IL-4 alone without the involvement of GM-CSF transforms human peripheral blood monocytes to a CD1a\textsuperscript{dim}, CD83\textsuperscript{+} myeloid dendritic cell subset

Keshab Chandra Roy\textsuperscript{*}, Gautam Bandyopadhyay, Srabanti Rakshit, Mitali Ray and Santu Bandyopadhyay\textsuperscript{‡}

The Division of Immunology, Indian Institute of Chemical Biology, 4 Raja S.C. Mullick Road, Jadavpur, Kolkata, 700 032, India
\textsuperscript{*}Present address: Department of Microbiology and Immunology, Indiana University School of Medicine, 635 Barnhill Drive, Indiana, IN 46202-5120, USA
\textsuperscript{‡}Author for correspondence (e-mail: santu2@iicb.res.in)

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Summary

Myeloid dendritic cells (DCs) are conventionally generated by culturing human peripheral blood monocytes in the presence of GM-CSF and IL-4. Here we report that IL-4 alone, in the absence of detectable endogenous GM-CSF, transforms human peripheral blood monocytes to a CD1a\textsuperscript{dim} DC subset that could be matured to CD83\textsuperscript{+} DCs. Absence of endogenous GM-CSF in IL-4-DC was demonstrated by RT-PCR and flow cytometry. With the exception of CD1a expression, surface marker, morphology and phagocytic activity of these DCs (IL-4-DC) were similar to myeloid DCs (GM-IL-4-DC) conventionally generated in the presence of GM-CSF and IL-4. Conventional GM-IL-4-DC produced less IL-12 compared with IL-4-DC after stimulation with anti-CD40 monoclonal antibody, or LPS plus IFN-\(\gamma\), although the difference was more prominent when LPS plus IFN-\(\gamma\) was used as the stimulus. The GM-IL-4-DC also induced less frequent IFN-\(\gamma\)+ T cells in a mixed leukocyte reaction (MLR) than that of IL-4-DC. Yields of IL-4-DCs were marginally lower than that of GM-IL-4-DCs. Our data indicate that peripheral blood monocytes can be transformed to CD1a-deficient myeloid DCs solely by IL-4, and these IL-4-DCs are likely to induce a stronger Th1 response than conventional GM-IL-4-DCs.

Key words: Dendritic cell, IL-4, Blood monocyte

Introduction

Dendritic cells (DCs) are the most potent antigen-presenting cells (APCs) in the immune system and are critically involved in the initiation of primary immune responses, autoimmune diseases, graft rejection, human immunodeficiency virus infection, and the generation of T-cell-dependent antibodies (Steinman, 1991). In humans, at least two types of DCs have been established: plasmacytoid DCs (pDCs) and myeloid DCs (mDCs). Plasmacytoid and myeloid DCs differ phenotypically and functionally. The pDCs have features of a lymphoid lineage, produce Type I/II interferons (IFNs) and elicit a Th2-type response (Chehimi et al., 2002; Rissoan et al., 1999). Following culture with GM-CSF or M-CSF, pDCs do not differentiate into macrophages and have little ability to phagocytose or macropinocytose antigens. The pDCs depend on IL-3, but not on GM-CSF, for their survival and maturation as they express low GM-CSF receptor and high IL-3 receptor. Conversely, mDCs express myeloid antigens, produce IL-12 and induce a Th1-type response (Chehimi et al., 2002; Rissoan et al., 1999). Human peripheral blood monocytes give rise to immature mDCs after culturing with GM-CSF and IL-4 (Mayordomo et al., 1995; Romani et al., 1994; Gallus and Lanzavecchia, 1994) or after transmigration through endothelial cells and phagocytosis (Randolf et al., 1998). These immature cells become mature mDCs after stimulation with CD40 ligand (CD40L) or endotoxin (Cella et al., 1996; Koch et al., 1996).

