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Monocyte-derived cells express CYP27A1 and convert vitamin D3 into its active metabolite

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Abstract

CYP27A1 catalyses hydroxylations in the biosynthesis of bile acids and the bioactivation of vitamin D3. We investigated the expression of CYP27A1 in human monocytes, monocyte-derived macrophages, and dendritic cells on mRNA and protein levels as well as its enzymatic activity in comparison with the expression of CYP27B1 and CYP24A1. Macrophages showed a strong expression of CYP27A1, whereas monocytes and dendritic cells expressed low levels of CYP27A1 mRNA. Immunohistochemistry revealed CYP27A1 and CYP27B1 protein expression in macrophages. Accordingly, macrophages converted vitamin D3 into the active metabolite $1,25(OH)_2D_3$. Dendritic cells also metabolized vitamin D3 although to a lesser extent. This could be due to the high expression of CYP24A1, the enzyme that degrades $25(OH)D_3$ and $1,25(OH)_2D_3$. Our results show that macrophages and dendritic cells are capable to perform both hydroxylation steps of the vitamin D3 metabolism suggesting a possible role of local $1,25(OH)_2D_3$ synthesis by myeloid cells in the skin and gut.

Keywords: Monocyte; Macrophage; Dendritic cell; Active vitamin D3; CYP27A1; CYP27B1; CYP24A1

Vitamin D3, as synthesized in the skin by photolysis from 7-dehydrocholesterol or ingested in the diet, is transported in the blood by the vitamin D binding protein (DBP) [1] to the liver where it is hydroxylated at the C-25-position. Subsequently, after transport to the kidney by DBP, it is hydroxylated at the C-1α-position to form the active metabolite 1α,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$). In the current understanding of vitamin D3 metabolism, the skin represents the unique site of vitamin D3 production and the liver is thought to be the main site of conversion of vitamin D3 to 25-hydroxyvitamin D_3 ($25(OH)D_3$) by sterol 27-hydoxylase (CYP27A1). The next step is the conversion of $25(OH)D_3$ to the active metabolite $1,25(OH)_2D_3$ by P450 cytochrome 25-hydroxyvitamin D_3 1,α -hydroxylase (CYP27B1) in the kidney. $1,25(OH)_2D_3$ is degraded by P450 cytochrome 25-hydroxyvitamin D_3 24-hydroxylase (CYP24A1) into the inactive metabolite $1,24,25(OH)_2D_3$. This enzyme is not only responsible for the catabolism of $1,25(OH)_2D_3$ but also converts $25(OH)D_3$ into $24,25(OH)_2D_3$ [2,3].

Several reports describe the extrarenal production of $1,25(OH)_2D_3$ from $25(OH)D_3$ and suggest a local paracrine immune regulatory function of $1,25(OH)_2D_3$ besides its well-known regulation of calcium homeostasis [4]. Endothelial cells [5] and epithelial cells [6] express CYP27B1 and produce $1,25(OH)_2D_3$. We and others have shown that macrophages (MAC) and dendritic cells (DC) convert $25(OH)D_3$ into $1,25(OH)_2D_3$ and have speculated that extrarenal $1,25(OH)_2D_3$ production is involved in the autocrine/paracrine regulation of monocyte differentiation into MAC or DC as it exerts an inhibitory effect on DC differentiation but induces MAC differentiation [7–10]. Accordingly, vitamin D receptor deficient mice which show an unresponsiveness to $1,25(OH)_2D_3$ have increased numbers

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of DC in lymph nodes suggesting a physiologically relevant inhibition of DC differentiation by $1,25(OH)_2D_3$ [11]. Both, MAC and DC are present in the skin as well as the gut, the only organs where high levels of the $1,25(OH)_2D_3$ precursor vitamin D3 are found. Therefore we were interested as to whether human primary MAC and DC can also perform the hydroxylation at position C-25 which represents the first step in the generation of the active vitamin D3 metabolite $1,25(OH)_2D_3$. Schuessler et al. [12] have reported that skin is an autonomous organ with respect to the synthesis of $1,25(OH)_2D_3$ and that primary human keratinocytes can convert vitamin D3 into hormonally active $1,25(OH)_2D_3$. In addition, Vantieghem et al. [13] reported recently that UVB induces $1,25(OH)_2D_3$ synthesis in the intestinal CaCo-2 cell line and the macrophage cell line THP-1. We found that besides keratinocytes, CaCo-2 cells, and myeloid cell lines, also human primary MAC and DC are a possible source of $1,25(OH)_2D_3$ from the precursor vitamin D3 and therefore can perform both hydroxylation steps which normally are thought to occur only in liver and kidney. Our results indicate a possible role of local $1,25(OH)_2D_3$ synthesis by myeloid cells from vitamin D3 in the skin and gut.

