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Differentiation-associated expression of prostaglandin G/H synthase in monocytic cells

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Significant progress in the investigation of the regulation of prostanoid formation has recently been made by cloning a second gene coding for prostaglandin G/H synthase (PGHS; EC 1.14.99.1). In this study we examined the expression of the two PGHS isoforms during phorbol ester induced monocytic differentiation of human myeloid leukemia cells (U937). Murine and ovine PGHS-1 probes hybridized to 2.8- and 5.5-kb mRNA species, whereas the murine PGHS-2 probe hybridized to a 5.3-kb species. Western blot analysis using antisera to mouse PGHS-1 and to a synthetic peptide derived from a mouse PGHS-2-specific region revealed a band of 70 kDa for PGHS-1 and a doublet of about 85 kDa for PGHS-2. Unlike PGHS-2, which was not expressed in U937 control cells, both PGHS-1 protein and mRNA were detected in untreated U937 cells. TPA strongly induced PGHS-2 protein and also increased the amount of PGHS-1 protein. Correspondingly, a marked induction of PGHS-2 mRNA was found, but virtually no change in the expression of the PGHS-1 2.8-kb mRNA occurred. The induction of both PGHS isoforms turned out to be dexamethasone-sensitive. The suppression of PGHS-2 induction was more pronounced. These results suggest that both PGHS-1 and to a larger extent PGHS-2 contribute to the upregulation of prostanoid synthesis during monocytic differentiation.

Prostaglandin G/H synthase; Prostanoid; Monocytic differentiation; Corticosteroid

1. INTRODUCTION

Prostanoids are local hormones acting at or near their sites of synthesis in both an autocrine and paracrine fashion, e.g. as proinflammatory agents secreted by activated macrophages [1,2]. Therefore, the regulation of the cellular capacity to secrete prostanoids both as rapid response to a stimulus or slowly induced during differentiation is of great interest.

A major regulatory step occurs at the level of PGHS. The enzyme has been purified from sheep seminal vesicles [3] and cDNA clones were obtained from various sources [4–7]. These hybridize to mRNAs of about 2.8 kb on Northern blots. Recently, a second PGHS gene (PGHS-2) has been cloned from sheep [8], mouse [9,10], and human [11], which hybridizes to a mRNA of about 4–5 kb and is not coordinately expressed with PGHS-1 [12]. In human peripheral blood monocytes the PGHS-2 gene was induced by LPS, TPA, or IL-1, while little or no change was observed in the expression of the 2.8-kb PGHS-1 mRNA [10,11]. Thus the upregulation of prostaglandin secretion during monocytic activation seems to be related to PGHS-2 induction. We were interested in whether changes in the induction of PGHS-2 also correlate with the increase in prostaglandin synthesis and PGHS activity observed during monocytic differentiation of human myeloid leukemia cells [13–15]. Therefore, the human monoblastoid cell line U937 was used as a model system. When treated with the phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate (TPA) these cells acquire a macrophage-like phenotype within two to three days [15–17]. The data presented here suggest that the glucocorticoid-sensitive increase in prostaglandin synthesis and PGHS activity in TPA-treated U937 cells [14,15] is due to an induction of both PGHS isoforms.

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Abbreviations: HEL, human erythroleukemia; HUVE, human umbilical vein endothelial; IL-1β, interleukin-1β; LPS, lipopolysaccharide; PG, prostaglandin; PGHS, prostaglandin G/H synthase; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; Tx, thromboxane.

2. MATERIALS AND METHODS

2.1. *Materials*

Dexamethasone phosphate (Decadron) and prednisolone hemisuccinate sodium salt (Solu-Decortin H) were obtained from Merck Sharp and Dohme, München, and progesterone was obtained from Sigma, München. TPA (Sigma, München) was kept as stock solution (10^{-3} M) in dimethyl sulfoxide and diluted in medium immediately before use.

2.2. *Differentiation of U937 cells*

Cells (5×10^5 cells/ml) were cultured in RPMI 1640 medium (Gibco, Karlsruhe, Germany) with 5% fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 1% L-glutamine, and differentiated with

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5 nM TPA for 3 days. Steroids (10^{-6} M) were added 4 h before the addition of TPA. Viability of the cells was determined by trypan blue exclusion.