GM-CSF appears to be instrumental in generating mDCs from peripheral blood monocytes and the role of IL-4 has previously been implicated to suppress generation of macrophages and to induce a more mature phenotype of DCs (Sallusto and Lanzavecchia, 1994). However, recent reports on the ability of IL-4 to induce dendritic-cell-specific ICAM-3 grabbing nonintegrin (DC-SIGN), a myeloid DC-specific lectin, suggest that IL-4 not only suppresses the monocyte and/or macrophage lineage but it actively promotes the differentiation of monocytes along the DC lineage (Relloso et al., 2002). Previous reports also suggest that IL-4 alone can activate accessory properties of monocytes and up-regulate MHC class-II molecules (Ruppert et al., 1991; Ulanova et al., 2001), co-stimulatory molecules (Ulanova et al., 2001) and down-regulate CD14 on monocytes (Lauener et al., 1990; Ruppert et al., 1991). Although previous reports studied the effects of IL-4 on monocytes, here we present the first comprehensive study demonstrating that IL-4 alone, without the involvement of endogenous or exogenous GM-CSF, transforms human peripheral blood monocytes to a CD1a\textsuperscript{dim} myeloid DC subset. These IL-4-DCs induce a stronger IL-12 response after stimulation, resulting in a stronger Th1 response than that of conventional GM-IL-4-DCs.

Materials and Methods

Reagents
Complete RPMI medium consisted of RPMI-1640, 1% L-glutamine, 1% penicillin/streptomycin, 1% essential amino acids and 10% heat-
inactivated FCS (all from Life Technologies, New Delhi). Recombinant human cytokines: rhGM-CSF, rhIL-4, rhTNF-α, and rhIL-2 (R and D Systems, Minneapolis, MN). RNA-extraction reagent, TRIzol and Superscript one-step RT-PCR kit (Life Technologies). FACSTM permeabilizing solution, rhIFN-γ and monoclonal antibodies (mAbs) (BD Biosciences, Mountain View, CA). Brefeldin A (Sigma-Aldrich, St. Louis, MO). Neutralizing mouse mAb to human GM-CSF without sodium azide and cytokine ELISA kits (R and D systems). Neutralization Dose50 for this antibody was determined to be approximately 0.3-0.5 μg/ml in the presence of 0.5 ng/ml of rhGM-CSF, using proliferation of a GM-CSF-dependent cell line TF-1. The mAbs used for flow cytometry analysis are listed in Table 1.

Generation of DCs

DCs were generated from adherent mononuclear cells in human blood. Blood was freshly drawn from healthy volunteers. Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll/Hypaque density gradient centrifugation, according to standard procedures. Monocytes were purified from PBMC using a two-hour-adherence step on bacteriologic plastic dishes coated with human IgG (Young et al., 1988). Non-adherent cells were flushed away by extensive washing with PBS. Adherent cells (>95% monocytes as determined by flowcytometric analysis of forward scatter/side scatter, surface staining for CD14, CD11c and intracellular staining for myeloperoxidase) were immediately subjected to the DC differentiation protocol as follows: monocytes were resuspended at 0.5×10^6 cells/ml and cultured in complete medium alone, or in complete medium containing IL-4 (1000 U/ml) plus neutralizing anti-GM-CSF antibody (10 μg/ml), or in GM-CSF (800 U/ml) plus IL-4 (1000 U/ml) in a total volume of 2 ml. Cells were cultured for 7 days, with cytokine addition every second day, to obtain immature cells. In selected experiments, graded concentrations (1.0-10.0 μg/ml) of neutralizing anti-GM-CSF mAb were added to monocyte cultures containing GM-CSF and IL-4 to evaluate the effect of GM-CSF neutralization on surface CD1a expression. For maturation, the initial presence of 0.5 ng/ml of rhGM-CSF, using proliferation of a GM-CSF-dependent cell line TF-1. The mAbs used for flow cytometry analysis are listed in Table 1.

Flow cytometry

Flow cytometry was performed to define the phenotypic characteristics of DCs generated in vitro from human peripheral blood monocytes, to assess phagocytic activity of DCs, and to analyse the intracellular cytokine and chemokine profiles in DCs and T cells. Analysis was performed using FACSCalibur flow cytometer and Cell Quest software (Becton Dickinson, San Jose, CA).