Materials and methods

Chemicals. All chemical reagents used were purchased from Roche (Mannheim, Germany) unless otherwise noted. 1α,25-Dihydroxyvitamin D_3 was a gift from Roche (Basel, Switzerland).

Cell separation and culture. Blood mononuclear cells of healthy donors were isolated by leukapheresis and gradient centrifugation over Ficoll/Paque (Pharmacia, Freiburg, Germany). Subsequently, a countercurrent centrifugation in a J6M-E centrifuge (Beckmann, München, Germany) was performed as described before in order to obtain purified monocytes [14,15]. The purity (>90%) of the monocytes was determined by morphology and the expression of CD14 antigen. After the isolation, monocytes were cultured with RPMI (Biochrom, Berlin, Germany) supplemented with antibiotics (0.5 U/ml penicillin and 0.5 mg/ml streptomycin), 1 mM sodium pyruvate, 50 mM mercaptoethanol, 1× MEM non-essential amino acids, 1× MEM vitamins and 0.22 mg/ml L-glutamin (Gibco BRL, Eggenstein, Germany). For the differentiation of MAC, monocytes were cultured for up to seven days at a density of 1×10^6 cells/ml in medium containing 2% human AB-serum (Sigma, Deisenhofen, Germany). Dendritic cells were generated by culturing monocytes for 7 days in the presence of 50 U/ml IL-4 (Promocell) and 200 U/ml GM-CSF (Leukine/Sargramostim, Immunex). Lymphocytes represent a fraction of the countercurrent centrifugation containing about 70% T-lymphocytes, 20% B-lymphocytes and 10% NK cells.

To exclude donor variation effects, all experiments were performed with cells from at least three different healthy donors.

RNA preparation, reverse transcription and real-time PCR. Total RNA was extracted from primary cells by the RNeasy Mini Kit (Quiagen, Hilden, Germany) and RNA (1 μg) was reverse transcribed using Superscript II MMLV-RT (Invitrogen). Real-time PCR was performed with a Lightcycler (Roche Molecular Biochemicals) using the Quantitect Kit (Quiagen) according to the manufacturer's instructions. Primers used were:

CYP27A1:
S(5′-GCTCTTGGAGCAAGTGATG-3′);
AS(5′-AGCATCCGTATAGAGCGC-3′).

CYP27B1:
S(5′-AAGCACACCCGGTGAACTC-3′);
AS(5′-ATGGAGTCAGCGAGGTGAG-3′).

CYP24A1:
S(5′-GATGCTACACTCAGGCACCC-3′);
AS(5′-GCACTCAGTCCGCTTCCC-3′).

β-Actin:
S(5′-CTA CGT CGC CCT GGA CTT CGA GC-3′);
AS(5′-GAT GGA GCC GCC GAT CCA CAC G-3′).

18S rRNA:
S(5′-ACCGATTGGATGGTTTAGTGAG-3′);
AS(5′-CCTACGGAAACCTTGTTACGAC-3′).

Cycling parameters were: 95 °C for 15 s, 57 °C for 20 s, 72 °C for 25 s; 45 cycles (CYP27A1, CYP27B1), 50 cycles (CYP24A1) and 42 cycles (β-actin, 18S rRNA).

Immunostaining. Macrophages were immunostained with anti-Cyp27A1 (T19, goat polyclonal antibody, Santa Cruz Biotechnology), anti-Cyp27B1 (anti-1α-hydroxylase, sheep polyclonal antibody, The Binding Site, UK) and the respective isotype controls (goat and sheep polyclonal antibodies) in combination with a APAAP-technique using Fast-Red as chromogen. Photographs were taken at an original magnification of 50×.

1α,25-dihydroxyvitamin D_3-ELISA. Freshly isolated monocytes and lymphocytes, monocyte-derived macrophages on day 7, and dendritic cells on day 7 were used. Cells were seeded in 12-well plates at a concentration of 1×10^6/ml (serum free) with 1×10^{-7} M vitamin D3 (VD_3) or 5×10^{-8} M 25-hydroxyvitamin D_3 ($25(OH)D_3$, Sigma, Deisenhofen, Germany). After 24 h, cells and supernatant were harvested, pooled, frozen and 1α,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) determined according to the manufacturer's instructions (Immundiagnostik, Bensheim, Germany).