2.3. *Western blot analysis of PGHS isoforms*

After sonication (3 × 10 s, 50 W) cytosol and membranes were obtained by differential centrifugation (10 min, 1,000 × g; 30 min, 100,000 × g). Microsomal protein (50 μg [PGHS-1] and 100 μg [PGHS-2]) was subjected to sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis according to Laemmli [18]. After electrophoresis, proteins were transferred to a nitrocellulose membrane (Schleicher & Schuell) by semidry blotting according to the manufacturers' instructions. After blocking with low-fat dry milk (5% w/v) the blot membrane was incubated overnight with a specific polyclonal rabbit anti-PGHS-1 antibody [19] or a polyclonal anti-PGHS-2 antibody generated by immunizing rabbits with a synthetic 17-mer peptide derived from a unique inserted region of murine PGHS-2 protein near the carboxyl-terminal, which is not present in PGHS-1 [20]. Blots were incubated with a goat anti-rabbit alkaline phosphatase conjugate. Color development at the sites of bound complexes was performed with Nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate [21]. As a control for non-specific binding of the second antibody, membranes were incubated without the specific antibodies. The stained bands were quantitated by laser densitometry.

2.4. *Northern blot analysis*

Northern blot analysis of total cellular RNA using the 1,156-kb *Eco*RI fragment from the 5'-end of the mouse PGHS-2 cDNA labeled with P-32 by random priming [22] was performed as previously described [15].

Northern blot analysis using the 2.3-kb coding sequence of the ovine PGHS-1 cDNA or the 2,767-kb PGHS-1 cDNA from mouse labeled with digoxigenin by in vitro transcription was essentially performed as described by the manufacturer (Boehringer Mannheim). Fluorographs and chemiluminographs were analyzed by densitometry.

3. RESULTS AND DISCUSSION

The kinetics of the TPA effect on PGHS expression in U937 cells were studied by Northern and Western blot analysis. In Figs. 1 and 2 the differentiation-associated changes in the levels of PGHS-1 and -2 proteins and mRNAs are quantitated and plotted.

3.1. *Regulation of PGHS-1 protein and mRNA levels*

Polyclonal antiserum against the murine PGHS-1 specifically recognizes a 70-kDa polypeptide, which was slightly expressed in untreated U937 control cells. Treatment with 5 nM TPA led to a steady increase in PGHS-1 resulting in a 13-fold induction after three days. Similar results obtained in promonocytic THP-1 cells [13] suggest that this induction generally occurs during monocytic differentiation.

Murine and ovine PGHS-1 probes hybridized to 2.8- and 5.5-kb mRNA species. Similar transcripts were also observed in human umbilical vein endothelial (HUVE) cells [11], erythroleukemia (HEL) cells [23], and in platelet and placenta samples [24], and were speculated to be splicing variants [23]. Similar to the occasional detection of the 5.5-kb PGHS-1 mRNA species in HEL cells [23], the expression pattern of the 5.5-kb PGHS-1 mRNA species was not as reproducible as that of the 2.8-kb transcript. At present, the significance and mechanism of generation of heterogeneity of PGHS-1 transcripts are not known. When normalized with respect to the ethidiumbromide fluorescence of the 28 S rRNA, the 2.8-kb PGHS-1 mRNA level in U937 cells was virtually unaffected by TPA (Fig. 1). The differences in the PGHS-1 expression at the protein and mRNA levels are discussed below.

3.2. *Regulation of PGHS-2 protein and mRNA levels*

Using the polyclonal antibody against a synthetic PGHS-2-specific peptide a double band at about 85 kDa appeared weakly after 16 h and could be strongly detected after 64 h of incubation with TPA (Fig. 2). The molecular weight of the human PGHS-2 has been determined by in vitro translation to be 70 kDa [11]. A lack of posttranslational modifications of PGHS-2 in rabbit reticulocyte lysates might account for this discrepancy. The nature of the 85-kDa PGHS-2 doublet, which was also found in LPS-primed rabbit alveolar macrophages at approximately 72 kDa [20], remains to be elucidated.