Detection of intracellular cytokines/chemokines

Cells were stimulated with LPS (1.0 μg/ml), or with LPS (1.0 μg/ml) plus IFN-γ (100 ng/ml) or with anti-CD40 mAb (10.0 μg/ml), for 18 hours as shown. Brefeldin A (10 μg/ml) was included in the culture for the last 4 hours. Cells were washed, surface stained with the chosen antibody, permeabilized by treatment with FACSTM permeabilization solution, stained with FITC- or PE-conjugated anti-cytokine/anti-chemokine mAbs (GM-CSF, IFN-γ, IL-4, IL-12, IL-13, IL-8, MIP-1α) or isotype-matched control mAbs, and were analysed in a flow cytometer (FACSCalibur). Intracellular cytokine analysis in T cells were performed in primary MLR and in allogeneic T cells after repetitive stimulation with DCs. For use as responders, the primary MLR were set up with non-adherent PBMC (NPBMC) after depletion of HLA-DR+ cells, B cells, NK cells [by mAb and complement-mediated lysis (Bandyopadhyay et al., 1986)], and allogeneic mature DCs were used as stimulators (1×10^6 NPBMC plus 1×10^6 DCs) in 24-well plates in 1 ml complete medium. Alloreactive T cells were expanded from primary culture with immature DCs from day 6 in the presence of 50 U/ml IL-2. Two weeks after priming, T cells were restimulated with immature DCs from the same donor as in the primary culture, with weekly repetitive stimulation. Six days after stimulation with DCs (after first stimulation or third stimulation), cultures were activated with 2.4 g/ml human IgG to prevent binding through Fc portion of the mAbs. Staining with anti-cytokine/anti-chemokine mAbs before permeabilization always resulted in <0.3% positive cells.

Phagocytosis

Cells were harvested from culture and resuspended at 5×10^5 cells/ml in complete medium. Five microliters of FITC-latex beads of 3 μm diameters (BD Biosciences) were added to the cells and mixed well. Five microliters of FITC-latex beads of 3 μm diameters (BD Biosciences) were added to the cells and mixed well. The cells were incubated with the beads for 30 minutes at 37°C. After incubation, the cells were washed five times with ice-cold PBS and then fixed overnight with 1% paraformaldehyde before analysis by flow cytometry.

Semiquantitative RT-PCR

Total RNA was isolated from DCs with RNA extraction reagent, TRIzol. Reverse transcription of mRNA into DNA and PCR were performed from 500 ng total RNA using the Superscript one-step RT-PCR kit. PCR amplification was performed with a thermal cycler (Applied Biosystems, Foster City, CA) for 30 cycles (30 seconds denaturation at 94°C, 30 seconds annealing at 50°C or 55°C, and 1 minute elongation at 72°C). A 10 μl portion of each PCR product was electrophoresed on a 2% agarose gel and visualized by ethidium bromide staining. Oligonucleotide primers (Axel et al., 2000; Hmama et al., 1998; Johansson et al., 2000; Pisa et al., 1992; Sato et al., 2000; 3436 Journal of Cell Science 117 (16)
mouse anti-human CD40 mAb (5 et al., 1999). Briefly, immature DCs were incubated in PBS containing ng/ml for 2 days) or by CD40 ligation as reported previously (Bianchi et al. 1999). Graded numbers of DCs were added to 1·10⁵ allogeneic NPBMC in 96-well, flat-bottom culture plates for 6 days. Proliferation was determined by the addition of 0.5 Ci [3 H]thymidine per well after the culture period and subsequent measurement of incorporated radioactivity in a liquid scintillation counter (PerkinElmer Life Sciences, Boston, MA).

Results

IL-4, without the involvement of endogenous GM-CSF, induces differentiation of adherent human peripheral blood monocytes to CD83⁺, CD1a⁻dim dendritic cells. Human peripheral blood monocytes were cultured in medium alone (Med-MO), or in medium containing IL-4 plus anti-GM-CSF antibody (IL-4-DC), or GM-CSF plus IL-4 (GM-IL-4-DC) for 7 days followed by a 2 day maturation period in the presence of TNF-α. Anti-GM-CSF antibody was included in monocyte cultures containing IL-4 alone to neutralize any endogenously synthesized GM-CSF that may have been present.