Results and discussion

Sterol 27-hydroxylase (CYP27A1) is of importance for cholesterol homeostasis in human macrophages (MAC) and its mRNA has been shown by several investigators to be present in MAC [16–18]. However, up to now these investigations focused on the role of CYP27A1 in bile acid metabolism whereas we show here that CYP27A1 is an active enzyme in terms of vitamin D3 metabolism. Monocyte-derived MAC, but also monocytes and monocyte-derived DC, express CYP27A1 mRNA and can perform both hydroxylation steps necessary to convert vitamin D3 into the active 1α,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$). We detected low CYP27A1 mRNA expression in freshly isolated monocytes (Fig. 1A), whereas monocyte-derived MAC showed a strong expression. This is in line with a recent publication by Hansson et al. who found a differentiation-dependent increase in CYP27A1 mRNA expression in MAC [16]. However, they investigated the enzymatic activity in terms of conversion of cholesterol to 27-hydroxycholesterol during human monocyte differentiation into MAC. In contrast to the CYP27A1 expression in human MAC, nothing is known about its expression during the differentiation from monocytes into DC. Therefore, we cultured monocytes for 7 days with IL-4 and GM-CSF and

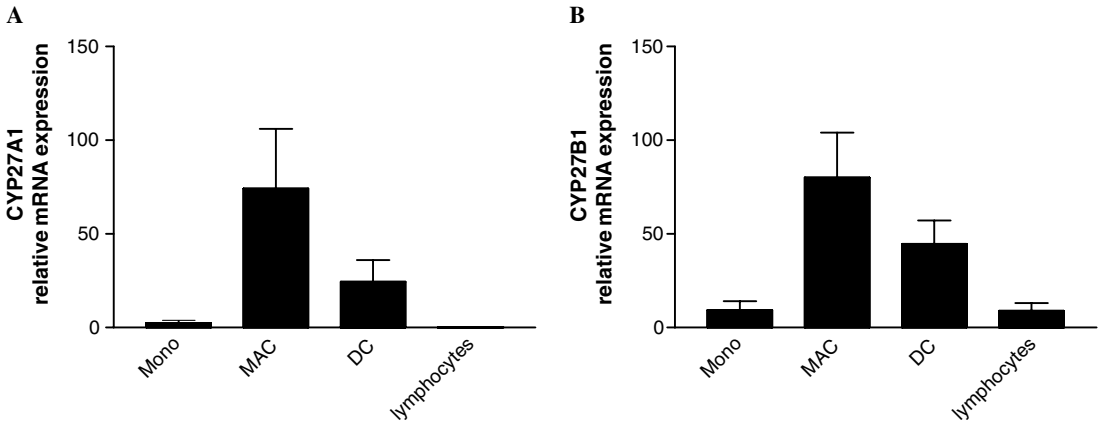

Fig. 1. Expression of CYP27A1 and CYP27B1 on mRNA level. Human primary monocytes (Mono) and lymphocytes were isolated by elutriation. Monocytes were cultured for 7 days to induce the differentiation into macrophages (MAC) and dendritic cells (DC). RNA was isolated and CYP27A1 (A) and CYP27B1 (B) expression was determined by LightCycler real-time PCR. The expression was calculated relative to β-actin mRNA expression (means ± SEM of triplicate runs of one representative experiment).

found an even lower expression of CYP27A1 mRNA compared to MAC (Fig. 1A). Terminally differentiated DC showed a similar expression (data not shown). No mRNA expression was detected in lymphocytes (Fig. 1A). Next we compared the expression of CYP27A1 with the expression of the CYP27B1, the enzyme which is necessary for the second hydroxylation step at position C-1α. As expected, MAC showed a strong expression of CYP27B1 and CYP27B1 expression was also detected in DC (Fig. 1B). These data show that MAC as well as DC express both enzymes important for the generation of the active metabolite $1,25(OH)_2D_3$. Immunohistochemical analysis revealed a specific staining for CYP27A1 and CYP27B1 in MAC as compared to the isotype control (Fig. 2). DC showed a lower CYP27A1 expression and no expression was found in lymphocytes (data not shown).

