

Correspondence

GM-CSF along with IL-4 but not alone is indispensable for the differentiation of human dendritic cells from monocytes

To the Editor:

López-Bravo et al.¹ reported that IL-4 blocks T_H1-polarizing/inflammatory cytokine expression during murine monocyte-derived dendritic cell (Mo-DC) differentiation. For their experimental set-up, authors cultured monocytes in the presence of GM-CSF alone for 24 hours to obtain control Mo-DCs or with IL-4 to derive IL-4-Mo-DCs. They concluded that IL-4 during murine Mo-DC differentiation prevent the ability of LPS-stimulated Mo-DCs to produce T_H1-polarizing and inflammatory cytokines. To obtain translational insight on this report, we investigated whether the Mo-DC differentiation condition and duration, and conclusion made by López-Bravo et al, is also pertinent in human Mo-DCs.

We differentiated CD14⁺ human monocytes with GM-CSF alone or with IL-4 for 24 hours (see the Methods section in this article's Online Repository at www.jacionline.org) as described by López-Bravo et al. In contrast to mice, human monocytes did not differentiate into DCs in 24 hours in either of the conditions (Fig 1, A and B). Most of the cells remained positive for CD14, and only 2% to 3% of the cells were CD1a⁺, a differentiation marker for human DCs. We then followed the kinetics of Mo-DC differentiation over 5 days.² Monocytes differentiated with GM-CSF and IL-4 progressively lost CD14 and CD16 during this period. At day 5, differentiated DCs were either negative or very low positive for the above markers and were highly positive for CD1a (Fig 1, C). In contrast, cells differentiated in GM-CSF alone never acquired the DC phenotype even after 5 days: about 20% of the cells retained CD14 and were highly positive for CD16. The expression of CD1a was less than 10% (Fig 1, C). Thus, GM-CSF-differentiated cells exhibited phenotype similar to those of M1-type macrophages rather than DCs.³ Of note, the expression of CD14 and CD16 was retained on these GM-CSF-differentiated M1-type cells even following LPS stimulation while they were negative on GM-CSF and IL-4-differentiated DCs (Fig 1, D and E).

As in the study by López-Bravo et al, GM-CSF-differentiated cells produced large quantities of inflammatory cytokines on LPS stimulation (Fig 1, F). But unlike mice, these data only indicate differences between human M1 macrophages and DCs but not 2 DC subsets in their ability to produce cytokines. The high proportion of inflammatory cytokines produced by GM-CSF-differentiated cells could also be attributed to CD14 molecules on these cells because CD14 acts as a coreceptor for LPS.⁴ However, no major differences were observed in IL-10 and IL-12 secretion by these 2 cell types.

Pathogen-associated molecular patterns (PAMPs) have differential abilities to polarize T cells on interaction with DCs.⁵ By using PAMPs of *Mycobacterium tuberculosis*, that is, mannosylated lipoarabinomannan (ManLAM), a glycolipid,⁶ and Rv3812, a cell wall-associated protein belonging to proline-glutamic acid (PE)-polymorphic GC-rich repetitive-sequences (PGRS) family,⁷ we examined the T-cell-polarizing capacity of GM-CSF-IL-4-differentiated Mo-DCs. While DCs stimulated with ManLAM polarized T_H2 responses, this polarization could

be skewed toward T_H1 when DCs were stimulated with a combination of ManLAM and Rv3812 (Fig 1, G). Thus, the presence of IL-4 during human Mo-DC differentiation does not block the ability of DCs to polarize T_H1 responses; rather, it is dictated by PAMP-pattern recognition receptor interaction.

To conclude, although murine DCs could be obtained by culturing monocytes with GM-CSF alone for 24 hours, this condition is not applicable for humans because it will yield M1-type macrophages. Thus, GM-CSF and IL-4 are both indispensable for the differentiation of human Mo-DCs and these cells retain the ability to polarize T_H1 responses depending on the stimuli they receive.

