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Regulation of 25-hydroxyvitamin D-1α-hydroxylase by IFNγ in human monocytic THP1 cells☆

Lut Overbergh, Katinka Stoffels, Dirk Valckx, Annapaula Giulietti, Roger Bouillon, Chantal Mathieu*

Laboratory for Experimental Medicine and Endocrinology (LEGENDO), University Hospital Gasthuisberg, Catholic University of Leuven, Leuven, Belgium

Abstract

1,25-DihydroxyVitamin D_3 ($1,25(OH)_2D_3$), a molecule with well-known actions in bone and mineral homeostasis, also plays a role in the immune system. Indeed, the receptor for $1,25(OH)_2D_3$ is found in most immune cells and important immunological effects have been described in vitro, reflected by its capacity to prevent autoimmunity and to prolong graft survival. The aim of this study was to elucidate the intracellular pathways used by the immune system to regulate $1,25(OH)_2D_3$ production. Therefore we studied the regulation of 25-hydroxyvitamin-D-1α-hydroxylase (1α hydroxylase) in THP1 cells by IFNγ, demonstrating that its induction is highly dependent on the activation/differentiation by PMA and occurred at a late time point (140-fold at 72 h, $P < 0.05$). Complete inhibition with actinomycin D indicated that the observed induction was, at least in part, a transcriptional event. Dose-dependent inhibition with cycloheximide demonstrated that the induction was dependent on "de novo" protein synthesis, a finding that correlates with the late time point of up-regulation. The data presented indicate a role for $1,25(OH)_2D_3$, activated by 1α hydroxylase, as a late down-tapering signal in the immune cascade.

Keywords: Macrophages; Vitamin D; Transcription factors; Real time PCR

1. Introduction

1,25-DihydroxyVitamin D_3 ($1,25(OH)_2D_3$), well-known for its action in bone and mineral homeostasis, also plays an important role in the immune system. Previously, we have demonstrated that in macrophages 1α-hydroxylase (CYP27B1), the enzyme responsible for the final activation of $1,25(OH)_2D_3$ production, is under the regulation of immune stimuli, such as LPS and IFNγ [1]. The aim of the present study was to analyze the pathways regulating $1,25(OH)_2D_3$ synthesis in macrophages, upon stimulation with IFNγ. The human monocytic/macrophage THP1 cell line was used as a model.

2. Materials and methods

2.1. Cell culture

THP1 cells (ATCC, Rockville, MD, USA) were grown in RPMI1640, supplemented with 100 μM β-mercaptoethanol, 10%FCS and antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin). PMA, actinomycin D and cycloheximide were purchased from Sigma (Bornem, Belgium). Human recombinant IFNγ was from Roche (Brussels, Belgium).

2.2. Real time RT-PCR

1×10^6 cells were used per condition. Total RNA was extracted using the High pure RNA isolation Kit (Roche). cDNA synthesis and real time quantitative PCR for β-actin and 1α hydroxylase were performed as described previously [1].

3. Results

THP1 cells were incubated with 100U/ml IFNγ, either in basal or in PMA-stimulated (20 ng/ml) conditions and cells

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* Corresponding author. Tel.: +32-16-34-59-70; fax +32-16-34-59-34.
E-mail address: chantal.mathieu@med.kuleuven.ac.be (C. Mathieu).

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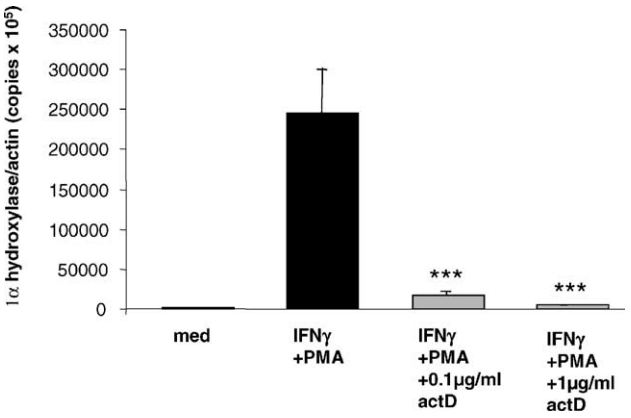

Fig. 1. 1α Hydroxylase mRNA expression in THP1 cells, dose-dependent inhibitory effect of actinomycin D. THP1 cells were incubated during 24 h with or without IFNγ (100U/ml) and PMA (20 ng/ml). Actinomycin D was added to the stimulated cells at two different concentrations, as indicated (0.1 μg/ml and 1 μg/ml). $***P < 0.005$.

were harvest after different time points of incubation, i.e. 6, 12, 24, 48 and 72 h. 1α Hydroxylase mRNA levels were quantified by real-time RT-PCR. Only in the combination treated cells, an induction of 1α-hydroxylase mRNA levels was evident, starting at 24 h (10-fold induction), increasing to 50-fold after 48 h and reaching maximal levels after 72 h of incubation (140-fold over baseline levels). No induction was seen in cells incubated with INFγ alone. Increasing the concentration of IFNγ to 2000 U/ml, did not induce 1α hydroxylase mRNA levels in undifferentiated THP1 cells. These data demonstrate that 1α-hydroxylase is highly inducible by IFNγ at the transcriptional level, although activation/differentiation of the THP1 cells by PMA seems to be a requirement for the observed induction.

