Brief report

Regulation of 25-hydroxyvitamin D_3-1α-hydroxylase and production of 1α,25-dihydroxyvitamin D_3 by human dendritic cells

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25-Hydroxyvitamin D_3-1α-hydroxylase (25(OH)D_3-1α-hydroxylase), the key enzyme of 1α,25-dihydroxyvitamin D_3 (1,25(OH)$_2D_3$) production, is expressed in monocyte-derived macrophages (MACs). Here we show for the first time constitutive expression of 25(OH)D_3-1α-hydroxylase in monocyte-derived dendritic cells (DCs), which was increased after stimulation with lipopolysaccharide (LPS). Accordingly, DCs showed low constitutive production of 1,25(OH)$_2D_3$, but activation by LPS increased 1,25(OH)$_2D_3$ synthesis. In addition, 25(OH)D_3-1α-hydroxylase expression was found in blood DCs but not in CD34$^+$-derived DCs. Next we analyzed the functional consequences of these results. Addition of 1,25(OH)$_2D_3$ at concentrations comparable with those produced by DCs inhibited the allostimulatory potential of DCs during the early phase of DC differentiation. However, terminal differentiation decreased the responsiveness of DCs to 1,25(OH)$_2D_3$. In conclusion, DCs are able to produce 1,25(OH)$_2D_3$ especially following stimulation with LPS. Terminal maturation renders DCs unresponsive to the effects of 1,25(OH)$_2D_3$, but those cells are able to suppress the differentiation of their own precursor cells in a paracrine way through the production of 1,25(OH)$_2D_3$. (Blood. 2003;102: 3314-3316)

Introduction

The active form of vitamin D, 1α,25-dihydroxyvitamin D_3 (1,25(OH)$_2D_3$), positively modulates the differentiation of monocyte-derived macrophages (MACs),[1,2] whereas the functions of lymphocytes and dendritic cells (DCs) are suppressed.[3,4] At least in vitro, monocytes are precursor cells of both MACs and DCs, and the specific microenvironment determines their fate (eg, differentiation into either DCs or MACs). Positive regulators of MAC differentiation such as macrophage colony-stimulating factor or 1,25(OH)$_2D_3$ inhibit DC differentiation in vitro.[5-9] Accordingly, vitamin D receptor–deficient mice that show an unresponsiveness to 1,25(OH)$_2D_3$ have increased numbers of DCs in lymph nodes, suggesting a physiologically relevant inhibition of DC differentiation by 1,25(OH)$_2D_3$.[10]

The normal serum level of 1,25(OH)$_2D_3$ is relatively low (10^{-10} M), whereas that of 25-hydroxyvitamin D_3 (25(OH)D_3), the major circulating vitamin D metabolite, is about 1000-fold higher. Synthesis of 1,25(OH)$_2D_3$ from its precursor 25(OH)D_3 normally occurs in the kidney, however several reports describe extrarenal production of 1,25(OH)$_2D_3$.[11,12] This production is believed not to exert systemic effects but rather suggests a local paracrine function of 1,25(OH)$_2D_3$. We and others have shown that MACs convert 25(OH)D_3 into 1,25(OH)$_2D_3$[13,14] and have speculated that extrarenal 1,25(OH)$_2D_3$ production is involved in the autocrine/paracrine regulation of MAC differentiation. In light of recent publications on the inhibitory effect of 1,25(OH)$_2D_3$ on DC differentiation, the absence or presence of 1,25(OH)$_2D_3$ within the microenvironment could determine whether a monocyte becomes a MAC or DC. Therefore we were interested as to whether, similar to MACs, DCs can also convert 25(OH)D_3 into 1,25(OH)$_2D_3$ and found that DCs are indeed a possible extrarenal source of 1,25(OH)$_2D_3$.