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Editor's note: There is no accompanying reply to this correspondence.

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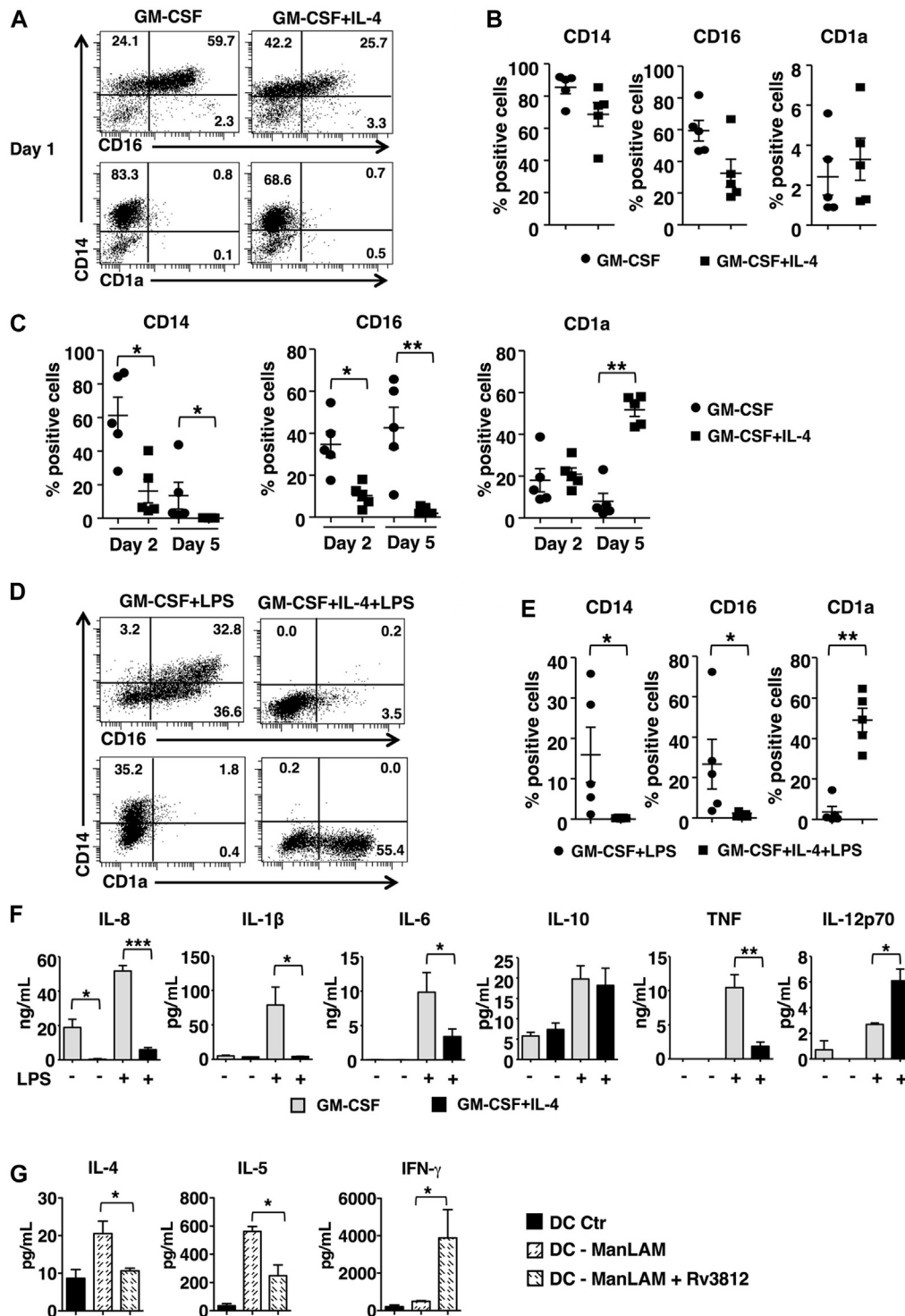