As monocytes were enriched by adherence to human IgG-coated plates, which might lead to cytokine production via cross-linking of Fc receptors, we investigated the endogenous production of GM-CSF of freshly isolated monocytes, and monocytes cultured for 1 and 7 days in the presence of IL-4 after stimulation with LPS. We also evaluated intracellular GM-CSF by day 1 to test whether GM-CSF is made by the cells at early time points in IL-4. Endogenous GM-CSF made at early time points might contribute to DC differentiation without being detectable at later time point (7 days). As shown in Fig. 1A, freshly isolated monocytes had only barely detectable intracellular GM-CSF before stimulation with LPS. However, upon stimulation with LPS, a significant proportion of fresh monocytes had intracellular GM-CSF. By contrast, when monocytes were cultured with IL-4 for 1 day or 7 days, intracellular GM-CSF was undetectable before stimulation with LPS. Even after stimulation with LPS, intracellular GM-CSF became virtually undetectable after 7 days. The sensitivity and specificity of intracellular detection of GM-CSF were demonstrated by analysing GM-CSF in non-permeable cells at early time points in IL-4. Endogenous GM-CSF made at early time points might contribute to DC differentiation without being detectable at later time point (7 days).

### Table 2. List of primers used for RT-PCR analysis

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show that IL-4 suppresses GM-CSF synthesis leading to undetectable GM-CSF in monocyte cultures containing IL-4, even at early time points. To rule out the involvement of endogenous GM-CSF in monocyte differentiation to DCs (in the presence of IL-4 alone), RT-PCR was performed to examine the expression of GM-CSF mRNA in these cells using varying concentrations of total cellular RNA. Using 500 ng total RNA, expression of GM-CSF was undetectable in all the cultures (Fig. 1Ci). However, when 2000 ng total RNA was used, expression of GM-CSF was detectable in monocytes cultured in medium (Med-Mo) but not in monocyte cultures containing IL-4 (IL-4-DC) or GM-CSF plus IL-4 (GM-IL-4-DC) (Fig. 1Cii). Although endogenous GM-CSF protein or mRNA was undetectable in monocyte cultures containing IL-4 alone, neutralizing anti-GM-CSF mAb was always included in these cultures to exclude a role for GM-CSF in DC transformation. For the determination of DC yields, the number of monocytes plated on day 0 was normalised to 100%. Percentages indicate the numbers of recovered DCs on day 7 (immature) or day 9 (mature) of cultures. IL-4 cultures (both immature and mature) contained approximately 20% fewer cells than the GM-CSF plus IL-4 cultures, as determined by counting in the haemocytometer with trypan blue (Fig. 1D). Morphologically, monocytes cultured in the presence of IL-4 alone, or in the presence of GM-CSF plus IL-4, were indistinguishable. Both cultures had DC-like veiled structures (Fig. 1E).

### Fig. 1. Endogenous GM-CSF is undetectable in IL-4-DC. (A) Detection of intracellular GM-CSF in monocytes (freshly isolated or cultured for 1 day and 7 days in the presence of IL-4) before and after stimulation with LPS. Relevant cells were surface stained for the expression of CD11c (FL2), permeabilized, stained for intracellular GM-CSF (FL1). Quadrants were set after staining with isotype-matched control mAbs. The proportion of positive cells are given in each quadrant. One representative experiment of three is shown. (B) Specificity and sensitivity of intracellular GM-CSF staining was demonstrated by surface staining fresh monocytes (stimulated with medium or LPS) with CD11c followed by staining for GM-CSF without permeabilization. (C) Detection of GM-CSF transcripts by RT-PCR. Total RNA was extracted from relevant cells, 500 ng (i) and 2000 ng (ii) RNA were used to analyse the expression of mRNA for GM-CSF and β-actin. Data are representative of three similar experiments. (D) Yields of immature and mature cells. The numbers of monocytes plated on day 0 was set equal to 100%. Percentages indicate the numbers of recovered cells on day 7 for immature and on day 9 of culture for mature cells. Data shown are from three separate experiments; error bars indicate s.d. (E) Phase contrast microscopy of TNF-α-stimulated (48 hours) mature cells. Note the numerous veiled processes in IL-4-DC and GM-IL-4-DC. Magnification ×300.
DC differentiation solely by IL-4