The ovine PGHS-2 cDNA probe hybridized to a 5.3-kb mRNA species clearly distinct from the 5.5-kb PGHS-1 transcript. Similar PGHS-2 transcripts have also been described in other human cells such as monocytes [10] or endothelial cells [11]. The PGHS-2 mRNA was not detectable in untreated U937 control cells, however highly significant induction occurred after one day. In addition, a transient induction of PGHS-2 mRNA occurred shortly after the onset of incubation with TPA (Fig. 2). This effect was observed as early as 30 min after addition of TPA (data not shown) suggesting that the protein kinase C pathway might be involved in the transcriptional regulation of this gene. The more pronounced differentiation-associated upregulation starting after one day of incubation presumably reflects a more complex interplay of different control mechanisms arising as part of the differentiation process. Remarkably, the rapid induction of PGHS-2 mRNA expression is not reflected by detectable early increase in expression at the protein level (Fig. 2). The small extent of the early mRNA induction relative to the late upregulation could account for the lack of detectable PGHS-2. Furthermore, it is interesting to note that the strong increase in the level of PGHS-2 measured after 64 h appeared approximately one to two days after the late effect of TPA on PGHS-2 mRNA. Therefore, TPA might induce additional posttranscriptional regulatory events.

3.3. *Effects of glucocorticoid hormone*

Dexamethasone, which almost completely suppressed the induction of PGHS activity in TPA-treated U937 cells [15], completely inhibited the upregulation of PGHS-2 protein and mRNA (Fig. 2). The rapid transient induction of PGHS-2 mRNA was only prevented when dexamethasone was added 4 h prior to TPA (data without preincubation not shown). Thus, the activated