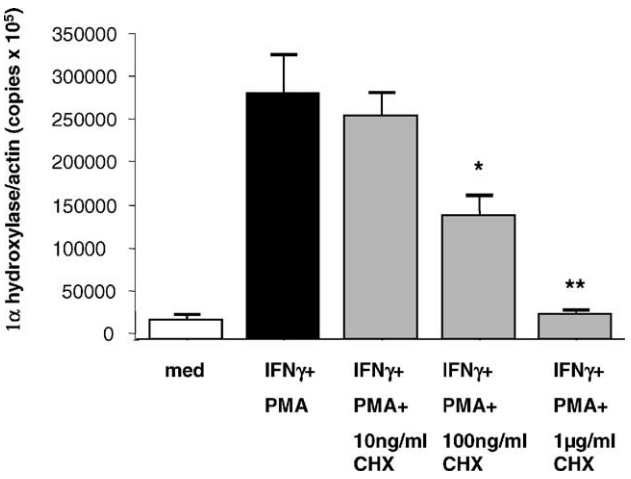

Fig. 2. 1α Hydroxylase mRNA expression in THP1 cells, dose-dependent inhibitory effect of cycloheximide. THP1 cells were incubated during 24 h with or without IFNγ (100 U/ml) and PMA (20 ng/ml). Actinomycin D was added to the stimulated cells at three different concentrations, as indicated (10, 100 and 1000 ng/ml). $*P < 0.05$ and $**P < 0.01$.

To gain a better inside into the mechanism of 1α hydroxylase regulation by IFNγ and PMA, the effect of different inhibitors on the synergistic induction was investigated. THP1 cells were incubated for 24 or 48 h with 100 U/ml IFNγ and 20 ng/ml PMA with or without the inhibitor.

Addition of two different doses of actinomycin D (0.1 or 1 μg/ml) resulted in a dose-dependent inhibition of 1α hydroxylase mRNA levels, indicating that the induction by IFNγ and PMA is, at least in part, a transcriptional event (Fig. 1). Addition of increasing doses of cycloheximide (ranging from 10 to 1000 ng/ml) to the cells resulted in a dose dependent inhibition of 1α hydroxylase induction, with an almost complete inhibition at the highest dose of 1000 ng/ml (Fig. 2). This indicated that "de novo" protein synthesis is involved in the synergistic induction.

4. Discussion

1α-Hydroxylase expression in macrophages is subject to a complex immune regulation. In this study we showed that the up-regulation of 1α hydroxylase occurs at a late time point, and is completely dependent on the simultaneous activation/differentiation of the cells by PMA. We demonstrated that the induction is a transcriptional event. Finally, we showed that the up-regulation is dependent on "de novo" synthesis, a finding that correlates with the late time point of induction.

The kinetics of up-regulation and the synergism between the IFNγ and PMA pathway point toward a physiological role for this system in inflammation. Indeed, it indicates that early after a bacterial infection, involving only macrophages, no induction of 1α hydroxylase is observed, and thus only a marginal production of $1,25(OH)_2D_3$ will take place. At a later time point however, when the immune cascade becomes more activated, and T lymphocytes induce their production of IFNγ, 1α-hydroxylase mRNA levels are induced to an enormous level. The time-point of up-regulation suggests a role for $1,25(OH)_2D_3$ as a late signal in the immune Based on previously published data that show an effect of $1,25(OH)_2D_3$ on macrophages, T cell proliferation and cytokine production, this role can best be described as a down-tapering signal of the whole immune cascade, thus preventing unrestricted immune reactions. In a previous study we showed a deficiency of this system in the NOD mouse, a model for type 1 diabetes. Moreover, our findings that administration of $1,25(OH)_2D_3$ prevents or delays insulitis and diabetes, and prolongs islet graft survival of transplanted islets, further strengthen this hypothesis [1–4]. In conclusion, the data presented here, combined with previously published data, point toward a major role for $1,25(OH)_2D_3$, activated by 1α-hydroxylase, as a late down-tapering signal in the immune cascade.

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