Study design

Cell separation and culture

DCs and MACs were derived from human blood monocytes or CD34$^+$ as described previously.[15,16] Terminal maturation was induced with 10 ng/mL tumor necrosis factor (TNF) or 10 ng/mL lipopolysaccharide (LPS) or a mixture containing 10 ng/mL TNF-α, 10 ng/mL interleukin-1β (IL-1β), 1000 U/mL IL-6, and 1 μg/mL prostaglandin E_2.[17]

Northern blot analysis

A cDNA fragment of 25-hydroxyvitamin D_3-1α-hydroxylase (25(OH)D_3-1α-hydroxylase) complementary to the nucleic acids +931 base pair (bp) to +2073 bp (GenBank/European Molecular Biology Laboratory [EMBL] accession no. AB005989) was used for hybridization. As a loading control, membranes were rehybridized with an 18S rRNA-oligonucleotide (5′-ACG GTA TCT GAT CGT CTT CGA ACC-3′).

Immunohistochemistry

DCs were fixed with glutaraldehyde and a standard alkaline phosphatase antialkaline phosphatase (APAAP) staining was performed with a polyclonal sheep antibody against mouse and human 25(OH)D_3-1α-hydroxylase (The Binding Site, Birmingham, United Kingdom), a secondary rabbit antisheep antibody (Abcam, Cambridge, United Kingdom), APAAP reagent (Dianova, Hamburg, Germany), and Fast Red as a detection substrate (BioGenex, San Ramon, CA).

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1α,25-Dihydroxyvitamin D_3 ELISA

Cells were seeded (10^6 cells/well/mL) in RPMI medium in a 6-well plate. After incubation for 24 hours with 5×10^{-8} M $25(OH)D_3$ (Sigma, Deisenhofen, Germany) with or without 100 ng/mL LPS (*Salmonella abortus equi*, kindly provided by Dr Chris Galanos, MPI, Freiburg, Germany), the supernatant and the cells were harvested. $1,25(OH)_2D_3$ was separated from other vitamin D metabolites by extraction columns (Immundiagnostik, Bensheim, Germany) and determined with a commercially available enzyme-linked immunosorbent assay (ELISA; Immundiagnostik). This ELISA is specific for $1,25(OH)_2D_3$ and does not recognize other vitamin D metabolites.

Mixed lymphocyte reaction (MLR)

T cells (10^5) were incubated with different amounts of immature and mature allogenic DCs in RPMI containing 5% T-cell autologous plasma. On day 6 of coculture, 1 μCi (0.037 MBq) of ^{3}H-methyl-thymidine/well was added, and incorporated radioactivity was determined after 20 hours. All samples were performed in triplicates and values represent mean ± SEM.

Results and discussion

$25(OH)D_3$-1α-hydroxylase, the mitochondrial cytochrome P450 enzyme that catalyzes the conversion of $25(OH)D_3$, was detectable only in the late stages of DC differentiation, and the level of expression was low independent of the culture condition (granulocyte-macrophage colony-stimulating factor [GM-CSF] plus IL-4 vs interferon α [IFNα]).[16] Terminal differentiation of DCs with LPS clearly up-regulated the expression (Figure 1A). In contrast, MACs cultured either in human AB-group serum or with fetal calf serum (FCS) plus GM-CSF showed a strong expression of $25(OH)D_3$-1α-hydroxylase mRNA. $CD34^+$-derived DCs[15] cultured with stem cell factor (SCF), GM-CSF, and TNF-α showed no $25(OH)D_3$-1α-hydroxylase mRNA expression (data not shown). At the protein level, immature DCs on day 7 of culture were weakly positive for $25(OH)D_3$-1α-hydroxylase (data not shown), and terminal differentiation of DCs markedly increased the expression (Figure 1B). $25(OH)D_3$-1α-Hydroxylase expression was also found in freshly isolated blood DCs (Figure 1E). The specificity of the $25(OH)D_3$-1α-hydroxylase antibody used has previously been demonstrated by Zehnder et al[18] who detected extrarenal expression of $25(OH)D_3$-1α-hydroxylase in different tissues (eg, lymph nodes). Parallel staining with CD68 indicated that $25(OH)D_3$-1α-hydroxylase is expressed in MACs and/or DCs as both cell types express CD68.[18,19]