Next, we tested the enzymatic activity of CYP27A1 and CYP27B1 and determined the production of $1,25(OH)_2D_3$ from its different precursors vitamin D3 and $25(OH)D_3$. When we added vitamin D3 (VD_3) to the medium, we found a strong production of $1,25(OH)_2D_3$ by MAC and a low production by DC (Fig. 3A). This corresponds to the expression of CYP27A1 mRNA, which is responsible for the conversion of VD_3 into $25(OH)D_3$. This hydroxylation at C-25 generates an intermediate product which therefore seems to be the limiting step in the production of $1,25(OH)_2D_3$ by MAC and DC. Monocytes and lymphocytes did not show an enzymatic conversion into $1,25(OH)_2D_3$. In the literature no data exist regarding the conversion of vitamin D3 by primary macrophages but recently Vantieghem et al. [13] reported that UVB induces $1,25(OH)_2D_3$ synthesis in the macrophage cell line THP-1. This indicates that myeloid cells have the capacity to metabolize vitamin D3. Next we analyzed the ability to produce $1,25(OH)_2D_3$ starting with the more proximal precursor $25(OH)D_3$. Consistent with the mRNA and protein expression of CYP27B1, monocytes and lymphocytes showed no synthesis of $1,25(OH)_2D_3$ while MAC produced

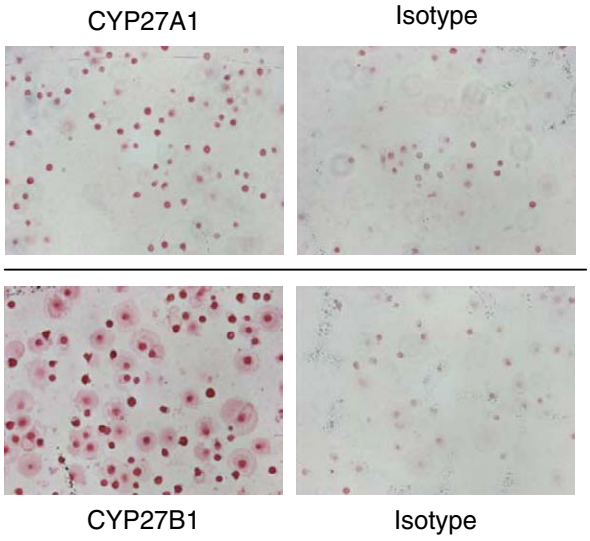

Fig. 2. Protein expression of CYP27A1 and CYP27B1 in macrophages. Monocyte-derived macrophages were immunostained with anti-Cyp27A1 goat polyclonal antibody, anti-Cyp27B1 sheep polyclonal antibody and the respective isotype controls. The staining was performed with a APAAP-technique using Fast-Red as chromogen. Photographs were taken at an original magnification of 50×.

high amounts of $1,25(OH)_2D_3$ (Fig. 3B). The production in DC compared to MAC (Fig. 3B) was much lower than we expected from the CYP27B1 mRNA expression data (Fig. 1B). One possible reason for lower amounts of $1,25(OH)_2D_3$ in DC could be differences in catabolism or degradation. We therefore analyzed the mRNA expression of cytochrome P-450 24-hydroxylase (CYP24A1), a regulatory multicatalytic enzyme, that directs the degradation of $1,25(OH)_2D_3$ [19]. As CYP24A1 expression is known to be induced by $1,25(OH)_2D_3$, which thereby regulates its own degradation, we analyzed its regulation in the presence or absence of $1,25(OH)_2D_3$. We found that CYP24A1 was only expressed in the presence of $1,25(OH)_2D_3$ (Fig. 4). This is in line with the published finding that