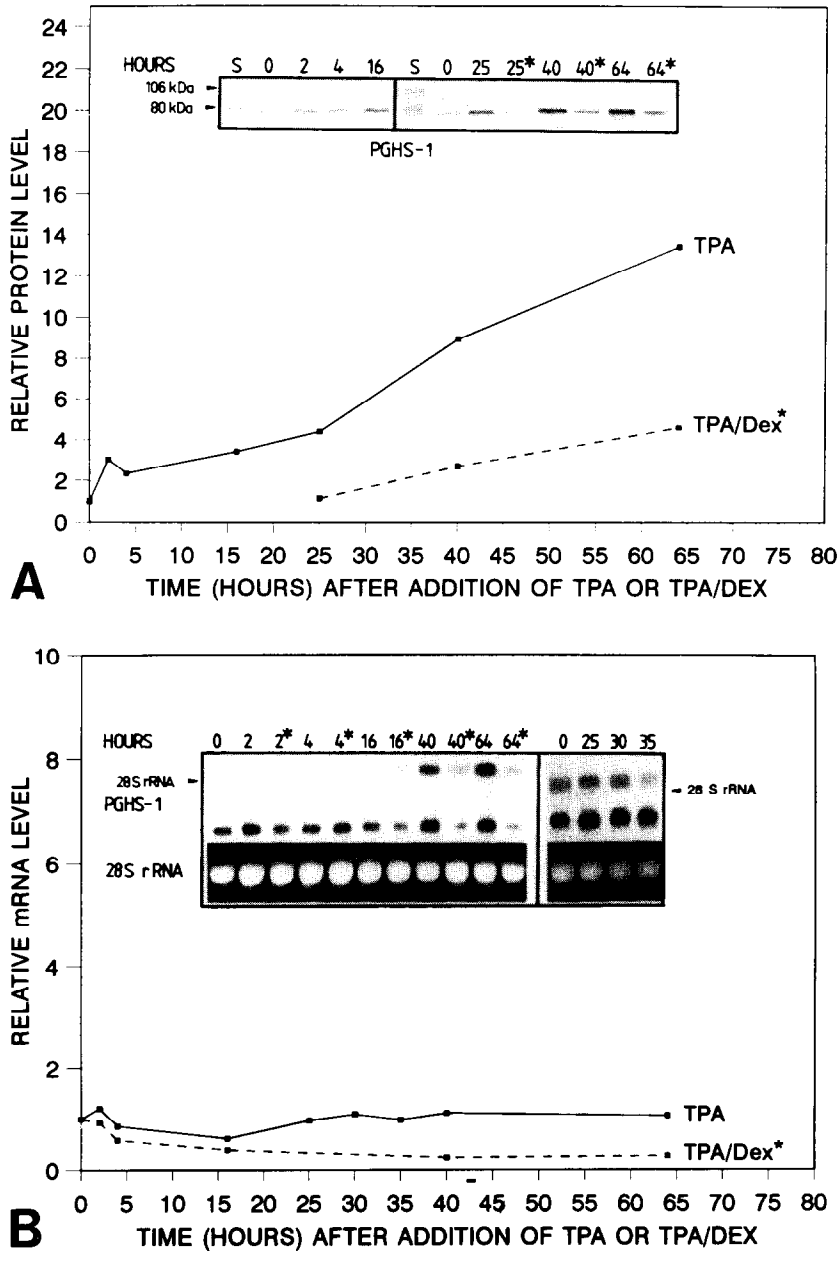

Fig. 1. Expression of PGHS-1 mRNA and protein in U937 cells. (A) U937 cells were treated with TPA at 5 nM or (*) preincubated with dexamethasone (Dex., 1 μM) for 4 h and then treated with 5 nM TPA for the indicated times. Cytosol and membranes were prepared by differential centrifugation. Microsomal protein (50 μg [PGHS-1] and 100 μg [PGHS-2, see Fig. 2]) was subjected to Western blot analysis as described. The stained bands were quantitated by densitometry. S, molecular weight standard. (B) Total RNA (10 μg per lane) from U937 cells treated as described in (A) were analyzed by Northern blot hybridization using an ovine digoxigenin-labeled PGHS-1 RNA probe essentially as described by the manufacturer (Boehringer Mannheim). The same results were obtained using a murine PGHS-1 RNA probe (data not shown). The signals were quantitated by densitometry and normalized with respect to ethidiumbromide-stained ribosomal RNA. Data are representative for two independent experiments.

glucocorticoid receptor does not seem to be directly involved in regulating PGHS-2 gene expression early after addition of TPA to U937 cells.

The effect of dexamethasone on PGHS-1 expression was less pronounced. It reduced the PGHS-1 mRNA level by about 75% and inhibited the TPA-induced upregulation of PGHS-1 protein by about 65%. The association of the total inhibition of PGHS-2 protein and mRNA induction with the nearly complete suppression of the upregulation of PGHS activity by dexa-