The efficacy of neutralizing anti-GM-CSF mAb to block endogenous GM-CSF was demonstrated by blocking the generation of CD1a+ cells when added to monocyte cultures containing GM-CSF plus IL-4. Freshly purified monocytes containing >90% CD14+ cells (top panel; dotted line represents staining with isotype-matched control Ig, unbroken line represents staining with anti-CD14 mAb) were stained for surface expression of CD1a and GM-CSF receptor α chain (CD116) immediately and after culture in media alone, or media containing GM-CSF (800 U/ml) plus IL-4 (1000 U/ml) and graded concentrations of neutralizing anti GM-CSF mAb (1.0 to 10.0 μg/ml), or media containing IL-4 alone. Data shown are from three separate representative experiments.

Mature IL-4-DCs expressed mRNA characteristic of mature GM-IL-4-DCs with the exception of CD1a

The mRNAs of CD1b, CD1c, CD83, HLA-DR and CXCR4 were detected in mature IL-4-DCs and were found to be as strong as mature myeloid DCs grown conventionally in GM-CSF plus IL-4 followed by a 48-hour maturation period by TNF-α. A band for CCR7 mRNA was also detected in mature IL-4-DCs and in GM-IL-4-DCs. CD1a mRNA was weakly detectable in mature IL-4-DCs but was strongly detectable in mature GM-IL-4-DCs supporting the flow cytometry data. Monocytes cultured in medium alone had barely detectable CD1a mRNA. As expected, faint mRNA bands of CD1c, CD83, CCR7, CXCR4, and strong bands of CD1b and HLA-DR mRNA were detected in monocytes cultured in medium alone (Fig. 3).

DCs generated solely by IL-4 have reduced phagocytic activity compared with that of myeloid DCs conventionally generated by GM-CSF plus IL-4

Previous studies have demonstrated that myeloid DCs rapidly lose phagocytic activity after in vitro culture (Reis e Sousa et al., 1993). As human peripheral blood monocytes after culture with IL-4 alone for 7 days developed a dendritic-cell-like morphology, and expressed surface markers preferentially expressed on dendritic cells we therefore wished to determine whether monocytes cultured in IL-4 were still capable of...
phagocytosis (like monocytes cultured in medium alone) or whether this property is lost like conventional myeloid DCs cultured in GM-CSF plus IL-4. Immature DCs were incubated at 37°C with FITC-latex beads and examined by flow cytometry to determine the proportion of cells phagocytosing latex particles. Freshly isolated monocytes, or monocytes cultured for 7 days in medium alone, were quite efficient in internalising FITC-latex beads (Fig. 4A). By contrast, monocytes cultured in IL-4 alone, or in GM-CSF plus IL-4, had greatly diminished phagocytic activity (Fig. 4A). Compared with freshly isolated monocytes, however, the expression of intracellular myeloperoxidase (MPO) was greatly reduced in monocytes when cultured in medium alone, or in medium containing IL-4, or in medium containing GM-CSF plus IL-4 (Fig. 4B).

IL-4-DCs produce cytokines and chemokines characteristic of conventional myeloid DCs upon activation with LPS

Conventional myeloid DCs produce a series of cytokines and chemokines implicated in the immune response. We wished to investigate whether a similar profile was detected in IL-4-DCs, so we examined the mRNA (RT-PCR) and the intracellular protein (flow cytometry) levels. Immature DCs were activated with LPS (1.0 μg/ml) for 6 hours for detection of mRNA, and 18 hours for detection of intracellular proteins by flow cytometry. IL-12 (p40) mRNA was detectable in unstimulated IL-4-DCs and GM-IL-4-DCs, which was not further up-regulated after stimulation with LPS. By contrast, this was undetectable in monocytes cultured in media (Med-Mo) but was strongly up-regulated in the presence of LPS. IL-10 and IFN-γ mRNA were undetectable in all unstimulated cultures but were strongly detected in IL-4-DCs and GM-IL-4-DCs in the presence of LPS. A faint band of TNF-α was detected in unstimulated Med-Mo, which was strongly up-regulated by LPS. By contrast, expression of TNF-α mRNA in IL-4-DCs and in GM-IL-4-DCs (detectable in unstimulated cells) was down-regulated by LPS stimulation (Fig. 5).