Next we measured the $1,25(OH)_2D_3$ levels in the supernatants of immature and mature DCs. Low constitutive production was found in monocytes and immature DCs up to day 4 of culture, but stimulation with LPS always up-regulated $1,25(OH)_2D_3$ synthesis (Figure 1C). Accordingly, terminal differentiation induced with LPS stimulated $1,25(OH)_2D_3$ synthesis, but other inducers of terminal differentiation had no effect on $1,25(OH)_2D_3$ production (Figure 1D). Therefore the up-regulation of $25(OH)D_3$-1α-hydroxylase by LPS represents an activation rather than a differentiation event. Accordingly, positive regulation of $25(OH)D_3$-1α-hydroxylase by LPS has already been shown by Reichel et al in human MACs.[20]

Mature DCs synthesize $1,25(OH)_2D_3$ at a maximal concentration of 5×10^{-9} M. To analyze the functional consequences of $1,25(OH)_2D_3$ production by DCs we added 5×10^{-9} M $1,25(OH)_2D_3$ or 5×10^{-8} M of the precursor $25(OH)D_3$ to DCs on day 1 and day 6, respectively, and performed mixed lymphocyte reaction. Even at this low concentration, $1,25(OH)_2D_3$ markedly inhibited antigen-presentation capabilities when added in the early stage of DC differentiation on day 1 (Figure 2A), but the precursor $25(OH)D_3$ had no effect in DCs cultured with 10% FCS. In contrast, DCs generated with 2% autologous plasma were also suppressed by the precursor (Figure 2B). This is in line with our finding that the production of $1,25(OH)_2D_3$ was extremely high under serum-free conditions or in the presence of autologous plasma but low in the presence of FCS (data not shown), indicating an inhibitory effect of FCS on $25(OH)D_3$-1α-hydroxylase activity.

