then with CM for 3 days (day 10) were collected and recultured for 5 more days (day 15) with CM (I), or in cytokine-free medium (II), or with M-CSF (III); percentages of CD1a<sup>+</sup> and CD14<sup>+</sup> cells in the populations recovered at the indicated time point are shown.
TNF-α/IL-1β-induced MDDC, only part of which were terminally differentiated, MDDC driven to maturation by LPS (a physiologic "danger" signal (26)) or by in vitro-produced MO-CM could not revert to Mϕ in the new cytokine environment. Since prolonged culture resulted in substantial cell loss, it may be argued that the potential of Mϕ to convert to MDDC and vice versa is confined to only a fraction of both cell types that are progressively selected in the course of experiments. However, it seems more likely that cell loss (noted under all conditions) is random and due to the experimental manipulation of highly activated cells. This would be particularly true for the ADH cell fractions, the detachment of which results in high mortality that may lead to a possible underestimation of cell counts.

Our results show that Mϕ and MDDC can readily interconvert into one another until the late stages of their respective differentiation/maturation process. Thus, it is conceivable that in a situation in which exogenous or endogenous tissue damage is beyond the capability of first-line innate immunity for in situ repair, resident Mϕ will convert to DC upon appropriate signals and travel to regional lymph nodes to present the Ag and induce acquired immunity responses. This could be particularly true in a situation in which no immunologic memory is available for an Ag, thus making the recruitment of naive T and/or B cells necessary. Indeed, it has recently been postulated that DC constitute a link between innate and acquired (adaptive) immunity (1, 2).

The concept of alternative MO developmental pathways leading either to "accessory" cells or Mϕ was already proposed in the mid-eighties (27). It was then observed, however, that Mϕ development is preceded by a transient differentiation stage of veiled cells with increased accessory function (27). It was not possible under any conditions for Mϕ to proceed through their development pathway without this stage, but they could remain at the veiled cell stage if appropriate differentiation signals were provided (27). Indeed, we have seen veiled cells that preceded adherence and the development of Mϕ morphology at early time points (up to day 7) in all M-CSF cultures. Veiled cells could also be seen early on (during the first 2–3 days) in GM-CSF/IL-4 cultures, and the allostimulatory capacity of such early cells was rather low, as previously reported (15). Taken together with the data presented here, these findings suggest that MO are immature precursors that go through a stage of veiled cells (immediate DC precursors), the ultimate fate of which is to become DC (Fig. 8). Accordingly, the local cytokine environment and the presence of maturation and other stimulatory signals may well be the final decision-making factors determining whether the cells will acquire DC or Mϕ characteristics and function.

FIGURE 7. The heterogeneity of MDDC pushed to maturation by TNF-α/IL-1β. This experiment is representative of three performed. Day 7 CD83hi and CD83low cells from the culture with GM-CSF/IL-4 for 5 days followed by 2 days with TNF-α/IL-1β were sorted to >95% purity and cultured further with M-CSF; NA CD83hi cells remained CD1a+CD14+, while CD83low cells readily converted to Mϕ. A two-color cytogram of day 5 MDDC labeled with FITC-CD1a and PE-CD83 mAbs is shown. A indicates the sort windows and the phenotype of the resulting sorted populations. B indicates the MLR-stimulating capacity for 5 × 10^3 allogeneic T lymphocytes (cpm) of day 7-sorted CD83hi and CD83low cells. Two-color cytograms and morphology (×400 magnification) of day 9 cells from the culture of sorted CD83hi (C) and CD83low (D) cells labeled with FITC-CD1a and PE-CD14 mAbs are shown.
FIGURE 8. A model of the MO differentiation pathway.

Acknowledgments

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