Intracellular IFN-γ, but not IL-4, was detected in IL-4-DCs and in GM-IL-4-DCs after stimulation with LPS. Neither IL-4 nor IFN-γ was detected in monocytes cultured in medium alone after LPS stimulation. IL-8- and MIP-1α-producing cells were marginally reduced in IL-4-DCs and in GM-IL-4-DCs when compared with monocytes cultured in medium alone followed by stimulation with LPS (Fig. 6A).

As we could not detect strong induction of IL-12 by LPS stimulation in GM-IL-4-DCs and in IL-4-DCs, additional stimuli were also tested. Intracellular IL-12-positive cells were detected in all IL-4-DCs and GM-IL-4-DCs after LPS stimulation (Fig. 6B).
detectable in IL-4-DCs (52.8%) and in GM-IL-4-DCs (58.3%) even in an unstimulated condition, supporting our RT-PCR data. When stimulation was induced by anti-CD40 mAb, intracellular IL-12-positive cells were drastically increased in GM-IL-4-DCs compared to IL-4-DCs (52.8% versus 91.8%). The differences in IL-12 production by IL-4-DCs versus GM-IL-4-DCs became more prominent when stimulation was induced by LPS plus IFN-γ. Intracellular IL-12-positive cells did not increase appreciably in GM-IL-4-DCs after stimulation with LPS plus IFN-γ (58.3% in unstimulated cells versus 57.0% in stimulated cells). By contrast, the same stimulus greatly increased IL-12-positive cells in IL-4-DCs (52.8% in unstimulated cells versus 81.8% in stimulated cells) (Fig. 6B). Intracellular IL-13-positive cells, which were marginally lower in unstimulated IL-4-DCs compared with GM-IL-4-DCs (4.5% versus 14.8%), did not increase significantly in both cases after stimulation with anti-CD40 mAb or LPS plus IFN-γ (Fig. 6B). Some of the cytokines and chemokines in the supernatants were quantified by commercial ELISA (Table 3).

**Fig. 6.** Intracellular cytokine/chemokine analysis of IL-4-DC by flow cytometry. (A) Cells were activated with LPS (1 µg/ml) for 18 hours. Brefeldin A was added to the cultures for the last 4 hours and then intracellular IFN-γ/IL-4 (upper row) and MIP-1α/IL-8 (lower row) were analysed. The percentages of the respective cytokine-producing cells are shown in each dot plot profile. Quadrants were set according to the fluorescence intensities of FITC- and PE-conjugated isotype-matched control Igs with irrelevant specificity. (B) Cells were left untreated or stimulated with anti-CD40 mAb (10 µg/ml) or LPS (1.0 µg/ml) plus IFN-γ (100 ng/ml) for 18 hours to detect intracellular IL-12/IL-13. Quadrants were set after staining with isotype matched control mAbs. The percent positive cells are given in each quadrant. The flow cytometry results are representative of three similar experiments.

**IL-4-DCs act as potent APCs in allogeneic mixed lymphocyte reaction (MLR)**

Because CD1a plays a role in presentation of antigens, at least to CD1-restricted T cells (Sieling et al., 1999), and IL-4-DCs had weaker expression of CD1a, we compared the efficacy of IL-4-DCs and conventional myeloid DCs in inducing allogeneic MLR. Both IL-4-DCs and GM-IL-4-DCs induced potent proliferation of allogeneic T cells (Fig. 7). The responses were higher when maturation of stimulator cells was induced either by TNF-α or by CD40 cross-linking. This is consistent with previous studies showing that the APC function of DC is up-regulated upon maturation (Zhou and Tedder, 1996). Induction of proliferation of allogeneic T cells by immature or mature IL-4-DCs was comparable to that of conventional GM-IL-4-DCs and the differences were not statistically significant at all 'stimulator to responder' ratios tested.