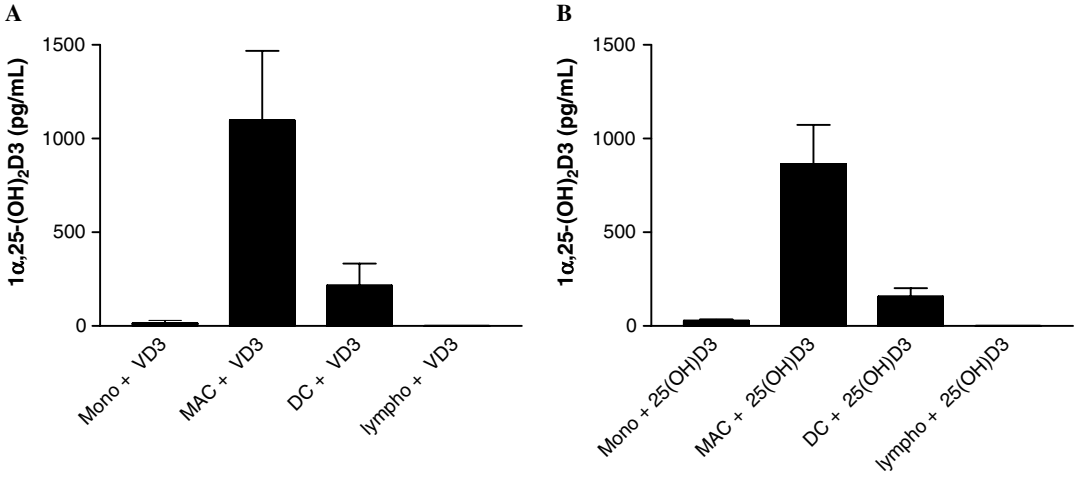

Fig. 3. Enzymatic conversion of vitamin D3 or 25(OH)D_3 by CYP27A1 and CYP27B1. Freshly isolated monocytes (Mono), monocyte-derived macrophages (MAC), monocyte-derived dendritic cells (DC), and freshly isolated lymphocytes (lympho) were used for the determination of the enzymatic activity of CYP27A1 (A) and CYP27B1 (B). The cell populations were further cultured for another 24 h under serum-free conditions with vitamin D3 or 25(OH)D_3. 1,25$(OH)_2D_3$ synthesis was determined by ELISA. The values represent means ± SEM of at least three experiments.

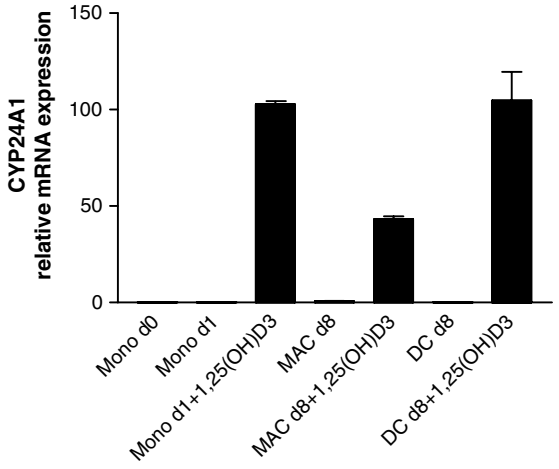

Fig. 4. CYP24A1 mRNA expression in monocytes, macrophages and dendritic cells. The expression of CYP24A1 mRNA was determined in the presence and absence of 1,25$(OH)_2D_3$. Freshly isolated monocytes (Mono d0), or monocytes cultivated with or without 1,25$(OH)_2D_3$ for 16 h (Mono d1 and Mono d1 + 1,25$(OH)_2D_3$). Macrophages and dendritic cells on day 7 were further cultured over night with or without 1,25$(OH)_2D_3$ (MAC d8 and MAC d8 + 1,25$(OH)_2D_3$), DC d8 and DC d8 + 1,25$(OH)_2D_3$). The CYP24A1 mRNA expression was analyzed by LightCycler real-time PCR and calculated relative to 18S rRNA. Given is the mean values ± SEM of triplicates of one representative experiment.

1,25$(OH)_2D_3$ leads to a concentration dependent increase in CYP24A1 mRNA in skin and the CYP24A1 promoter contains a 1,25$(OH)_2D_3$-response element [20]. In addition we found that DC on day 8 showed a higher relative expression of CYP24A1 than MAC. Accordingly, Hewison et al. [9] have reported that long term incubation of DC with 1,25$(OH)_2D_3$ for 3–9 days also induced CYP24A1 mRNA. This strong expression of CYP24A1 most likely leads to a rapid degradation of 1,25$(OH)_2D_3$ and contributes to the low amounts of 1,25$(OH)_2D_3$ detected in DC in comparison to MAC.

In summary, our results clearly show, that MAC express the enzyme CYP27A1 and are able to convert vitamin D3 into 25(OH)D_3, which is further converted into 1,25$(OH)_2D_3$. Vitamin D3 is present in high amounts especially in the skin where it is photochemically produced from 7-dehydrocholesterol, or in the gut, where it is taken up from the ingested diet. In both organs the direct conversion of vitamin D3 into 1,25$(OH)_2D_3$ could be of some importance for immune regulatory processes since skin and the gastrointestinal tract represent important immunological "barriers". Production of 1,25$(OH)_2D_3$ from vitamin D3 by MAC could suppress DC differentiation and lymphocyte activation in a paracrine way and thereby suppress adaptive immune responses.

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