FIG 1. Both GM-CSF and IL-4 are indispensable for the human Mo-DC differentiation. **A** and **B**, Flow cytometric analysis of cells following 24 hours differentiation of monocytes in GM-CSF or GM-CSF + IL-4. **C**, Kinetics of expression of markers over 5 days of differentiation ($n = 5$). Phenotype (**D** and **E**) and cytokines (**F**) secreted by LPS-stimulated GM-CSF or GM-CSF+IL-4-differentiated cells ($n = 5$). **G**, T-cell-polarizing ability of GM-CSF+IL-4-differentiated DCs stimulated with ManLAM or ManLAM + Rv3812 ($n = 3$). * $P < .05$, ** $P < .01$, and *** $P < .001$.

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METHODS

Human monocyte differentiation

Human PBMCs were isolated from buffy coats of healthy blood donors purchased from Centre Necker-Cabanel (Etablissement Français du Sang, Paris, France). Ethical committee permission was obtained for the use of buffy coats of healthy donors (No. 12/EFS/079). Circulating monocytes were isolated from these PBMCs using CD14 microbeads (Miltenyi Biotec, Paris, France). The purity was more than 98%. Monocytes were cultured in RPMI-1640 medium containing 10% FCS for indicated days in the presence of recombinant human GM-CSF (1000 IU/10⁶ cells) alone or with recombinant human IL-4 (500 IU/10⁶ cells)^{E1} (both from Miltenyi Biotec) and used for subsequent experiments and analysis.

Stimulation of cells with LPS

Cells were harvested following differentiation of monocytes for 5 days either in GM-CSF alone or in GM-CSF and IL-4. The cells were washed and further cultured in respective cytokines and stimulated with LPS (100 ng/mL/0.5 × 10⁶ cells, from *Escherichia coli*, Sigma-Aldrich, Lyon, France) for 24 hours. Cell culture supernatants were collected for analyzing cytokines, and the phenotype of cells were analyzed by flow cytometry.

DC:CD4⁺ T-cell cocultures

DCs were derived from differentiation of monocytes for 5 days in GM-CSF and IL-4. The cells were washed and were cultured (0.5 × 10⁶ cells/mL) with GM-CSF and IL-4 alone or GM-CSF, IL-4, 5 μg/mL of ManLAM (BEI resource, purified from H37Rv, NR-14848) or GM-CSF, IL-4, 5 μg/mL of ManLAM, 5 μg/mL of Rv3812 for 48 hours. Recombinant Rv3812 protein was obtained as previously described.^{E2} Following extensive washings, DCs were cocultured with CD4⁺ T cells at 1:20 for 96 hours. T-cell cytokines in DC:T cell cocultures were analyzed in cell-free culture supernatants.

Flow cytometry

Surface staining of cells was carried out by using fluorochrome-conjugated mAbs to CD14, CD16, and CD1a (all from BD Biosciences, Le Pont de Claix, France). Cells were further processed for flow cytometry wherein 5000 gated events were recorded for each sample acquired and the data were analyzed using FACSDiva software (BD Biosciences).

Cytokine analysis

Cytokines were quantified in cell-free culture supernatants using BD Cytometric Bead Array human inflammatory cytokine kits and human T_H1/T_H2 cytokine kits (BD Biosciences). The data were analyzed using FCAP Array software (BD Biosciences).

Statistical analysis

Levels of significance for comparison between samples were determined by 2-tailed nonparametric Mann-Whitney test (for data depicted in figures as the median along with an appropriate range values) or by Student *t* test (for data depicted as means and SDs).^{E3} Values of *P* < .05 were considered statistically correlated (**P* < .05, ***P* < .01, ****P* < .001). All statistical analyses were performed using Prism 5 software (GraphPad Software, Inc, La Jolla, Calif).

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