**Mature IL-4-DCs induce stronger Th1 response than GM-IL-4-DCs in primary MLR**

The polarized Th1 cytokine production profile induced by mature IL-4-DCs and GM-IL-4-DCs was evident by the detection of intracellular cytokines by means of flow cytometry. However, as shown in a representative experiment (Fig. 8), mature IL-4-DCs induced more IL-2-producing (23.06% versus 13.17%) and IFN-γ-producing (23.10% versus 14.94%) cells than GM-IL-4-DCs. In both cases, IL-4- and IL-10-producing cells were only barely detectable.

**Table 3. Secretion of cytokines and chemokines by IL-4 DC*\**

<table>
<thead>
<tr>
<th>Cytokine/Chemokine</th>
<th>Unstimulated (pg/ml)</th>
<th>Stimulated (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Med Mo</td>
<td>GM-IL-4-DC</td>
</tr>
<tr>
<td>IL-4</td>
<td>&lt;10.0</td>
<td>&lt;10.0</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>&lt;20.0</td>
<td>&lt;20.0</td>
</tr>
<tr>
<td>IL-13</td>
<td>50.0</td>
<td>&lt;32.0</td>
</tr>
<tr>
<td>IL-8</td>
<td>930.0</td>
<td>415.0</td>
</tr>
<tr>
<td>MIP-1α</td>
<td>350.0</td>
<td>85.0</td>
</tr>
</tbody>
</table>

*Cells were stimulated or not with LPS plus IFN-γ as described in Materials and Methods, supernatants were collected and assayed for indicated cytokines and chemokines using commercial ELISA kits. Data are representative of two similar experiments.
Induction of IL-10-producing T cells after repetitive stimulation with immature IL-4-DCs or GM-IL-4-DCs

To analyse the cytokine profile of alloreactive T cells after repetitive in vitro stimulation with immature IL-4-DCs and GM-IL-4-DCs, intracellular cytokine staining of the lymphocytes was performed. The alloreactive T cells induced by IL-4-DCs or GM-IL-4-DCs had reduced capacity to synthesize IL-2 or IFN-γ (Fig. 9) but an enhancement in IL-10 production was detected (Fig. 9).

Discussion

DCs are recognized as the most efficient APCs for the induction of primary immune responses. Several previous studies showed that DC could develop from CD14+ blood monocytes cultured with GM-CSF plus IL-4 (Mayordomo et al., 1995; Sallusto and Lanzavecchia, 1994), IL-3 plus IL-4 (Ebner et al., 2002), Flt-3L plus IL-4 or by CD40-CD40 ligand interactions (Brossart et al., 1998). IL-4 was shown to down-regulate CD14 and CD16 antigens, up-regulate MHC class II molecules and enhance the allo-stimulatory properties of monocytes (Ruppert et al., 1991; Velde et al., 1988). Exposure to aluminium hydroxide led to a significant activation of accessory properties of monocytes and an increase in expression of MHC class-II, CD40, CD54, CD58, CD83 and CD86 molecules (Ulanova et al., 2001). The enhanced accessory property of monocytes, and increased cell surface expression of co-stimulatory molecules on monocytes by aluminium hydroxide, was attributed to IL-4 released by Th2 cells. Expression of dendritic-cell-specific ICAM-3-grabbing non-integrin (DC-SIGN), considered as a monocyte-derived DC differentiation marker, was also shown to be dependent on IL-4 (Relloso et al., 2002). All these previous studies suggest that IL-4 modulates phenotypic and functional properties of human peripheral blood monocytes towards dendritic lineage.

