Regulation of 25-hydroxyvitamin D₃-1α-hydroxylase and production of 1α,25-dihydroxyvitamin D₃ by human dendritic cells

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25-Hydroxyvitamin D₃-1α-hydroxylase (25(OH)D₃-1α-hydroxylase), the key enzyme of 1α,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) production, is expressed in monocyte-derived macrophages (MACs). Here we show for the first time constitutive expression of 25(OH)D₃-1α-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)₂D₃, but activation by LPS increased 1,25(OH)₂D₃ synthesis. In addition, 25(OH)D₃-1α-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)₂D₃ 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)₂D₃. In conclusion, DCs are able to produce 1,25(OH)₂D₃ especially following stimulation with LPS. Terminal maturation renders DCs unresponsive to the effects of 1,25(OH)₂D₃, 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)₂D₃.

Study design

Cell separation and culture
DCs and MACs were derived from human blood monocytes or CD34⁺ as described previously. 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 E₂.

Northern blot analysis
A cDNA fragment of 25-hydroxyvitamin D₃-1α-hydroxylase (25(OH)D₃-1α-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 GA T 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)D₃-1α-hydroxylase (The Binding Site, Birmingham, United Kingdom), a secondary rabbit antiseep antibody (Abcam, Cambridge, United Kingdom), APAAP reagent (Dianova, Hamburg, Germany), and Fast Red as a detection substrate (BioGenex, San Ramon, CA).

Introduction

The active form of vitamin D, 1α,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃), positively modulates the differentiation of monocyte-derived macrophages (MACs), whereas the functions of lymphocytes and dendritic cells (DCs) are suppressed. 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)₂D₃ inhibit DC differentiation in vitro. Accordingly, vitamin D receptor-deficient mice that show an unresponsiveness to 1,25(OH)₂D₃ have increased numbers of DCs in lymph nodes, suggesting a physiologically relevant inhibition of DC differentiation by 1,25(OH)₂D₃.

The normal serum level of 1,25(OH)₂D₃ is relatively low (10⁻¹⁰ M), whereas that of 25-hydroxyvitamin D₃ (25(OH)D₃), the major circulating vitamin D metabolite, is about 1000-fold higher. Synthesis of 1,25(OH)₂D₃ from its precursor 25(OH)D₃ is expressed in CD34⁺-derived DCs. Next we analyzed the functional consequences of these results. Addition of 1,25(OH)₂D₃ 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)₂D₃.

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Supported by the Deutsche Forschungsgemeinschaft Kr1418/4-2.

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1α,25-Dihydroxyvitamin D₃ ELISA

Cells were seeded (10⁶ cells/well/mL) in RPMI medium in a 6-well plate. After incubation for 24 hours with 5 × 10⁻⁵ M 25(OH)D₃ (Sigma, Deisenhofen, Germany) with or without 10 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)₂D₃ was separated from other vitamin D metabolites by extraction columns (Immunodiagnostik, Bensheim, Germany) and determined with a commercially available enzyme-linked immunosorbent assay (ELISA; Immunodiagnostik). This ELISA is specific for 1,25(OH)₂D₃ and does not recognize other vitamin D metabolites.

Mixed lymphocyte reaction (MLR)

T cells (10⁵) 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 ³H-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)D₃-1α-hydroxylase, the mitochondrial cytochrome P450 enzyme that catalyzes the conversion of 25(OH)D₃, 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]). 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)D₃-1α-hydroxylase mRNA. CD34⁺-derived DCs cultured with stem cell factor (SCF), GM-CSF, and TNF-α showed no 25(OH)D₃-1α-hydroxylase mRNA expression (data not shown). At the protein level, immature DCs on day 7 of culture were weakly positive for 25(OH)D₃-1α-hydroxylase (data not shown), and terminal differentiation of DCs markedly increased the expression (Figure 1B). 25(OH)D₃-1α-Hydroxylase expression was also found in freshly isolated blood DCs (Figure 1E). The specificity of the 25(OH)D₃-1α-hydroxylase antibody used has previously been demonstrated by Zehnder et al. who detected extrarenal expression of 25(OH)D₃-1α-hydroxylase in different tissues (e.g., lymph nodes). Parallel staining with CD68 indicated that 25(OH)D₃-1α-hydroxylase is expressed in MACs and/or DCs as both cell types express CD68.

Next we measured the 1,25(OH)₂D₃ 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)₂D₃ synthesis (Figure 1C). Accordingly, terminal differentiation induced with LPS stimulated 1,25(OH)₂D₃ synthesis, but other inducers of terminal differentiation had no effect on 1,25(OH)₂D₃ production (Figure 1D). Therefore the up-regulation of 25(OH)D₃-1α-hydroxylase by LPS represents an activation rather than a differentiation event. Accordingly, positive regulation of 25(OH)D₃-1α-hydroxylase by LPS has already been shown by Reichel et al. in human MACs.

Mature DCs synthesize 1,25(OH)₂D₃ at a maximal concentration of 5 × 10⁻⁹ M. To analyze the functional consequences of 1,25(OH)₂D₃ production by DCs we added 5 × 10⁻⁹ M 1,25(OH)₂D₃ or 5 × 10⁻³ M of the precursor 25(OH)D₃ to DCs on day 1 and day 6, respectively, and performed mixed lymphocyte reaction. Even at this low concentration, 1,25(OH)₂D₃ markedly inhibited antigen-presentation capabilities when added in the early stage of DC differentiation on day 1 (Figure 2A), but the precursor 25(OH)D₃ 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)₂D₃ 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)D₃-1α-hydroxylase activity.
Terminal differentiation has been reported to render DCs unresponsive to the actions of 1,25(OH)2D3 with regard to the expression of the maturation-associated antigen CD83.6 and only these DCs produce high amounts of 1,25(OH)2D3 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 D3 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)2D3. The high local 25(OH)D-1α-hydroxylase production of 1,25(OH)2D3 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.

Acknowledgments

We thank Ute Ackermann, Alexandra Müller, and Alice Peuker for excellent technical assistance.

References