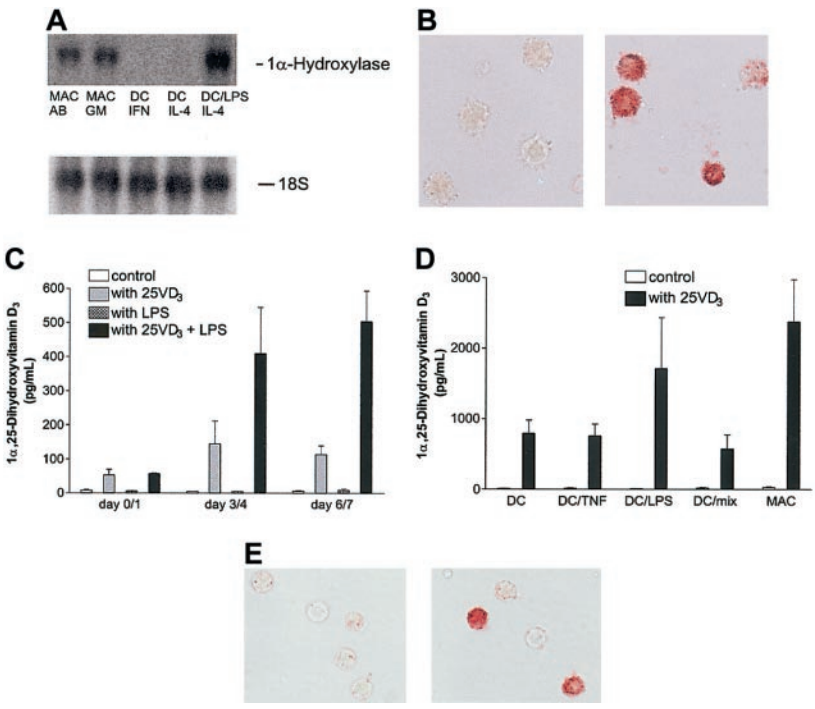

Figure 1. Expression of $25(OH)D_3$-1α-hydroxylase and production of $1,25(OH)_2D_3$ by different types of immature and mature DCs. (A) RNA of monocyte-derived DCs (GM/IL-4, GM/IFNα) and MACs (AB, GM) cultured for 5 days was used for Northern blot analysis. Terminal differentiation of DCs was induced by LPS (DC/LPS). The expression of $25(OH)D_3$-1α-hydroxylase protein in DCs matured by stimulation with LPS (DC/LPS) was determined by immunohistochemistry. As negative control, staining without the primary antibody is shown (B, left panel). (C) $1,25(OH)_2D_3$ synthesis was measured by ELISA. Freshly isolated monocytes or monocytes differentiated along the DC pathway for 3 or 6 days were harvested and cultured for another 24 hours under serum-free conditions with $25(OH)D_3$ in the absence or presence of 100 ng/mL LPS. The values represent mean ± SEM of at least 3 experiments. (D) The maturation of DCs was induced on day 4 of culture for 72 hours with tumor necrosis factor (DC/TNF-α) or LPS (DC/LPS) or a mixture containing TNF-α, IL-1β, IL-6, and prostaglandin E_2 (DC/mix). The values represent mean ± SEM of 4 experiments. (E) Blood DCs were isolated from mononuclear cells,[15] and the expression of $25(OH)D_3$-1α-hydroxylase was investigated by immunohistochemistry. Original magnification of panels B and E, × 400. In B and E, left panels show negative control (without primary antibody) and right panels show the positive sample (with primary antibody).

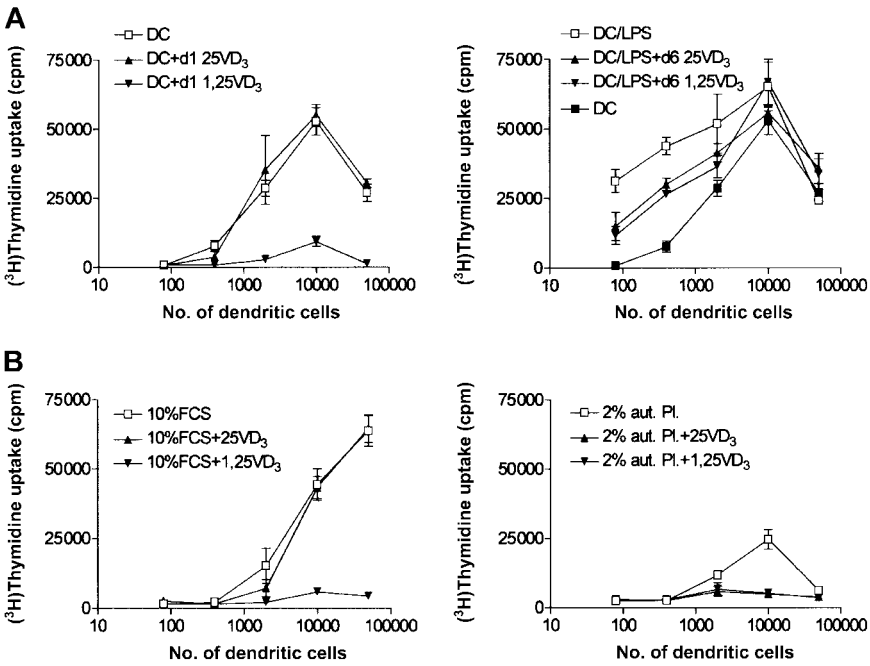

Figure 2. The effect of $1,25(OH)_2D_3$ and $25(OH)D_3$ on DC function. On day 1 or day 6 of DC culture, 5×10^{-8} M $25(OH)D_3$ or 5×10^{-9} M $1,25(OH)_2D_3$ (kindly provided by Hoffmann-La Roche, Basel, Switzerland) was added with (right) or without (left) induction of terminal differentiation (A; n = 3). In panel B (n = 2), we compared DCs generated with either 10% FCS (left) or 2% autologous plasma (aut PL; right). After 8 days, antigen presentation was determined by MLR.

Terminal differentiation has been reported to render DCs unresponsive to the actions of $1,25(OH)_2D_3$ with regard to the expression of the maturation-associated antigen CD83,[6] and only these DCs produce high amounts of $1,25(OH)_2D_3$ comparable with MACs. Accordingly, we found a stable expression of maturation-associated antigens on terminally differentiated DCs (data not shown), but exposure to both vitamin D_3 metabolites still slightly influenced their capacity for antigen presentation (Figure 2A).

In summary, our results clearly show that myeloid DCs are a possible extrarenal source of $1,25(OH)_2D_3$. The high local $25(OH)D_3$-1α-hydroxylase production of $1,25(OH)_2D_3$ by DCs (and MACs) after LPS stimulation may serve as a paracrine signal during bacterial infection and favor MAC differentiation but suppress DC differentiation and lymphocyte activation.

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