Here we demonstrate, for the first time, that CD1a(dim) DC can develop from peripheral blood monocytes solely upon treatment with IL-4. Involvement of endogenous GM-CSF in IL-4-induced transformation of monocytes to DCs was ruled out by showing that GM-CSF mRNA or protein was...
undetectable in monocytes when cultured in the presence of IL-4. A number of laboratories have reported that IL-4 inhibits the production of GM-CSF in a variety of cells including human monocytes (Sato et al., 1994; Dechanet et al., 1995; Akashi et al., 1991; Trindade et al., 1999; Sawada et al., 1995; Galy and Spits, 1991) by down-regulation of RNA precursor (Akahane and Pluznik, 1992). These reports explain our findings that GM-CSF mRNA or protein is undetectable in IL-4-DCs. Additionally, neutralizing anti-GM-CSF antibody was included in monocyte cultures containing IL-4 alone to take care of any endogenously synthesized GM-CSF, if at all. The efficacy of the antibody to neutralize GM-CSF was demonstrated by its ability to block the generation of CD1a+ cells when added to cultures containing GM-CSF plus IL-4. With the exception of CD1a, these cells showed all the phenotypic and functional characteristics of conventional myeloid DC cultured in the presence of GM-CSF plus IL-4. Weak expression of CD1a on IL-4-DC was expected because CD1a expression on monocytes is dependent on GM-CSF (Kasinrerk et al., 1993). DC with reduced CD1a expression could be generated by culturing human peripheral blood monocytes in the presence of IL-3 and IL-4 (Ebner et al., 2002), or GM-CSF plus IL-4 in Yssel’s medium (Chang et al., 2000), GM-CSF, IL-4 and IFN-β (Huang et al., 2001) or GM-CSF, IL-4 and prostaglandin-E2 (Kalinski et al., 1997). Others have previously shown that CD1a+ and CD1a– myeloid DC may actually arise from different precursors (Sanchez-Torres et al., 2001). These CD1a–DCs, unlike conventional myeloid DCs expressing CD1a, shifted Th-responses toward a Th2 cytokine pattern (Chang et al., 2000; Ebner et al., 2002; Huang et al., 2001; Kalinski et al., 1997). Surprisingly, in our studies CD1a–DCs generated by culturing monocytes in IL-4 alone produced more IL-12 after stimulation, resulting in a stronger Th1 response in MLR compared with that of conventional GM-IL-4-DCs, which contain a mixture of CD1a+ and CD1a– cells. However, a potential problem exists in comparing data from a heterogenous (with respect to CD1a expression) DC population (GM-IL-4-DC) with the relatively more homogeneous (CD1a–) IL-4-DCs. Conversely, IL-10-producing T cells emerged at almost equal frequency by both GM-IL-4-DCs and IL-4-DCs after repeated in vitro stimulation. Alloreactive T cells with IFN-γ- and IL-2-producing Th1 characteristics, and IL-10-producing non-proliferating T cells with regulatory properties, were generated previously by conventional mature and immature myeloid DCs, respectively (Jonuleit et al., 2000).

LPS stimulates monocytes to produce IL-8 and MIP-1α. Moderate reduction in IL-8 and MIP-1α, but enhanced production of IL-10 and IFN-γ were observed both in IL-4-DCs and in conventional myeloid DCs after LPS stimulation. Our results are in accordance with previous studies where enhanced production of IFN-γ and IL-10 by peripheral blood DCs and in conventional myeloid DCs after LPS stimulation. Our results are in accordance with previous studies where enhanced production of IFN-γ and IL-10 by peripheral blood DCs and in conventional myeloid DCs after LPS stimulation. Our results are in accordance with previous studies where enhanced production of IFN-γ and IL-10 by peripheral blood DCs and in conventional myeloid DCs after LPS stimulation. Our results are in accordance with previous studies where enhanced production of IFN-γ and IL-10 by peripheral blood DCs and in conventional myeloid DCs after LPS stimulation.

In conclusion, our findings provide evidence that IL-4 alone, without the involvement of GM-CSF, can transform human peripheral blood monocytes to myeloid DCs with deficient surface CD1a expression. In spite of deficient CD1a expression, these DCs induce stronger a Th1 response in MLR than that of conventional myeloid DCs generated in the presence of GM-CSF plus IL-4. A functional implication of the lack of CD1a expression may relate to the function of CD1a molecules as efficient presenting molecules for microbial lipid antigens (Seiling et al., 1999). IL-4-DC may therefore be deficient in presenting microbial lipid antigens.

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References