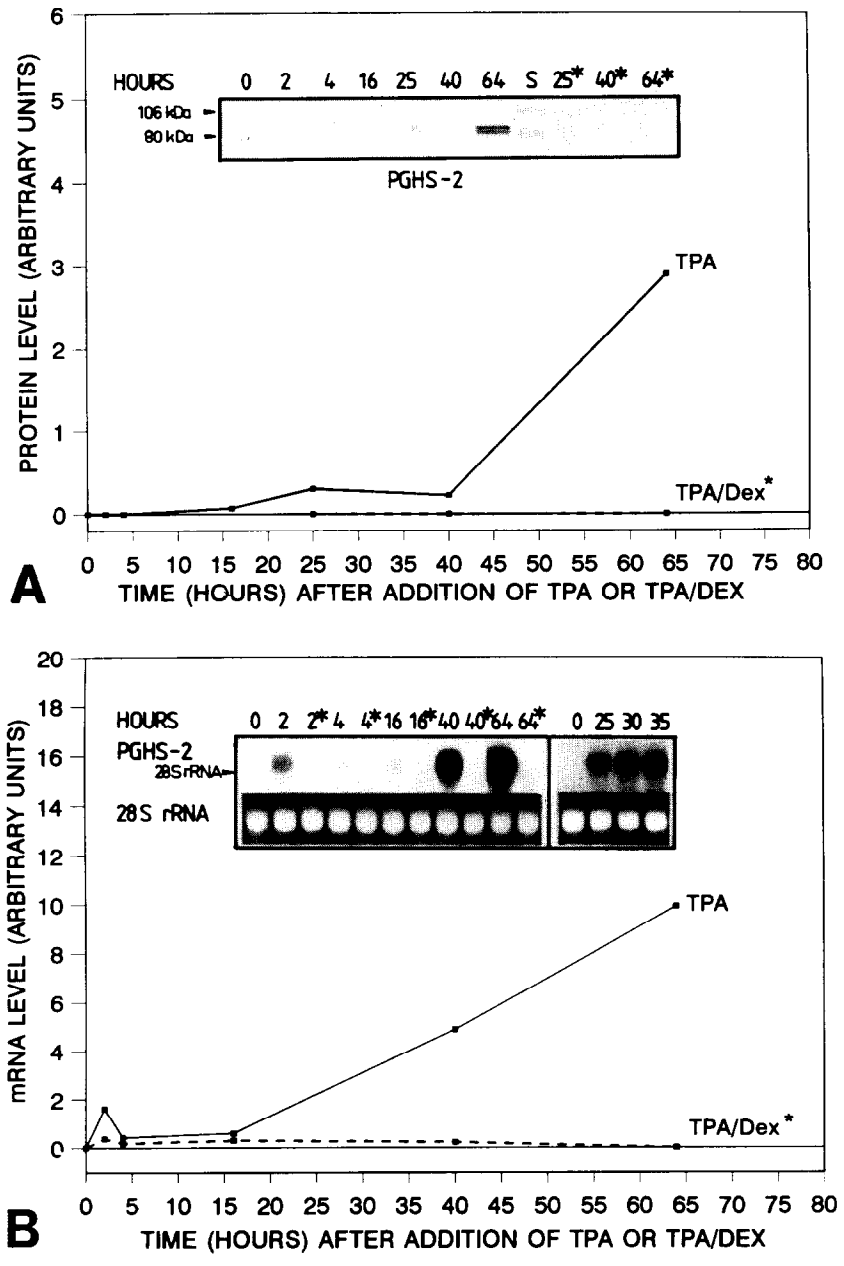

Fig. 2. Expression of PGHS-2 mRNA and protein in U937 cells (A) [see legend of Fig. 1A] (B) [see legend of Fig. 1B for the conditions]. Northern blot analysis using the 1.156-kb mouse cDNA fragment was performed as previously described [15]. The right panel of the inserted fluorographs represents two separate experiments performed to define the time course of PGHS-2 induction with improved resolution. The signals of the left inserted panel were quantitated by densitometry, normalized with respect to ethidiumbromide-stained ribosomal RNA and plotted.

methasone suggests that upregulation of PGHS activity in TPA-treated U937 cells is largely due to induced PGHS-2 expression.

Regarding the highly significant induction of PGHS-2 mRNA and the negligible increase in 2.8-kb PGHS-1 mRNA, one is tempted to conclude that PGHS-2 is the predominant isoform mediating the differentiation-associated upregulation of PGHS activity in U937 cells. On the other hand, Western blot analysis reveals that there is no quantitative correlation in the TPA or dexamethasone effects on the PGHS-1 mRNA level and polypeptide expression. Although the level of the PGHS-1 mRNA is not affected by TPA, the 70-kDa PGHS-1 protein is induced nearly 5-fold. Furthermore, despite the reduction in PGHS-1 mRNA expression dexamethasone did not completely prevent the increase in PGHS-1 polypeptide level. The underlying posttranscriptional control mechanisms remain to be character-

ized. Thus, examination of PGHS-1 transcript levels are likely to be insufficient to estimate the contribution of PGHS-1 enzyme to the overall PGHS activity. Therefore, the hypothesis that PGHS-2 predominantly mediates the inflammatory response [10] supported by correlations in PGHS activity and PGHS-2 mRNA level in LPS-treated monocytes [11], should be tested by examining PGHS-1 and -2 protein levels. The development of PGHS isoform-specific inhibitors should enable the determination of the relative contributions of both synthase isoforms in these systems. In summary, these data indicate that the expression of both PGHS-1 and PGHS-2 is increased during TPA-induced monocytic differentiation of U937 cells in a glucocorticoid-sensitive manner. This suggests that both isozymes, but predominantly PGHS-2, contribute to the concomitant increase in the capacity to secrete prostanoids.

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